CNS infections

P1667

Neonatal meningitis-bacteraemia from *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a Gram-positive microorganism that causes infection mainly to pregnant women and immunocompromised patients. Infection during pregnancy can lead to abortion or premature birth of a dead or infected baby. Infection during or after birth may lead to a late onset disease, septicemia or meningitis.
Methods: Blood cultures and CSF cultures were done by conventional methods as Bact Alert (Biomerieux) and the identification of Listeria by API Coryne (Biomerieux). Sensitivity tests against antibiotics involved the determination of MIC (E-Test) in Mueller Hinton agar with blood according to the guidelines of NCCLS.
Case-results: A case of a 13-day newborn female with fever (T:380C) is presented. Laboratory tests were done. Blood: Hct = 38.5% WBC = 27,200 (Neu. = 65%) TKE = 43 mm CRP = 1.69 mg/dl CSF: WBC = 1293/mm (Neu = 53%) Glucose = 45 mg/dl Total protein = 140 mg/dl Gram stain: negative. A Gram-positive bacterium, catalase positive, oxidase negative, was isolated by blood and CSF cultures. API Coryne identified *Listeria monocytogenes*. The microorganism was susceptible to penicillin, ampicillin, gentamicin, erythromycin, vancomycin and resistant to second and third generation cephalosporins. The patient was treated successfully (intravenous therapy with ampicillin, gentamicin).
Conclusion: *L. monocytogenes* may cause severe infections as bacteraemia, meningoencephalitis to the newborns. Early laboratory diagnosis and initiation of treatment has been associated with successful therapy. Clinical microbiologists have to apply the appropriate methods for the rapid isolation and accurate identification of *Listeria monocytogenes*.

P1668

Acute bacterial meningitis: epidemiological pattern in a paediatric hospital

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Objectives: To establish the incidence, epidemiological pattern and etiology of ABM.
Methods: A prospective epidemiological study of patient with ABM diagnosis hospitalized in Ricardo Gutierrez Children Hospital from Jan/92 to Dec/03 inclusive (12 years), was performed.
Results: A total of 631 cases of ABM were studied, 61.6% were male, median of age were 10 month (range: newborn-14 years old) and 69.6% were from the suburbs. One hundred and sixty-one patients (25.5%) received antibiotic treatment previously to admission (parenteral 47.2%). The isolation rate was 58.8% (371/631). The organism were: *Neisseria meningitidis* (Nm) 39.9% (148/371), *Haemophilus influenzae* (Hib) 19.1% (71/371), *Streptococcus pneumoniae* (Sp) 23.5% (87/371) and other agents 17.5% (65/371). The annual incidence was: 1992 (n = 32) Nm:15 (46.9%) serotype B:15, Hib:10 (31.3%), Sp:6 (18.8%); 1993 (n = 63) Nm:34 (54%) B:30, C:1, no typied:3, Hib:19 (30.2%), Sp:8 (12.7%); 1994 (n = 42) Nm:23 (54.8%) B:22, C:1, Hib:10 (23.8%), Sp:6 (14.3%); 1995 (n = 46) Nm:12 (26.1%) B:7, C:5, Hib: 9 (19.6%), Sp:11 (23.9%); 1996 (n = 45) Nm:14 (31.1%) B:3, C:9, no typied:2, Hib:12 (26.7%), Sp: 12 (26.7%); 1997 (n = 21) Nm:4 (19%)B:2, C:2, Hib:3 (14.3%), Sp:3 (14.3%); 1998 (n = 23) Nm:6 (26.1%) B:2, C:3, Y:1, Hib: 7 (30.4%), Sp:5 (21.7%); 1999 (n = 31) Nm:14 (45.2%) B:5,C:6,W135:2 no typied:1, Hib:0 (0%), Sp:10 (32.3%); 2000 (n = 17) Nm:10 (58.8%) B:5, C:5, Hib: 0 (0%), Sp:2 (11.8%); 2001 (n = 21) Nm:8 (38.1%) B:6, C:1, no typied:1, Hib:1 (4.8%), Sp:12 (57.1%); 2002 (n = 15) Nm: 6 (40%)B:3,C:2, no typied:1; Hib:0 (0%), Sp:4 (26.7%); 2003 (n = 15) Nm:2 (13.3%) C:1, no typied:1, Hib:0 (0%), Sp:8 (53.3%). School and day care contacts were found in 26.4% Nm cases; 2.7% were secondary cases. Most of cases were children <2 years: Nm 54.7%, Hib: 88.7% and Sp : 63.2%. Neurological complications depended upon agent: Nm: 9.5%, Hib: 38% and Sp 54% [Sp vs Nm RR 5.71 (3.35–9.75)]; and letality was 6.1%, 5.6% and 16.1% respectively [Sp vs Nm RR 2.65 (1.20–5.86)].
Conclusions: (1) There was a peak of Nm in 1993–94 with a decreasing incidence in 1995–03. Although Nm B is the most prevalent, Nm C incidence was increasing since 1995, and Y and W135 cases were sporadics. (2) After the Hib vaccine incorporation , since 1999 there are no cases, except 1 case in 2001. (3) Sp pattern has not changed through the years, and it had a higher morbimortality than the other organisms.

P1669

Bacterial meningitis guidelines and risk-factors: initial microbiological laboratory registration

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Objectives: The Dutch national guidelines for empiric treatment of bacterial meningitis (BM) takes into account age, specific risk-factors, and recent neurosurgery, and this information should be available for relevant microbiological advice. As antibiotic sensitivity patterns are similar in Danish bacteria and thus relevant for these guidelines, we explored if initial knowledge of these risk-factors, initial biochemical results, hospital- or community acquisition, and the advice given to the clinician were documented in the microbiologist's notes in a national 2-year survey.
Methods: In 2002 and 2003 all Danish departments of clinical microbiology reported data on culture-positive spinal fluids judged not to be contamination. Personal identification data, results of microscopy and culture, antibiotic sensitivity tests, and the 4 mentioned parameters (initial knowledge of risk-factors, initial biochemical results, whether hospital- or community acquisition, and documentation of the advice given) were reported.
Results: 427 episodes of BM were registered in the 2-year period. In 235 cases, the microbiologist's notes were not reported and thus not available. Concerning the 4 parameters, knowledge of risk-factors was documented in 31% (59/191; presence in 21); the initial biochemical results in 54% (103/192); the place of acquisition in 45% (72/192; nosocomial in 24); and documentation of the advice given in 93% (176/190).
Conclusion: Documentation in the microbiologist notes was incomplete in terms of place of acquisition, knowledge of risk-factors and initial biochemical results. The design of the survey was retrospective and the information may have been available, although not documented. Data availability is a prerequisite for optimal and relevant microbiological consultation in BM.

P1670

An evaluation of bacterial antigen detection tests in CSF of children in developing communities

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Acute childhood meningitis is a life threatening condition often leaving the survivors with various degrees of neurological impairments including learning problems. Rapid diagnosis is imperative for timely and specific treatment. Bacterial antigen detection tests by latex agglutination (L.A. tests) have been available for several years for this purpose.

Objective: To analyze the available data on L.A. tests retrospectively to observe their performance as to their sensitivity specificity and cost effectiveness in a resource poor setting.

Methods: Data was collected from CSF samples analysed over 10 years(1993–2003). Sources were A. five post graduate thesis projects supervised by the author in teaching hospitals 1993 (360CSFs) 1995 (420 CSFs) in GTB hospital, Delhi, India. 1999, 2001, 2003 in Kenyatta National Hospital, Nairobi (480 CSFs) B.983 CSFs data was contributed by two private sector hospitals in Nairobi, the Aga Khan Hospital and the Gertrude Garden children's hospital where the author is a sessional pathologist. Data from A and B were separately analysed for comparison purposes. Selected case files were reviewed to compile the outcomes.

Results: A striking difference was seen between the data from A and B. Data A found the L.A test an important diagnostic tool, the high cost being seen as the only limiting factor. In contrast data B brought out serious draw backs as follows: 1. these tests were negative in early meningitis cases, 2. important and frequent bacterial pathogens like enterric bacilli and *staphylococci* cannot be diagnosed, 3. Haemophilli other than type B were missed. 4. 5% of positive L.A tests were non specific agglutinations 5. 8% were non specific cross reactions 6. sensitivity on the whole was no greater than that of gram staining 7.for detection of *Strpt. pneumoniae* and *H. influenzae* b gram stain was found to be more sensitive than L.A test.

Conclusions: To justify the high costs of L.A tests must be used very selectively. The tests are cost effective if used in situations like 1. high clinical suspicion of bacterial meningitis where gram stain is negative 2. partially treated meningitis where culture is likely to be negative 3. the results of L.A test can only augment other parameters of diagnosis rather than replacing them. 4. routine use of L.A tests on all CSFs is not cost effective in a resource poor setting. Author is deeply indebted to the post graduate students involved in this work.

P1671

The prognostic value of cerebrospinal fluid procalcitonin in acute bacterial meningitis in children

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Background: It was shown that serum procalcitonin (PCT) was a better marker than serum C – reactive protein and cerebrospinal fluid (CSF) parameters in differentiating between acute bacterial meningitis (ABM) and viral meningitis in children. The production of PCT during inflammation is linked with a bacterial endotoxin and with inflammatory cytokines (TNF, IL-6). In children PCT is generally not detectable in CSF.

Objectives: The aim of our prospective study was to evaluate if a detectable level of PCT in CSF might be correlated with severity of disease and can therefore be used as a prognostic marker in ABM in children.

Methods: PCT levels was measured in serum and CFS of 33 children patients diagnosed with ABM admitted in our department. Meningitis was considered to be bacterial by a positive result on gram staining and/or bacterial culture of CFS or a positive blood culture in combination with clinical evidence of meningitis and CFS parameters (PMN counts, protein levels, ratio of glucose in CFS to glucose in serum, lactat CFS levels). PCT was estimated by an immunochromatographic assay (Brahms PCT – Q).

Results: The median age of the patients was 4.1 years (range, 3 months to 8 years). The microbiologically results yielded *Neisseria meningitidis* in 18 patients, *Streptococcus pneumoniae* in 9, *Haemophilus influenzae* type b in 4 and *Escherichia coli* in 2. A high serum PCT concentration (>10 ng/ml) identified in all 33 studied patients, except in two patients with previous antibiotic therapy; these had a lower serum levels of PCT (>5 ng/ml), that demonstrated the PCT decrease during treatment for ABM. In 16 children we found a detectable level of CFS procalcitonin: >10 ng/ml in 4, >2 ng/ml in 7 and >0.5 ng/ml in 5 patients. Levels of CFS procalcitonin >2 ng/ml (11 patients) were correlated with severity of disease (two children died, 5 recovered with serious neurological sequelae and 4 developed complications and required more than 21 days of treatment) and with young age (the median age was 2.2).

Conclusions: A CFS procalcitonin concentration >2 ng/ml, in MAB, was highly associated with a poor prognosis. We considered that measured of PCT in CFS will enable accurate prediction of mortality risk in children with ABM, particularly younger than 3 years age and can be included in a prognostic scoring system.

P1672

PCR-based surveillance in five districts in Burkina Faso and Togo during the 2003–04 epidemic season

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Objectives: To determine in selected districts of Burkina Faso (BF) and Togo during the 2003–04 meningitis epidemic season epidemiological characteristics of acute bacterial meningitis (ABM) and the sensitivity and specificity of cerebrospinal fluid (CSF) testing using polymerase chain reaction (PCR) versus culture.

Methods: We conducted a prospective health centre and hospital-based surveillance study. We collected CSF specimens and clinical/demographical information from suspected cases of ABM detected within all health facilities in three districts of BF and two district referral hospitals in Togo. A confirmed case was defined as detection of bacteria in CSF using latex agglutination, culture or PCR. Routine microbiological techniques were strengthened (only in BF) and we established for both countries appropriate specimen transport systems to National Reference Laboratories in BF for PCR testing or culture.

Results: A total of 329 clinical cases of ABM were included from BF and 207 from Togo. Among 117 (36%) confirmed cases from BF, we identified 53 (45%) *Streptococcus pneumoniae* (Sp), 49 (42%) *Neisseria meningitidis* (Nm) and 15 (13%) *Haemophilus influenzae* (Hi). Among 143 confirmed cases from Togo, we identified 66 (46%) Nm, 52 (36%) Sp and 25 (17%) Hi. In BF and Togo, Nm A represented 55% and 83%, and Nm W135 31% et 8% of Nm cases, respectively. In BF, most isolated Sp were serotype 1 (8/22) or 6 (5/22) (preliminary results, genotyping under process). The case fatality ratio in BF was 54%, including 44% for Sp, 6% for Nm and 27% for Hi. In BF, PCR sensitivity

and specificity varied from 94–96% and 97–100%, respectively, depending on the etiology. In Togo, PCR allowed confirmation of an additional 65% of cases compared to culture or latex agglutination.
Conclusion: Most ABM cases in selected areas of BF and Togo were due to Sp or Nm A; the proportion of W135 varied depending on the country and was lower in BF when compared to the previous epidemic season. The continued confirmed circulation of Sp serotype 1 emphasizes the need to include this serotype in any vaccine formulation designed for use in Sub-Saharan Africa. PCR performs well relative to culture and allows identification of substantially more cases than culture or latex agglutination. We recommend expanding similar surveillance to other areas of the African meningitis belt.

P1673

Invasive meningococcal disease in children in 1999–2003

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Objectives: Invasive meningococcal disease (IMD) is still life-threatening state. The aim of our study was to analyze clinical course and possibilities of diagnostics of IMD in children.
Methods: Retrospective analysis of IMD in children hospitalized at Department of Infectious Diseases of University Hospital in Prague in 1999–2003. Along with demographic data we evaluated also risk factors, clinical course, therapy and role of various diagnostic methods in clinical praxis.
Results: In monitored time period there were 29 children hospitalized at the clinic (11 boys and 18 girls), average age 11 years (6M-18Y). 43% of patients were admitted from another hospital. Average time of hospital stay was 15 days (9–31 days). Majority of children suffered from purulent meningitis with sepsis (14), meningitis (9), sepsis (2) and acute meningococcemia (4). Petechial rash was observed in 82% of cases, other skin symptoms in 27%, 10% of patient came for diarrhoea. 86% of patients showed neck stiffness at the time of admission. Fever was recognized as the most frequent symptom (72%), often accompanied by vomiting (45%) and headache (45%). Intravenous antibiotics were used in average 9.8 days. The first choice was penicillin G (31%) or 3rd generation cephalosporin (69%). Antibiotics in prehospitalisation care were used in 51% of cases. *Neisseria meningitidis* was detected in 34% by both culture and PCR, in 39% only by PCR, in 14% only by culture and once only by latex agglutination. 10% of cases were suspected only on base of clinical symptoms. Prevalence of serogroups: 54% B, 31% C, 15% non-determined.
Conclusion: We observed an increasing number of adolescent in last 3 years. It is related to the epidemiological situation in Czech Republic. The culture showed positive results only in 48%, it is related to the fact that in 51% of patients the antibiotic therapy was started before admission to hospital. In more than one third of cases the aetiology was confirmed only by PCR method. PCR is possible to increase percentage of laboratory confirmed cases. Culture still remains an important method with respect to the fact that it serves for determination of sensitivity to antibiotics.

P1674

Invasive meningococcal disease: a review of 33 recent episodes in a general hospital

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Objectives: The aim of study was to evaluate retrospectively the laboratory features of invasive meningococcal disease in patients admitted to 'Thriasio' General hospital.
Methods: During a 6-year period (1999–2004), 33 specimens from clinical suspected cases of meningococcal disease,(26 children and 9 adults), were received for laboratory confirmation. The diagnosis was established on CSF cytochemical characteristics, positive Gram stain, CSF or /and blood culture and non-cultural methods (detection of antigens, PCR).The antimicrobial sensitivity was determined by MIC (E-test). PCR and E-test were determined by the National Meningitis Reference Laboratory.
Results: 17 out of 33 specimens (51.5%), were positive for *Neisseria meningitidis*. The combinations of cultures and PCR results were as follows: 16-culture negative and PCR negative, 7-culture negative and PCR positive and 10- culture positive.13/17 strains were classified as serogroup B, 3/17 as serogroup C and 1/17 as serogroup A (polyvalent group).Resistance to sulphamethoxazole (MIC greater than or equal to 16 mg/l) was found in 4 out of 10 strains. All strains were sensitive to penicillin, rifampicin, cefaclor and ciprofloxacin.
Conclusions: Bacteriological culture remains the main laboratory method for detection of *Neisseria meningitidis* and for determination of antimicrobial resistance. In cases that Gram stain and culture are negative, PCR is a useful tool for diagnosis of invasive meningococcal disease.

P1675

The outbreak of nosocomial sepsis and meningitis caused by *Escherichia coli* in a neonatal intensive care unit

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Objectives: In three premature babies, treated in the Neonatal Intensive Care unit (NICU) in a period of 37 days of the year 2004, late-onset *Escherichia coli* sepsis and meningitis were recorded. Because babies were treated in the same room, the possibility of an outbreak was postulated and bacterial typing was requested.
Methods: Three babies were treated for some time together in the same six-bed room of NICU. Their gestational age was from 24–29 weeks and their birth weight from 660 to 1070 g. Two babies needed ventilatory support. The acute onset of illness occurred in the first baby at the age of 21 days. In the second and the third baby the illness occurred 32 and 37 days later at the age of 26 days and of 16 days, respectively; at this time the first baby has already left the room. *E. coli*, susceptible to tested antibiotics, was isolated from blood and CSF of all neonates. It was also isolated from tracheal aspirate of the first and nasopharynx and urine of the third neonate, but in none of the other samples. All babies were successfully treated with cefotaxime. Capsular antigen of invasive isolates was determined by agglutination, using bacterial antigen kit (Wellcogen). Typing was performed by macrorestriction analysis of chromosomal DNA in pulsed-field gel electrophoresis (PFGE). Bacterial DNA was isolated in agarose plugs and digested by the rare cutting enzyme Xba I (Roche) during overnight incubation. DNA fragments were separated in molecular biology-grade agarose (Bio Rad) in a CHEF DR III drive module in 0.5 M TBE running buffer.
Results: *E. coli* isolates from all babies carried K1 capsular antigen. Macrorestriction analysis of chromosomal DNA showed that all isolates had identical PFGE pattern.
Conclusions: Typing confirmed the outbreak of nosocomial sepsis and meningitis caused by *E. coli* in three neonates. Unfortunately, staff and environmental samples were not taken for investigation. By reinforcement of strict hygienic precautions, the outbreak terminated.

P1676

Microbiology of meningitis in hospitalised patients of Ural region, Russia: 7-year surveillance

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Objectives: To study microbiology of meningitis in hospitalised patients of Ural region (Regional Children's Hospital, Yekaterinburg) in a 7-year period (1995–2001).
Methods: CSF of meningitis suspected patients from 1 month to 14 years old hospitalized at Regional Children's Hospital were studied at Microbiology Laboratory. Complete cultures, biochemical identification, API and automated media as well latex agglutination with CSF were used to classify isolated organisms.
Results: In studied period 568 samples of CSF were submitted to Microbiology Laboratory. From this total, 135 (23.77%) had bacterial growth. Most isolated organisms were: *Neisseria meningitidis* (39.26%), *Streptococcus pneumoniae* (22.22%), *Haemophilus influenzae* (14.81%), *Staphylococcus aureus* (8.15%), *Acinetobacter calcoaceticus* (5.93%), *Pseudomonas aeruginosa* (2.22%), *Candida albicans* (2.22%), *S. pyogen* (2.22%) and others (2.96%).
Conclusions: Most cases of bacterial meningitis (76.30%) in children were caused by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. The most common cause of meningitis were *Neisseria meningitidis* of different types. Gram-negative bacilli isolation (*Acinetobacter calcoaceticus*, *Pseudomonas aeruginosa*), was due to a nosocomial infections.

P1677

Bacterial meningitis in infants under 1 year of age – our experience

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Objectives: To determine the clinical, bacteriological and therapeutic profile of this type of infection in newborns and sucklings.
Material and Method: Eighty less than one-year-old infants (15 newborns and 65 infants older than 1 month) with bacterial meningitis, admitted in our hospital between January 2000 and December 2003 were retrospectively studied.
Results: Most of the children were boys (67.5%), had significant co-morbidities (75%); the mean duration from the onset of the disease to hospital admission was 2.75 days and 32.25% of them already received antibiotics. Symptoms and signs were unspecific and in 23.75% cases the diagnosis was suspected only after the onset of generalized seizures. Only 60.1% of the CSF specimens were purulent. Other septic foci were present in 32.5% cases. The etiology of the disease could be established in only 48.75% of cases. In newborns, from 10 cases with known etiology, we isolated gram positive cocci: group *B. Streptococcus* in 3 cases, *S. aureus* – 2 cases, gram negative bacilli – 3 cases and gram negative cocci: *Neisseria meningitides* – 2 cases. After 1 month of age, from 29 patients with known etiology, the main pathogen was *Neisseria meningitides* (12 cases) followed by *H. influenzae* (6 cases) and *S. aureus* (4 cases). Most organisms were sensitive to beta-lactam antibiotics (76.92%). The empirical etiological treatment used an association of two antibiotics (ampicilin 11.63% cases, a third generation cephalosporin in 39.54% cases, a fluoroquinolone 12.7% cases, Vancomicin 4.65%). Dexamethazone was used, for a mean duration of 5.62 days, in 32.5% of cases. The mortality rate was 21.25% (33% in newborns).
Conclusion: During the studied period and for this age group, bacterial meningitis was an elusive disease, still with a poor prognosis, despite vigorous etiological and supportive care.

P1678

Chitotriosidase activity in cerebrospinal fluid of children in diagnosis of serous and purulent meningitis

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Mammalian chitotriosidase belonging to chitinase family, plays the defense role as universal protector against chitin-containing pathogens (Fusetti et al., 2002). It was suggested that humans deficient in chitotriosidase activity are more susceptible to infections. The aim: to evaluate chitotriosidase activity of cerebrospinal fluid of children and teenagers with serous and purulent meningitis.
Objectives: 53 persons of 5 months to 14 years old, including 17 patients studied – 2–4 times in dynamics of serous and purulent meningitis.
Methods: Chitotriosidase activity was measured against fluorogenic substrate according to method of Guo et al. (1995).
Results: Low chitotriosidase activity was registered in cerebrospinal fluid of children with serous meningitis (similar to activity in serum). In purulent meningitis this index was increased 5-times in the beginning of disease (correlation with increased PMN number, young PMN forms and protein concentration) and 2-times after effective treatment course (with normalization of peripheral blood leukocytes number). The cellular origin of elevated chitotriosidase in cerebrospinal fluid is unknown, possibly – from activated macrophages. The dramatic (up to 1000-times) increase of serum chitotriosidase (originated from overloaded macrophages) was registered in Gaucher disease, lysosomal storage disease. We have observed that macrophage activation in rat and mice models was followed by several times increase of serum chitotriosidase activity.
Conclusion: One can conclude that chitotriosidase activity in cerebrospinal fluid can be used in differential diagnostic of serous and purulent meningitis in children and enzyme is involved into pathogenesis of purulent meningitis in children.

P1679

A case of ventricular drainage infection with a rare pathogen in cerebrospinal fluid: vancomycin-resistant *Enterococcus faecium*

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Objectives: To describe a patient, 7-month-old child with ventriculoperitoneal shunts for hydrocephalus who developed ventriculitis caused by VREF.
Methods: Two ventriculoperitoneal shunts were inserted just after birth and on the 2nd month of age. On the 6th month, both of them were removed because of dysfunction and external drainage was inserted. Then, he developed fever and lumbar puncture revealed high leucocyte count and protein concentration. CSF cultures yielded *E. faecium* which was resistant to ampicillin, erythromycin, gentamicin, penicillin G, vancomycin, teicoplanin and susceptible to chloramphenicol, ciprofloxacin, streptomicin, levofloxacin and rifampin by disk diffusion method. On the antimicrobial susceptibility tests, multidrug antibiotic therapy was changed from vancomycin and ceftazidime to chloramphenicol, rifampin and meropenem, and also rifampin–clindamycin-impregnated bactericeal shunt (Codman-USA) was inserted.
Results: He become a febrile and CSF cultures was sterile after 15 days of yielding *E. faecium*.

Conclusion: Implanting rifampin–clindamycin-impregnated bactericeal shunt and timely use of appropriate antibiotics for 10 days according to antimicrobial susceptibility testing seemed to be important in the resolution of VRE infections, especially in countries where linezolid and quinupristin–dalfopristin are not in use yet.

P1680

Linezolid in patients with pneumococcal meningitis

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Efficacy of Linezolid in patients with meningitis sustained by methicillin-resistant strains of *Staphylococci* and *Enterococci* has been reported, but only a few data are available about its efficacy in the treatment of pneumococcal meningitis. We enrolled consecutive adult comatose patients affected with severe pneumococcal meningitis, referred to our tertiary care centre during a period of 12 months, at risk for carriage of penicillin-nonsusceptible strains of *S. pneumoniae*. Patients with clinical pictures consistent with community-acquired bacterial meningitis were managed as follows: (i) *S. pneumoniae* antigen was searched on the Cerebro-spinal fluid (CSF) specimen by rapid immunochromatographic membrane assay; (ii) Ceftriaxone (100 mg/kg/day) and Linezolid (1200 mg/day) were administered to the pneumococcal antigen positive cases; (iii) linezolid was withdrawn when a penicillin-susceptible strain of *S. pneumoniae* grew form cultures. Combined treatments with Linezolid and Ceftriaxone or Cefriaxone alone were administered for 20 days. Thirteen patients with positive CSF and/or blood culture were included in the study. Median age was 57 years (range 27–79). Eight patients had chronic underlying diseases. All cases had low CSF glucose and high CSF proteins and cells. *S. pneumoniae* strains were penicillin-susceptible in 7 cases, penicillin-tolerant in 3 and penicillin-resistant in 3. On presentation, 11 cases had nuchal rigidity, 10 fever, 7 hypotension. Five had generalized seizures before our observation. Six cases required mechanical ventilation within 48 hours from appearance of symptoms. Eight patients (3 infected with penicillin-susceptible strains of *S. pneumoniae*) had 2 or more factors associated with adverse clinical outcome. Forty-eight hours after admission, all cases showed increase of CSF glucose and reduction of CSF proteins and cells, none tested positive for CSF *S. pneumoniae* antigens, blood and CSF cultures were negative. Overall, 2 patients died, 3 reported sequelae. Evaluating only patients infected with penicillin-nonsusceptible strains of *S. pneumoniae*, one died, one reported severe disability, 4 fully recovered. No haematologic, nefro- or hepato-toxicity was reported. Combined therapy with III generation cephalosporin and Linezolid should be considered in patients with severe meningitis, particularly when a MDR strain of *S. pneumoniae* is suspected and factors associated to high mortality rates are present.

P1681

Severe polymicrobial central nervous system infections and potential therapeutic role of linezolid

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Introduction: The emerging of resistant gram-positive cocci in the setting of surgery and intensive care units, recommends particular attention in the selection of effective therapeutic choices, leading to overcome microbial resistances, and blood-brain barrier.
Case reports: Three representative case reports (2 brain abscesses and 1 post-surgical meningitis), are discussed: in all patients (p) a salvage linezolid (L) therapy proved resolutory. In a patient (p),we could not rely on culture isolations of clinical, probably due to the prolonged antimicrobial therapy preceding neurosurgery. An empirical post-operative combination included imipenem and L. After improvement of neuroradiology, treatment was continued with oral L, avoiding i.v. administration and accelerating p discharge. From another p, a rare gram-negative oxidase-negative bacillus was isolated from local purulent drainage. The absence of immunosuppression, and combined surgical-antibiotic therapy, prompted clinical-bacteriologic success of this unusual localization of *Capnocytophaga* spp disease. Should the first-line therapy allowed microbial eradication, L achieved complete resolution of CNS abscess, which did not ameliorate after over 3 weeks of combined imipenem–amikacin–fluconazole administration, preceded by surgical drainage. With our third p, we underline the clinical-therapeutic evolution of a p who developed a post-surgical nosocomial purulent meningitis, failed to respond to a 3-drug association of vancomycin,imipenem,and gentamicin, but had a very favorable and rapid (10-day) course after L administration, in absence of relapses or sequelae. A post-surgical infection caused by multiresistant gram-positive agents may be strongly hypothesized, based on prior therapeutic failure, and immediate L response.
Discussion: Therapeutic options for CNS infection are still limited, and the prognosis remains severe, especially when gram-positive infection is of concern. In the treatment of post-neurosurgical cerebral abscess and meningitis, a key issue is represented by the low CSF concentration of glycopeptides, usually recommended as first-line therapy of resistant gram-positive organisms. When severe CNS infections are of concern, L is a safe and effective choice for the management of ascertained or highly suspected infections caused by Gram-positive pathogens.

P1682

Epidemiological features of meningococcal disease in Belarus

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Objectives: Group B *Neisseria meningitidis* has been the leading cause of meningitis in Belarus over the past 5 years, and has also been a frequent cause of meningococcemia and other generalised forms of disease. We present an updated appraisal of meningococcal disease in this country.
Methods: All data refer to the period 2000–2004, and were collected at the Medical State University, Reference Center for Infectious Diseases of the Belarus Ministry of Public Health.
Results and Conclusion: The correlation between a local meningococcal colonisation and the meningococcal disease shows one patient with purulent meningitis per 100–200 patients with pharyngitis and per 2000–3000 nasopharyngeal carriers (in practice, 2–5% of the population are healthy carriers of meningococci). Belarus shows, together with Russia, one of the highest rate of generalized meningococcal infection among all the former-USSR republics, namely 2.8 cases per 100,000 inhabitants in 2003. The age distribution for this infection has been quite unusual since from many years. So in 2004 the rates of meningococcal infection were 56.7 cases per 100,000 infants, 30.3 cases in the age from 1 to 2 years old, and 3.8 in the age from 3 to 6 years old, respectively. This distribution would suggest that parents and other household contacts are the main

source of infant infections. In 2004, both morbidity and mortality from meningococcal disease seem to have increased. The observed group consisted of 17 children that all died from bacteriologically-confirmed meningococcemia. 7 out of the 17 cases were infants and presented thymomegalia, which is known as one of the adverse comorbidity factors. All *N. meningitidis* isolates showed high and moderate susceptibility to penicillin, as determined by standard disk method, but penicillin therapy was not effective in these cases. All cases were accompanied by a strongly marked tendency to cardio-circulatory failure, with death ensuing within the first twenty-four hours of the onset of meningococcemia, and being accompanied by adrenal hemorrhage in the infants with thymomegalia.

P1683

Human ehrlichiosis in a group of patients with aseptic meningoencephalitis

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Objective: Human Ehrlichiosis is usually an uncomplicated tick-borne infection with unspecific symptoms that can be very similar or identical to common viral or aseptic meningoencephalitis. In patients hospitalized with aseptic meningitis and reporting the recent withdrawal of a tick, specific antibodies and PCR were examined.

Patients and methods: During the spring season 46 inpatients diagnosed with aseptic meningoencephalitis were enrolled into the study. The included criteria were: (1) a tick having been removed 2–6 weeks before the onset of the symptoms; (2) clinical symptoms of an acute viral-like disease; (3) CSF findings typical for viral infection. All patients with abnormal liver markers were tested for hepatitis A, B, C and all suspect cases were excluded, as well as all patients with laboratory positive Tick-borne encephalitis and *Lyme borreliosis* (one patient had low titre of IgG anti-TBE antibodies after vaccination). Antiehrlichial antibodies (*E. chaffeensis*, *A. phagocytophylum*) were detected by commercial kits (MRL Diagnostics, Germany). A nested PCR technique described elsewhere was used for detection of DNA. The target sequence was the Ank gene of *A. phagocytophylum*. Positive controls were isolated from the culture *A. phagocytophylum* (KlinLab, Prague, CZ).

Results: Anti-*A. phagocytophylum* antibodies IgM were detected in 1 patient (2%), IgG in 6 (13%) and both of them in an additional 6 cases (altogether 28%). IgG anti-*E. chaffeensis* antibodies were found in 5 (11%) patients. In any case antibodies against both agents were not found. Specific DNA (*A. phagocytophylum*) was found in the plasma of 5 patients. In all PCR positive cases, plasma anti-*A. phagocytophyllum* antibodies were found – once IgM, two times IgG and in two cases IgG and IgM antibodies were detected together. In four cases the clinical symptoms of meningoencephalitis were found, and in one patient mild meningitis was diagnosed. In two cases a macular rash was described with an elevation of liver enzymes. Hematologic abnormalities were found in three patients – leukopenia, leukocytosis and anemia. Four patients recovered quickly after two-week oral tetracycline treatment, one remained without treatment due to rapid improvement and was discharged.

Conclusion: The Czech population is exposed to ehrlichia infection and more intensive laboratory examination is advisable for endangered peoples.

Experimental CNS infections

P1684

Experimental study of fosfomycin combined with ceftriaxone, teicoplanin or vancomycin in the treatment of cephalosporin-resistant pneumococcal meningitis

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Objectives: The optimal therapy of cephalosporin-resistant (CepR) pneumococcal meningitis is still a problem and new antimicrobial combinations deserve to be tested. The aim of this work was to assess the in vitro and in vivo efficacies of fosfomycin (F) tested alone and in combination with: ceftriaxone (C), teicoplanin (T) or vancomycin (V) against a CepR pneumococcus.

Methods: we used the HUB 2349 strain (serotype 23F) with MICs (mg/l): C 2; F 16; T 0.03; V 0.25. Time-kill curves were performed using achievable CSF concentrations. Synergy, additive and indifference of a combination were respectively defined as >2, between 1 and 2, ±1 log ^{10} CFU/ml decrease compared to the most active agent alone. Meningitis in rabbits was induced by intracisternal inoculation of 10E6 CFU/ml. Eighteen hours later, therapy (Tx) was given (n > 8/group) over 26 h. Controls (n = 10) were inoculated and not treated. Tx groups (dose in mg/kg/d) were: F (1200), C (100), T (15), V (30), F + C, F + T, and F + V, and the standard regimen was C + V. Emergence of resistance to F was studied at 24 by performing E-test. ANOVA test was used in statistical analysis.

Results: In vitro studies at 6 h showed that F + T presented indifference and some combinations of F + C and F + V were synergistic or additive. In vivo, all combinations improved the activities of any antibiotics tested alone, being F + C and F + V statistically significant versus F monotherapy. Statistical data and bacterial killing rates (KR) in CSF calculated as delta-log10 CFU/ml (±SD) at 6 and 24 h, are resumed in the table. Emergence of resistance to F was detected in vitro when F alone and combined was studied at 24 h. In the animal model after 24 h of Tx, development of resistance was only found when F was administered alone (33.3%), all combinations prevented it.

Tx group	KR 0–6h	KR 0–24h
Control	+0.34 (±0.83)	+1.63 (±1.96)
F	−1.80 (±0.81)	−2.46 (±1.77)
C	−2.73 (±0.72)	−3.38 (±1.38)
T	−2.33 (±0.79)	−3.83 (±0.86)
V	−2.99 (±0.99)	−4.20 (±1.37)
C+V	−3.07 (±0.54)*	−4.95 (±1.21)*
F+C	−3.50 (±0.96)*	−4.52 (±0.84)*
F+T	−2.38 (±0.59)	−4.30 (±0.65)
F+V	−3.60 (±0.66)*	−4.30 (±0.97)

*$p < 0.05$ vs F alone (ANOVA)

Conclusions: The addition of fosfomycin to monotherapies (ceftriaxone, teicoplanin or vancomycin) improved the efficacy of antibiotics tested alone in the therapy of CepR experimental meningitis. F + C showed to be the most effective alternative, with

a similar activity of the standard empirical therapy (C + V). All combinations prevented the emergence of resistance to fosfomycin in the animal model unlike what occurred in time kill curves.

P1685

Efficacy of imipenem, sulbactam and rifampin in an experimental meningitis model caused by multiresistant *Acinetobacter baumannii*

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Objectives: Postsurgical meningitis due to multiresistant (resistant to all clinically used antibiotics except colistin) *Acinetobacter baumannii* (MrAb) is an important therapeutical problem. It is necessary to know the antimicrobials or synergistic active-combinations against MrAb in this clinical process. The aim of this study was to evaluate the efficacy of rifampin (RF), sulbactam (SB), and imipenem (IMP) in monotherapy and in combinations in an experimental meningitis model due to MrAb.

Methods: A multiresistant bacteremic strain was used. In vitro studies: MIC and MBC; bactericidal activity and synergy of the combinations (time-killing curves). PK parameters (Cmax, AUC, $t_{1/2}$) were measured after administration of a single dose of each antimicrobial. In vivo studies: 1st phase, characterization of a meningitis experimental model due to MrAb in New Zealand rabbits (2–3 Kg), with an intracisternal inoculum of 6–7 log cfu/ml. 2nd phase: Rabbits were grouped as CON (controls, without treatment), IMP (120 mg/Kg/d), SB (240 mg/Kg/d), RF (100 mg/Kg/d), IMP + SB, IMP + RF and SB + RF; animals received a single dose of treatment. Measures: bacterial clearance in CSF; WBC, brain edema, and lactate concentration in CSF. Statistical tests: Friedman and Kruskal-Wallis.

Results: MIC/MBC (mg/l): SB 32/32, IMP 32/32, RF 4/8. IMP (MIC) showed bactericidal in vitro activity; no synergies were found. The PK parameters were: Cmax (mg/l), 16.62, 75.33, 25.35; AUC (mg·h/l), 40.28, 57.07, 50.45; $t_{1/2}$(h), 3.38, 0.54, 1.74; for RF, SB and IMP respectively. All of the rabbits presented high levels of lactate, WBC and an increased brain weight due to the infection and brain edema. The treatments that showed a reduction of the bacterial concentration in CSF were: RF ($p < 0.001$), RF + IMP ($p < 0.01$), RF + SB ($p < 0.05$) and IMP + SB ($p < 0.05$).

Conclusions: In the experimental meningitis caused by multiresistant *A. baumannii*, rifampin in monotherapy and the combinations of rifampin plus sulbactam or imipenem, and imipenem plus sulbactam were effective in the clearance of bacteria from CSF.

P1686

Vancomycin versus teicoplanin in the therapy of experimental methicillin resistant *Staphylococcus aureus* meningitis

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Objective: MRSA is an emerging problem in bacterial meningitis. The main therapeutic choices in MRSA meningitis are glycopeptides. There is no human or animal study comparing teicoplanin and vancomycin in MRSA meningitis. In this study we aimed to compare antibacterial activity of teicoplanin and vancomycin in the treatment of MRSA meningitis in rabbit meningitis model.

Methods: Meningitis was induced by direct inoculation of ATCC 43300 MRSA strain into cisterna magna of New Zealand rabbits. After 16 h of incubation time, rabbits were separated into three groups vancomycin (V), teicoplanin (T) and control. (C). Group V received 20 mg/kg vancomycin q12 h, group T received 6 mg/kg teicoplanin q12 h and group C did not receive any treatment. Drug levels were measured by bioassay technique.

Results: At 0 h, CSF bacterial counts were similar in all groups ($p > 0.05$). When three groups were compared (table 1), bacterial counts in both treatment groups at either 12th or 24th h were significantly lower ($p < 0.05$) than control group. When two treatment groups were compared bacterial counts at either 12th or 24th h (table 1) and serum drug levels at 24th h (V: 7.9 ± 3.64 mg/l, T:10.8 ± 5.6 mg/l) were similar ($p > 0.05$).Number of living animals during the study period were similar in three groups (table 2). Drug level in CSF was higher than lowest limit of sensitivity (1–2 mg/L) in only 6 rabbits (4 animals in group T, 2 animals in group V) CSF/serum ratio ranged between 20 and 48% in group T and was 32% and 47% in the 2 rabbits in group V.

Table 1. Bacterial counts in CSF of rabbits during study period

	Bacterial count (log 10 cfu/ml)		
Treatment group	0th h	12th h	24th h
Control	4,539±0,576	5,396±0,569	6,147±0,578
Vancomycin	4,696±0,764	3,928±1,378	3,867±2,171
Teicoplanin	4,931±0,808	4,474±0,548	3,798±1,696

Table 2. Number of living

Number of living animals	Control group	Vancomycin group	Teicoplanin group
0th h	13	13	13
12th h	10	12	13
24th h	10	11	12

Conclusion: These data suggest that antibacterial activity of vancomycin and teicoplanin are similar in the treatment of experimental MRSA meningitis of rabbits

P1687

Increased expression of BDNF and proliferation of dentate granule cells after bacterial meningitis

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Objectives: Proliferation and differentiation of neural progenitor cells is increased after bacterial meningitis. In order to identify endogenous factors involved in neurogenesis, expression of BDNF, NGF and GDNF was investigated in a mouse model of pneumococcal meningitis.

Methods: Male C57BL/6 mice were infected by injection with log 4 CFU of *S. pneumoniae* into the right forebrain. Control animals received saline only. Mice were killed 30 h later (each

group n = 19) or treated with CRO (100 mg/kg twice daily) and killed 4 days after infection (each group n = 19). Gene expression levels of neurotrophins were measured by real-time PCR and BDNF protein synthesis was determined by Western blot. Immunohistochemistry was performed for BDNF and TrkB.
Results: Four days after infection, hippocampal BDNF mRNA levels were increased 2.4-fold ($p = 0.026$). Similarly, BDNF protein levels in the hippocampal formation were higher in infected mice compared to control animals ($p = 0.0003$). This was accompanied by an elevated proliferation of dentate granule cells as indicated by BrdU-incorporation ($p = 0.0002$). Conversely, NGF mRNA levels at 30 hours after infection were reduced by approximately 50% ($p = 0.004$). No significant changes in GDNF expression were observed. BDNF protein was located predominantly in the hippocampal CA3/4 area and the hilus. The density of dentate granule cells expressing the BDNF receptor TrkB was increased 4 days after infection ($p = 0.027$).
Conclusion: The increased synthesis of BDNF and TrkB suggests a contribution of this neurotrophic factor to neurogenesis after bacterial meningitis.

P1688

Anti-herpetic activity of phosphorylated derivative of plant polyprenols

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Objectives: This study was aimed at the assessment of anti-herpetic activity of phosphorylated derivatives of plant polyprenols.
Methods: In vitro experiments were carried out in the continuous culture of VERO cells. Polyprenyl immunostimulant (PI, phosphorylated polyprenyl with 10–14 prenyl residues) was added as 0.4% solution into culture at days –1, 0, and +1 in respect to herpes simplex virus type I (HSV-1) inoculation (strain L2, titre 107 TCID50/ml). HSV-1 was added into culture at a dose of 0,1 TCID50/ml. Then the effect of PI upon HSV-1 cytopathogenicity was assessed. In vivo experiments were carried out in mice and guinea pigs. Suckling mice (10–12 g) were inoculated i.p. with 10% of HSV-1-containing brain suspension of mice (dose 100 LD_{50}). PI at a dose of 400 mcg was inoculated i.p. at days –1, or 0, or +1 in respect to HSV-1-containing suspension inoculation. Anti-herpetic activity was assessed by increase in average life expectancy (ALE). In parallel the levels of IFN-a, and -g were determined in the spleen cells of mice inoculated with PI and HSV-1. Male guinea pigs (250–300 g) were infected with HSV-2, strain EC, by rubbing of HSV-2-containing suspension on scarified penile surface. PI was inoculated i.p. at days - 1, 0, +1, and +2 in respect to infection. The animals were monitored for 15 days.
Results: PI at a dose of 100 and 200 mcg/ml inhibited HSV-1 reproduction in the VERO cells culture approximately 100-fold when added into culture 1 day previous to HSV-1, or simultaneously. PI at a dose of 400 mcg exerted protective effect in mice following inoculation at day –1 in respect to HSV-1 (protective effect was 40%, and ALE 14.6 ± 0.29 days, compared with 10.0 ± 1.31 days in control, ($d < 0.01$); at day 0 (protective effect was 50%, and ALE 14.1 ± 0.85 days, $d < 0.05$). The protective effect was accompanied by increase in duration and a level of the IFN-a, but not the IFN-g synthesis. In parallel experiment with testing of HSV-1-containing brain suspension of mice in VERO cells PI treatment was found to decrease the HSV-1 titres by 2.0–3.5 lg. PI was very effective ($d < 0.01$) against genital herpes infection of guinea pigs. The maximal protective effect of PI (39.4%) was revealed at day 5 following infection. In the PI-treated group average duration of the disease was reduced up to 4.3 days in comparison with non-treated controls.
Conclusion: PI exerted pronounced antiviral effect against HSV-1 and HSV-2 in various in vitro and in vivo models.

P1689

Antiviral activity of phosphorylated derivatives of plant polyprenyls in different tick-born encephalitis models

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Objectives: This study was aimed at the assessment of antiviral activity of phosphorylated derivatives of plant polyprenyls in the in vivo experimental murine tick-born encephalitis (TBE) model, and in the in vitro TBE virus (TBEV) experiments.
Methods: In vivo studies employed BALB/c male mice, 12–14 g body weight, and unbred 2–3-day-old suckling mice. In vitro experiments were carried out in PEKF cell culture. The following substances were tested: 0.4% solution of polyprenyls of different chain lengths, including Phosprenyl (PP) with 14–18 prenyl residues, and polyprenyl immunostimulant (PI) with 10–14 prenyl residues. TBEV (a highly pathogenic Absettarov strain) was used as a culture fluid containing 1010 lg PFU_{50}/ml. Antiviral effects of PP and PI were assessed by numbers of mice surviving a combination of i.p. inoculation with 100 LD_{50} in 0.2 ml of TBEV, with the simultaneous i.m. administration of 100 mcg of PP or PI in 0.2 ml. Blood samples were collected daily from all infected mice for 7 consecutive days following the infection, and the sera were added to PEKF in order to reveal infectious TBEV. In the in vitro experiments TBEV titres in the culture were determined after enumeration of negative colonies in flasks with PEKF cells, and calculation of PFU. The experiments in PEKF were performed in the presence of PP or PI.
Results: Intracerebral inoculation of suckling mice with 0.03 ml of suspension containing the TBEV and 60 mcg PP resulted in 1000-fold inhibition of TBEV replication in the CNS of suckling mice: TBEV titres in the brain tissues were 3 lg LD_{50}, whereas in control mice TBEV titres reached 6 lg LD_{50}. Administration of 100 mcg PP and PI per mouse protected up to 80% mice against lethal TBEV infection. Infectious TBEV was recovered from blood sera of PP-treated mice only at days 6–7 (1 and 2 lg PFU_{50}, correspondingly) following the infection with TBEV and PP administration, while in the blood sera of control mice (without PP treatment), infectious TBEV was recovered at days 2–7 (2.5–4.5 lg PFU_{50}). PP and PI in concentrations 200 and 400 mcg/ml were shown to inhibit replication of TBEV in the PEKF culture 30-fold and 100-fold, respectively.
Conclusion: 1. Polyprenyl phosphates of different chain length (PP and PI) protected up to 80% of mice against TBEV-associated lethality in vivo. 2. PP and PI inhibited TBEV replication in PEKF cultures. The antiviral effect may occur at a stage of TBEV penetration into target cells.

Bloodstream infection

P1690

Time trend and seasonality of community-acquired bacteraemia in a Danish county as assessed from hospital registers

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Objectives: Origin of infection is an important distinction to make in bacteraemia research. Time trend and seasonality of community-acquired bacteraemia (CAB) have been ascertained in Denmark only for selected groups of pathogens. Therefore, we have done an 8-year register based study including all pathogens.
Methods: This retrospective study was done in North Jutland County (app. 490,000 inhabitants). Blood cultures (BCs) were processed by the Dpt. Clinical Microbiology, Aalborg Hospital. BCs were recorded as positive only if believed clinically relevant. Data on all BCs processed during 1995–2002 were retrieved from a departmental database and linked to date of admission by the unique Danish personal identification number. BCs drawn within 2 days of admission were deemed related to a community-acquired infection; likewise we took positive BCs within this time frame to indicate CAB. We analysed data graphically and by a log-linear model where time trend was fitted as a linear function and seasonality as a trigonometric function with a period of 1 year.
Results: The study base included 100,023 BCs. Of these 63,347 were potentially related to a community-acquired infection. Time trend was statistically significant with an annual increase of 6.9% (95% confidence interval (CI): 6.5–7.2%). There was a consistent seasonal pattern with 8–14% more BCs being obtained during the 1st quarter than during any other quarter of the year. A total of 6298 positive BCs (from 4833 patients) were obtained during the first 2 days of admission. Time trend was statistically significant with an annual increase of 6.0% (95% CI : 4.9–7.2%). Compared to the 1st quarter 7.7% more positive BCs were recorded in the 4th quarter and 5.3% less in the 2nd quarter; the 3rd quarter was nearly at level with the 1st (+2.3%). The peak-to-trough ratio was 1.10 (95% CI : 1.03–1.18). This had the consequence that the rate of positive BCs was lowest in the 1st and highest in the 3rd quarter.
Conclusion: We demonstrated rising trends both for total numbers and numbers of positive BCs. Patterns of seasonality were markedly different: most BCs were drawn during the 1st quarter, most positives were detected during the 4th quarter and the rate of positive BCs was highest during the 3rd quarter. Thus, both occurrence of CAB and the diagnostic efforts showed distinct annual variation.

P1691

Severe sepsis and septic shock: recent trends in the intensive care unit of a university hospital

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Objectives: Severe sepsis and septic shock are associated with mortality in the range of 20–35% and 40–80%, respectively. Yet, a recent US survey among patients with sepsis has suggested that mortality may be declining and etiology is changing (NEJM 2003;348:1546). The aim of this 3-yr study was to review the etiology and outcome of patients with severe sepsis and septic shock admitted to the MICU of a university hospital in Western Switzerland.
Methods: Retrospective analyses of demographic, clinical, microbiological and laboratory data and therapeutic interventions of all consecutive patients admitted for severe sepsis and septic shock in the MICU. Patients referred from other hospitals were excluded.
Results: From Jan 2001 to Dec 2003, 218 patients developed severe sepsis (n = 78, 35.7%) or septic shock (n = 140, 64.3%)(mean: 6 cases/month). Mean age was 60 ± 16 yr., M/F ratio was 62/38%, median APACHE II score was 22.5 ± 9.5. Sepsis was due to Gram-positive bacteria in 41.6% of cases, Gram-negative bacteria in 25.4%, fungi in 4.4%, malaria in 0.9% and was culture-negative in 27.7%. All-cause day 28 mortality was 25.6% (severe sepsis: 5.5%, septic shock 37.1%). While APACHE II score increased from 24.1 to 26.3, mortality of septic shock decreased from 43% (2001) to 31% (2003), possibly in relation with the introduction of new strategies (early goal-directed therapy and lung protective ventilation, only 2 patients were treated with activated protein C) during the study period.
Conclusion: Gram-positive are a leading cause of severe sepsis and septic shock in the MICU of a Swiss university hospital. All-cause day 28 was much lower than anticipated and decreased during the study period confirming some recent trends.

P1692

A comparative study of risk factors and outcome among outpatient-acquired and nosocomial-acquired candidemia

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Objectives: To describe all cases of candidemia that occurred in the outpatient setting and to compare risk factors and outcome among patients with outpatient-acquired and nosocomial-acquired candidemia.
Methods: Retrospective cohort study conducted in Santa Casa Complexo Hospitalar, Brazil, during 1995 and 2003.
Results: During the period of study, 210 patients developed candidemia at our Institution, and 9.0% were outpatient-acquired. Most of these outpatients were male, median age was 51 years old, and major underlying diseases were cancer (47.4%) and chronic renal failure (36.8%). Most of the candidemias in the outpatient group occurred within 24 h of hospitalization (63.2%) and 83.7% were caused by species other than *Candida albicans*, mainly *Candida parapsilosis* (36.8%). *Candida* was isolated from catheters in 21%, and most of them (52.6%) had been admitted to the hospital in the 60 days preceding candidemia. Compared to patients with nosocomial-acquired candidemia, chronic renal failure was more frequent in the outpatient group, who were also more commonly exposed to hemodialysis. Ileus, gastrointestinal bleeding, previous bacteraemia, use of proton pump inhibitors, previous stay in the ICU and requirement for antibiotics, blood transfusion, vasopressors and invasive medical procedures were more frequent in the nosocomial group. Overall mortality rate was higher than 50% in both groups.
Conclusion: Candidemia must be remembered as a potential etiology for sepsis in the community, and it is associated with a high mortality.

P1693

Soluble haemoglobin scavenger receptor is a prognostic marker in patients with bacteraemia

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Objective: Soluble haemoglobin scavenger receptor (sCD163) is a macrophage-specific marker and elevated levels have been observed in patients with pneumonia and sepsis. The objective of our study was to evaluate sCD163 as a diagnostic and prognostic marker in patients with bacteraemia.
Methods: 42 adult patients with community acquired bacteraemia admitted at a department of internal medicine at a tertiary hospital were included in a prospective manner. Daily blood sampling for sCD163 analysis was performed for up to five days. Laboratory analyses were performed with a sandwich ELISA using polyclonal rabbit anti-CD163 as the catching antibody and monoclonal anti-CD163 as the secondary antibody. The patients were classified according to SIRS criteria. Demographic data, co-morbidity, microbiological etiology, routine biochemical parameters, focus of infection, severity score and mortality on day 28 were recorded. The patient sCD163 levels were compared with a non-infected control group which consisted of 130 healthy blood donors.
Results: The median age was 71 years (range 20–97). The male-female ratio was 1.2. 23 patients had gram positive bacteraemia. 19 patients had gram negative bacteraemia. The mortality rate on day 28 was 16.6%. 5 patients had bacteraemia without SIRS. 17 patients had sepsis. 20 patients had severe sepsis. sCD163 concentrations are presented as median and range: 5.27 mg/L (1.13–35.95) in the 42 patients with bacteraemia, 4.78 mg/L (1.13–17.62) in patients with sepsis, 8.27 mg/L (2.01–35.95) in patients with severe sepsis. These measurements were significantly higher compared to the control group (median 1.87 mg/L)($P < 0.0001$). The survivors had a median of 4.4 mg/L (range 1.13–35.95). The non-survivors had a median of 15.74 mg/L (range 4.41–20.8). This was a significant difference ($P = 0.009$). No correlation was found between levels of sCD163 and C-reactive protein, leukocytes or polymorphonuclear leukocytes.
Conclusion: Higher levels of sCD163 were observed in patients with bacteraemia compared to a non-infected control group. A high level of sCD163 indicated a poorer prognosis in patients with bacteraemia. Further studies are needed to unravel the role of sCD163 as a prognostic marker of severe infections.

P1694

Bacteremia in neonatal intensive care units: bacterial isolates and susceptibility patterns

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Objective: To determine the frequency of bacterial isolates from neonatal blood cultures and investigate their susceptibility patterns.
Methods: Blood cultures were performed using the BacTAlert 30 automated system. Identification of microorganisms and susceptibility testing was performed using the Vitek 2 automated system (bioMerieux, France).
Results: From January 2001 to October 2004, 304 bacterial strains were isolated from neonatal blood cultures. 60% were Gram-positive and 40% were Gram-negative. The bacterial pathogens encountered were: coagulase-negative *Staphylococci* (CoNS) 45%, *Klebsiella* spp 19.4%, *Enterobacter cloacae* 7.9%, *Enterococcus* spp 6.2%, *Staphylococcus aureus* 5.6%, *Escherichia coli* 5.2%, *Pseudomonas aeruginosa* 4.2%, *Streptococcus* group B 2.3%, other Gram-negative 4%, other Gram-positive 0.2%. The Gram-negative organisms demonstrated high degree of resistance to ampicillin and ceftazidime (92.7% and 73.7% respectively), moderate degree of resistance to tobramycin, cefotaxime, cefoxitin and gentamicin (66.4%, 58.7%, 50% and 49% respectively) and low resistance to cotrimoxazole and amikacin (29.3% and 14.7% respectively). All were susceptible to imepenem. According to resistance phenotypes more than half of *Klebsiella* spp isolates (57.8%) and three *E.coli* isolates were characterized as extended spectrum beta-lactamase (ESBL) producers. Resistance rates for *S. aureus* were: oxacillin 58.8% (MRSA), gentamicin and tobramycin about 55%, erythromycin 5.8%. Full susceptibility was reported for clindamycin. CoNS presented 90% resistance to oxacillin, 75% to gentamicin and tobramycin and 50% to clindamycin and erythromycin. Nearly all *Staphylococci* were resistant to penicillin, while all were susceptible to glycopeptides. Resistance rates for *Enterococcus* spp were: ampicillin 21% and imipenem 29.4%. High-level resistance to gentamicin and to streptomycin was observed to 15.7% and 42.1% of isolates, respectively. One isolate was resistant to glycopeptides expressing a Van A phenotype. All group B *Streptococci* were susceptible to penicillin.
Conclusion: Periodic bacteriologic surveillance in neonatal units is necessary leading to effective empiric treatment of neonatal bacteraemia.

P1695

Epidemiology and clinical features of bacteraemia in a tertiary care hospital over a twelve-year study period

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Introduction: During the past three decades, a dramatic shift in the incidence of certain bacterial infections in both the community and hospital settings has been observed. In particular, the percentage of *Staphylococcus aureus* strains resistant to methicillin (MRSA) is continuously increasing as well as coagulase-negative *Staphylococci* resistant to methicillin and a considerably increasing number of infections due to gram negative bacteria.
Objectives: The objectives were to determine the rates and secular trends in bacteraemia at a tertiary care hospital, and the trends in antimicrobial resistance. Furthermore, relative risk factors for death by univariate analysis were evaluated.
Methods: During a 12-year period, from January 1992 to December 2003, a retrospective study was performed at the University Hospitals of Geneva (HUG) to obtain the profile of patients with positive blood cultures using data available at the HUG. These data included patient demographic characteristics, microbiological results, in-hospital patient hospitalization and outcome. All bacteremic patients with a positive blood culture were included in the study.
Results: During the study period, 469,418 patients were admitted to the HUG, averaging 39,119 patients per year representing a total of 8,947,654 patient-days of care. Over this period, 220,491 blood cultures were performed. Of these, 11,327 episodes of bacterial bloodstream infections were identified in 8,735 patients. The patient's mean age was 57.6 years. Fifty-eight per cent (n = 6595) of the episode occurred in men (4732 in females). The most commonly identified species were: coagulase negative *Staphylococcus* (CNS) followed by *Escherichia coli*, *S. aureus* and *Streptococcus pneumoniae*. The overall incidence of bacteraemia

was 0.04 episodes per 1000 admissions, or 0.078 episodes per 10,000 patient-days. Among the bacteremic episodes, 85.4% were caused by a single organism, and 47.3% were community-acquired. No significant changes in resistance patterns for the different bacterial species were observed, except for an increasing number of bacteraemia due to MRSA. The crude mortality rate was significantly higher in patients with nosocomial bacteraemia compared to community-acquired bacteraemia.
Conclusion: A progression in MRSA was observed and with a slight progression in CNS. There were no significant changes in resistance patterns. A predominance of nosocomial septicemia with a higher mortality rate was also documented.

P1696

Significance of staphylococcal bacteruria in the course of *S. aureus* bacteraemia

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Introduction: It has been reported that about 30% of patients with SA bacteraemia, have simultaneously a positive urine culture without a clear correlation with an apparent urinary tract infection. We performed a retrospective study to assess the significance of SA bacteriuria in patients with bacteraemia.
Materials and methods: From 2001 to 2004, 55 patients with SA positive blood cultures in whom urine cultures were obtained within 24 h of drawing of blood were identified from the Clinical Microbiological Laboratory. SA bacteraemia was defined by the presence of one or more positive blood cultures, and bacteriuria by the presence of any colonies of SA in the urine culture. Demographic variables, comorbidities, immunosuppression, urinary tract pathology or manipulation, renal function, proteinuria, pyuria, hematuria, source of bacteraemia and mortality were obtained from the medical charts. High grade bacteraemia was defined by positivity of all blood culture bottles taken in which bacterial growth was became apparent in less than 24 h.
Results: In 18/55 (32.7%) patients, blood and urine were positive for SA (group A) versus 37/55 (67.3%) where SA was present only in blood (group B). The two groups were similar in gender (men: 77% vs 76%), mean age (63 vs 61 y), immunosuppression (39% vs 50%), chronic renal failure (50% vs 31%), diabetes (11% vs 30%) and mortality (39% vs 28%). Urinary tract abnormalities or manipulation were present in 50% vs 30%(NS) and 17% vs 27%, (NS) respectively. Means of Charlson scores between groups were 2.28 and 2.74 (NS); mean renal creatinine clearance values were 51 and 57.7 ml/min (NS); pyuria, proteinuria and hematuria were present in 76% of group A vs 42% of group B ($p = 0.022$), 87% vs 53% ($p = 0.18$), 81% vs 56% ($p < 0.001$), respectively. In those patients with a clearance >40 mL/min, high grade bacteremia was present in 55.5% (5/9) of group A vs 20% (5/25) in group B ($p = 0.081$). In multivariate analysis only high grade bacteremia was related to the presence of SA in urine culture (OR = 22.1, (1.34–363.7; $p = 0.03$).
Conclusions: Pyuria, hematuria proteinuria and high grade bacteraemia are related to the simultaneous presence of SA in blood and urine. In patients with SA bacteraemia, the presence of the organism in the urine does not necessarily mean intravascular infection and /or higher mortality. However, in patients with preserved renal function the high grade bacteraemia could explain the presence of a positive urine.

P1697

Ten-year experience in bloodstream infections in liver transplant recipients. Changes in microbiological patterns between 1991–1995 and 1996–2000

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Objective: Bacteremia is a major cause of death in recipients of solid organ transplantation. After liver transplantation, bacteraemia has been documented in 24–35% of patients and accounts for 19–30% of all major infections. Mortality ranges between 24% and 36% in bacteriemic liver transplant recipients. The most frequent sources for bacteraemia in liver transplant patients is catheter related, pneumonia, and biliary. The aim of this study is to evaluate prospectively all cases of bacteraemia and fungaemia in liver transplant patients, source, mortality, and changes in microbiological isolations among 1995–1996 and 1996–2000 periods.
Methods: We prospectly collected all episodes of bloodstream infections in liver transplant patients since 1991 to 2000. In each episode, we collected the following variables: sex, age, date of transplantation, source of infection, type of microorganism, and outcome.
Results: In this period of time, 673 liver transplants were done in 628 patients, and 189 episodes of bloodstream infections in 149 patients occurred (24%). Male sex was 65%. Mean age was 49.6 (SD: 12.4, R:19–72). Crude mortality was 18% (34 patients). Nosocomial origin occurred in 135 (71%). Of 203 isolations, the most frequent were: *Escherichia coli* 47 (23%), plasmocoagulase-negative *Staphylococcus* 34 (17%), *Staphylococcus aureus* 30 (14 MRSA) (15%), *Enterococcus faecalis* 19 (9%), and *Pseudomonas aeruginosa* 16 (7%). Fungemia was present in 7 patients, 3 by *Candida albicans*, 2 by *Candida glabrata*, 1 by *Candida parapsilosis*, and 1 by *Cryptococcus neoformans*. We compared the periods 91–95 and 96–00 to analyze differences between groups. Middle age of patients was significantly lower in the period 91–95. We found no differences in mortality between the 2 groups. Nosocomial origin was more frequent in the first period ($p < 0.001$). In the second period, we found a higher number of abdominal source ($p = 0.001$), and the number of catheter-related bacteremia decreased ($p = 0.014$). In the second period, *E. coli* bacteraemia was higher (17 vs 30, $p = 0.035$) and *S. aureus* isolations lower (25 vs 5, $p < 0.001$).
Conclusions: Mortality in bacteremic liver transplant patients is high, and we found no differences between 1991–1995 and 1996–2000 periods. Nosocomial infections are lower in the last 5 years, with an increase in *E. coli* and a decrease in *S. aureus* bacteraemia.

P1698

Invasive listeriosis in Denmark

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Objectives: To review epidemiological, clinical, and microbiological data on 299 cases of invasive listeriosis, excluding materno-fetal cases, occurring in Denmark during the last 10 years.
Methods: Data regarding age, sex, isolation site, underlying diseases or conditions, immunosuppressive treatment, and clinical outcome within 1 month of diagnosis of infection, were analysed. Strains were submitted from 294 cases. The strains were serogrouped using antisera and further serotyped by PCR.

Results and conclusions: The annual incidence of invasive listeriosis ranged from 0.42 to 0.73 per 100,000 inhabitants. Of the 299 invasive cases in 298 patients, 221 were blood stream infections, 71 CNS infections, and seven focal infections (prosthetic infections in bones and joints plus peritonitis). Conditions known to predispose to infection were found in 70% of cases. Patients were evenly distributed throughout Denmark, and half of them were older than 70 years. Death occurred in 21% of cases. Blood stream and CNS infections carried the same mortality, while there were no deaths in patients with focal infections. Multivariate analysis disclosed the following: conditions known to predispose to infection were strongly related to death in patients below 70 years of age; above this age underlying conditions were not related to increased risk of death; and strains of serotype 4b caused higher case fatality than strains of serotypes 1/2a and 1/2c.

P1699

Morphological changes of *Listeria monocytogenes* when subjected in mild alkaline conditions

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Objectives: To investigate the effect of marginal alkaline conditions on the morphology of *Listeria monocytogenes* and its derived (sigB deficient) mutant strain.
Methods: Overnight cultures were resuspended and grown for 12 h in buffered and non buffered media at pH 7.4 (control), 9.0, 9.2, 9.5 and 9.7. Appropriate samples were taken at 3, 6 and 12 hours and the cellular dimensions (length, width and volume) were calculated using Scanning Electron Microscope (SEM) photomicrographs.
Results: SEM studies revealed that sublethally stressing alkaline treatment of *Listeria monocytogenes* induced cell elongation and the development of populations containing a wider diversity of cell sizes than non-sublethally stressed cells. At pH values above 9.0, atypical forms of *Listeria* were observed as single or paired filaments. The neutralisation of the medium appeared to reduce the increased cell size in non-buffered media while in buffered media there was a relationship between the exposure and the corresponding morphological changes. Filamentation was independent of the presence or absence of the sigB factor. Such effects were reversed by neutralisation of the suspension medium in buffered and non-buffered media, with septation within filaments, and division/separation of resultant cells within 3 hours.
Conclusion: Such alkaline induced sublethal stress may occur during a number of clinical circumstances, and during the usage of alkaline cleaning agents in food production and processing environments. The occurrence, persistence and recovery of filamentous and or other atypical forms of this undesirable pathogen may lead to failure to detect, or underestimation of the incidence/numbers of this organism in such environments, with significant implications for its control in clinical circumstances and public health.

P1700

Immunological and microbiological study of abortion samples for serotype determination of *Listeria monocytogenes* in Iran

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Listeria monocytogenes is a small gram positive rod which shows beta-hemolysis in sheep blood agar. The host range is wide, including mammals, birds, crustaceans, ticks and fish. Transmission from animal to man occurs by handling of newborn calves, infected foods and drinking infected milk. A wide variety of clinical syndromes are caused by *Listeria monocytogenes* ranging from a mild influenza-like illness to fulminant neonatal listeriosis associated with mortality rates of 54–90%. Under sterile conditions, 200 abortion samples and sera from patients were collected and stored at −70°C. The abortion samples were tested bacteriologically and the sera of these pregnant women tested by immunofluorescence method (IF). Samples were cold enriched at 4°C and subcultured in common and selective media. The results showed that 72 pathogenic bacterial strains were isolated and 5 of them were *L. monocytogenes*, which had caused abortion. The mother's sera was tested against 12 serovars of *L. monocytogenes* separately(IF test). Among the tested cultures, only 2.5% of samples were positive, but IF tests showed the existence of antibody against *L. monocytogenes* in 70.7% of sera. From positive aborted samples, strains of bacteria such as *Staphylococci*, *Streptococci*, *Enterobacteriace*, and *Bacillus* spp. were also isolated. False-positive reactions are caused by cross-reactions between *L. monocytogenes* and these bacterial strains. On the contrary, it is possible that many of these patients were contaminated by *L. monocytogenes* for a short period of their life before aborting, and as a result they had positive IF tests without true infection. Also Some reports shows fecal carriage of *L. monocytogenes* in about 0.6–16% of the population at any time .All the sera showed negative IF reaction for serovars 4a, 6a and 6b. A faint (weak) result was obtained with serovars 2,4d, 4e, 5 and 7.Serovars la,3 and 4b showed strong reactions with titres of 3200 in bacteriologically positive samples. So, the results showed that the most dominant serovars of *L.monocytogenes* which cause listeriosis in Iran are 4b, la ,and 3.The obtained results from this investigation compare with other researchers who determined serogrops of 1, 3 and 4 in UK , serogroups of 1, 3 and 4 in Europian contries (1 is the predominant type whereas in the mid-1960s, the dominant type was 4b). and serotypes of 1 and 4b in United States (but types 2 and 3 are rare). Type 4b comprises approximately two-thirds of all cultures.

P1701

Pseudobacteraemia due to contaminated normal saline sponges

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Objectives: During 2004, *Enterobacter* species were isolated from blood cultures of 2 patients in Chosun University Hospital. One was *Enterobacter aerogenes* and the other was *Enterobacter amnigenus*. But, two patient bacteria culture sensitivity and MIC were nearly same. We tried to find the origin and cause of *Enterobacter* spp bacteraemia outbreak. We retrospectively reviewed clinical record of two patients. One patient is healthy woman, but, the other woman is Liver cirrhosis patient. Both patients are asymptomatic, their vital and lab findings were within normal range. No common exposures other than venipuncture correlated with positive blood cultures were seen. *Enterobacter* spp. was cultured from Normal saline sponge used for wiper of skin disinfectant prior to venipuncture for blood culture.
Methods: Two clinical isolate and Normal saline sponge isolate were identified by phenotypic method and a nucleic acid based confirmatory test (i.e. 16S rRNA gene sequencing).
Results: Two clinical isolates and Normal saline sponge isolate were identified as *Enterobacter amnigenus*.
Conclusions: When unusual human pathogen such as *E. amnigeneus* was isolated with no clinical signs of sepsis. A psedobacteraemia should be suspected especially in case bacteria culture sensitivity and MIC were nearly same. Correct procedures for

blood culture were immediately reinstituted, No further cases of *E amnigenus* bacteraemia occurred after prohibition of the use of Normal saline sponge.

P1702

Prediction of bloodstream infections using TREAT. A causal probabilistic network for diagnosis and treatment of infections

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Background: Predicting the existence of bloodstream infection at sepsis onset allows for appropriate and economical management decisions. Withholding blood cultures may reduce both direct costs and those related to contamination, but can be guided only by highly specific criteria. TREAT is a computerized system modeled to diagnose the probability of sepsis, source of infection, microbiology, and recommend therapy based on its coverage and costs. Calibration to different locations is inbuilt.
Methods: Derivation: The system is a causal probabilistic network modeled from risk factors through pathogens to disease. Blood cultures were modeled using data derived from literature and local data (table). Validation: TREAT was calibrated to 3 sites (Israel, Germany, Italy) and tested in a prospective observational trial. All consecutive adults fulfilling criteria for sepsis were included. Opportunistic infections were excluded. Clinical, laboratory, and radiological variables available prior to prescription of empirical therapy were entered to the system. We evaluated TREAT's ability to predict clinically significant bloodstream infections. Discriminative power was assessed using a ROC curve.
Results: We included 1202 patients with a mean age of 59.4 years. Infection was community-acquired in 70.5%. Overall 30-day mortality was 10.2%. True bacteraemia was detected in 10.0%, while contamination occurred in 2.2% of patients. The most prevalent pathogens were *Escherichia coli* (27.5%), *Staphylococcus aureus* (14.2%) and *Klebsiella pneumonia* (7.5%). The area under the ROC curve generated using the probability for bacteraemia given by TREAT was 0.702 (95% CI 0.654–0.750). We identified 3 TREAT bacteraemia probability categories, significantly associated with documented bacteraemia ($p < 0.001$, Chi-Square test). The highest probability category identified a group of 240 patients of whom 22.9% were bacteremic. The lowest category identified 240 patients of whom only 1.3% were bacteremic (table), while contaminant growth was detected in 2.1%.
Conclusions: Using simple variables available within a few hours after sepsis presentation TREAT accurately predicted the occurrence of bacteraemia. Performance was demonstrated in 3 different countries. Using TREAT clinicians can rule out bacteraemia in 20% of patients presenting with sepsis with a specificity of 98.7%. TREAT identified a large group of inpatients evaluated for sepsis that can be safely managed without obtaining blood cultures.

TREAT Database	Calibration Database
Probability of bacteremia per site of infection and pathogen	Overall contamination rate
Probability of growth pre pathogen in aerobic and anaerobic bottles	Pathogen specific contamination rate
Probability of growth in multiple bottles, within same or different sets	Pathogen specific contamination rate per bottle
Automated detection sensitivity	
Previous antibiotic treatment effect	
Blood letting technique- associated contamination rates	

	Israel	Germany	Italy	All
N patients	348	611	243	1202
% bacteremia	11	8.9	9.1	10.0 (N = 1202)
% in category 1	1.7	0	1.5	1.3 (N=240)
% in category 2	9.7	6	9.1	8.6 (N=720)
% in category 3	27.3	18.5	24.2	22.9 (N=240)

P1703

Controlled clinical comparison of VersaTREK® versus BacT/ALERT blood culture systems

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Objectives: To assess the relative yield of bacteria and yeasts isolated from the blood of adult patients with suspected sepsis, we compared the new VersaTREK (TREK Diagnostic Systems, Cleveland, Ohio) automated continuously monitoring blood culture system to the BacT/ALERT 3D system.
Methods: Identical protocols were followed for both systems. Paired standard aerobic and anaerobic media were used; each of the 4 culture bottles was filled with 6–9 ml of blood. All bottles flagged positive by the instruments were subcultured to determine both true- (growth) and false- (no growth) positive cultures. Additionally, to assess false-negative bottles, terminal subcultures were done on all negative companion bottles to true-positive bottles. All isolates were identified by standard methods.
Results: All 4 bottles were adequately filled in 5,468 (79%) of the 6,884 4 bottle sets obtained. Of 413 clinically significant (based on previously published criteria) isolates detected in one or both blood culture systems, there was no overall difference in recovery of microorganisms; however, *Streptococci* and *Enterococci* ($P < 0.05$) were detected more frequently with VersaTREK. Of 233 clinically significant positive cultures detected in the aerobic bottles of both systems within 72 hours, growth was detected sooner in the VersaTREK (mean of 14.9 h) versus the BacT/ALERT 3D (17.9h) system. The reverse was seen in 181 comparisons with the anaerobic bottles: VersaTREK (mean of 21.9 h) and BacT/ALERT 3D (15.7 h). Both systems were comparable (P = NS) in detecting the 179 unimicrobial episodes of bacteraemia seen: 137 (77%) were detected in both systems, 23 (13%) only in VersaTREK and 19 (11%) only in BacT/ALERT 3D. False-positive rates for aerobic and anaerobic bottles, respectively, were 1.6% and 1.0% for VersaTREK and 0.7% and 0.8% for BacT/ALERT 3D. The number of false-negative aerobic bottles found were 3 with VersaTrek and 4 with BacT/ALERT 3D, whereas the corresponding numbers for anaerobic bottles were 3 with VersaTREK and 23 with BacT/ALERT 3D.
Conclusion: We conclude that the VersaTREK and BacT/ALERT systems are comparable for detection of bloodstream infections with bacteria or yeasts.

P1704

Solitary blood culture rate as a measure of institutional total quality

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Objectives: It is generally accepted that the solitary blood culture rate represent a good measure of routine institutional quality control. Our objective was to determine the frequency with which solitary blood culture samples obtained from adult

inpatients and outpatients were submitted to our laboratory during the first six months of 2004, as a measure of institutional total quality.
Methods: We measured the rates with which our laboratory received only solitary blood culture sets on adult inpatients and outpatients with suspected sepsis in the first six months of 2004. We did not evaluate the consequences of solitary blood cultures on clinical outcomes. The solitary blood culture rate was defined as the number of instances in which only 1 blood culture was performed on an individual patient during a 24-hour period divided by the total number of cultures performed during the study period in the outpatients and inpatients. Outpatient blood cultures came not only from community strictly, but also from nursing homes and residential home care centers.
Results: During the study period (the first six months of 2004), a total of 1884 blood culture sets was obtained from adult inpatients, 269 of which were solitary (rate:14.3%) and 1615 of which consisted of 2 or more sets (rate: 85.7%). In the same period, a total of 262 blood culture sets was obtained from adult outpatients, 75 of which were solitary (rate:28.7%) and 187 of which consisted of 2 or more sets (rate: 71.3%).
Conclusion: Data showed that solitary blood culture rates were lower in hospital (14.3%), where standard practice protocols to improve the efficiency of sepsis diagnosis exist, where these kind of measures are monitored as part of routine institutional quality control programmes and where workers are specifically trained to perform specialized tasks. Instead, solitary blood culture rates are higher in community setting, where standard practice protocols and solitary blood culture rates are not monitored routinely. We think that the frequency with which solitary blood culture samples were obtained from community patients should stimulate the community institutions to initiate specific corrective actions and continuous quality improvement programmes.

P1705

Clinical factors associated with contamination of blood cultures in the emergency room

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Objective: Contamination of blood cultures has important consequences in hospital dynamics since it may complicate the diagnosis and treatment of patients and delay hospital discharge. Some studies have compared different skin disinfectants or withdrawal techniques however there is another factor which should be taken into account, the patient. The objective of this study was to identify the clinical profile of adult patients with contaminated blood cultures (CBC) obtained in the emergency room (ER).
Methods: we retrospectively analysed all the adult patients with CBC performed in the ER in a 4-month period (June–Sept. 2003) and compared their features with a randomised sample of non contaminated blood cultures (NCBC) in the same period. We collected demographic data, underlying diseases, temperature, level of consciousness (LOC) and final diagnosis.
Results: 2979 blood cultures were submitted to the laboratory with 187 CBC (6.3%),corresponding to 151 episodes in 148 patients. We could analyze 146 of these episodes (96.7%). Mean age was 64.5 years,82 (56.2%) being males. The main bacteria implicated were: coagulase-negative *Staphylococci* (85.8%), *Corynebacterium* spp. (5.7%), *Bacillus* spp (3%) and polymicrobial (1.5%). Among the underlying diseases the presence of cognitive deterioration was of note in 27% of cases, diabetes mellitus (24.8%), neoplasm (13.1%), cirrhosis (8%), HIV infection (7.3%) and COPD (5.1%).No underlying diseases were found in 13.1% of these patients. A decrease was detected in the LOC in 44.5% of patients and only 36.6% had fever (T > 38°C).The main final diagnoses of these episodes were: urinary tract infections (25.7%) and respiratory infections (24.3%). Infection, fever or blood cultures were not described in 21.4% of the discharge reports. When we compared this group with NCBC group, CBC patients were older (64.5 vs 59.3), more often had cognitive deterioration at baseline (27% vs 7.3%), cirrhosis (8%vs2.4%) or any underlying disease (86.9% vs 61.4%) and more frequently showed a decrease in the LOC (44.5% vs 20%). In contrast, NCBC more often had neoplasm (26.8% vs 13.1%) and COPD (18.3% vs 5.1%). No other statistical significant differences were found.
Conclusions: Contamination of blood cultures affect older patients, almost all with underlying diseases, specially dementia, and often with a decrease in the level of consciousness. Thus, special care should be taken when performing blood withdrawal and interpreting blood cultures in these patients.

P1706

Evaluation of quantitative antibiotic susceptibility testing as a routine method to predict strain relatedness of coagulase-negative *Staphylococci* isolated from blood cultures

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Objectives: To date no precise criteria exist to predict genetic identity of coagulase-negative *Staphylococci* (CoNS) isolates recovered from consecutive blood cultures based on routine antibiotic susceptibility testing (AST) results to help differentiate true bacteraemia from contamination.
Methods: A total of 119 consecutive CoNS isolate pairs from patients with 2 or more blood cultures positive for CoNS that had been obtained within 7 days were selected from stored blood culture isolates recovered during a recent 2-years period. Isolates were identified to species level and tested for antimicrobial susceptibility by VITEK2 (BioMerieux, , France). Based on MIC data and the resistance category (S vs R) of each of the antibiotics – penicillin, oxacillin, ciprofloxacin, erythromycin, clindamycin, cotrimoxazol, tetracycline, gentamicin, fosfomycin, fusidic acid, and rifampicin – we classified pairs of isolates as concordant or discordant (i.e. ≥3 log 2 dilutions difference in MIC). The results were compared with molecular analysis of isolate pairs using pulsed-field gel electrophoresis as the gold standard.
Results: Concordant AST results were predictive for genetic identity: 100% sensitivity, 100% specificity, 100% positive predictive value (PPV), 100% negative predictive value (NPV). With a threshold of two or more antibiotic discrepancies to define non-identity, discordant AST results were predictive for genetic diversity: 100% sensitivity, 100% specificity, 100% PPV, 100% NPV. If discrepancy in just one AST result was used to define non-identity of isolates (n = 6), AST was only 50% predictive of genetic diversity.
Conclusions: AST determined by VITEK2 is a reliable, easy to perform, time and cost efficient tool to predict genetic relatedness of CoNS isolates from blood cultures. Our criteria may help distinguish true bacteraemia from contamination when confronted with 2 or more blood cultures yielding CoNS.

P1707

Typing of *Staphylococcus epidermidis* using multiple-locus variable-number tandem repeat analysis

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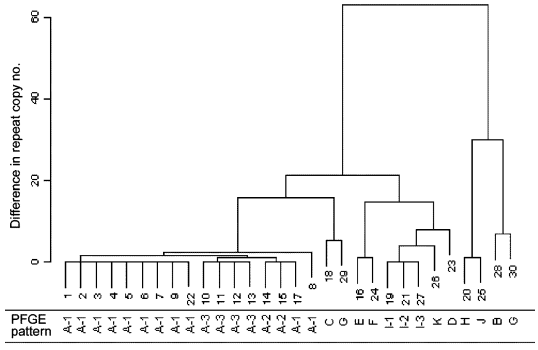
Objectives: The emergence of *Staphylococcus epidermidis* as an important nosocomial pathogen is related to advances in modern medicine. There is a lack of methods to distinguish clinically significant *S. epidermidis* from contaminant strains and rapid and reproducible methods for epidemiological studies. Multiple-locus variable-number tandem repeat analysis (MLVA) has proved efficient for high-resolution typing of several bacterial species. The aim of the present study was to develop a MLVA system for molecular typing of *S. epidermidis*.

Methods: The genome sequence of *S. epidermidis* ATCC 12228 was analysed for the presence of tandem repeated sequence using software programmes. Genomic DNA of 30 clinical isolates of multi-resistant *S. epidermidis* was subjected to PCR amplification of multiple genomic regions that exhibited tandem repeats. Variation in repeat copy numbers among isolates was determined by sizing of PCR fragments on agarose gels and verified by sequencing. All *S. epidermidis* isolates were typed using *Sma*I digestion and pulsed–field gel electrophoresis. Diversity values (D) were calculated using the formula $D = 1 - \text{SUM}(\text{allele frequency})^2$.

Results: The analysis of the genome of *S. epidermidis* ATCC 12228 identified 137 genomic regions with tandem repeated sequences. Fourteen regions were analysed in a subset of *S. epidermidis* isolates. Five regions showed variable numbers of tandem repeats and were further analysed in 30 clinical *S. epidermidis* isolates from a University Hospital. The variable repeat motifs displayed repeat sizes ranging from 18 bp to 58 bp in length. The number of detected alleles at individual repeat loci ranged from three to ten. Calculated D-values ranged from 0.50 to 0.82. The D-value for the combination of five repeat loci (all loci treated as a single allele) was 0.87. MLVA revealed 16 genotypes among 30 isolates. Observed PFGE genotypes resulted in a D-value of 0.84. It was apparent that MLVA and PFGE resulted in very similar clustering of isolates (Figure 1). Three out of five identified polymorphic tandem repeat loci were located in genes predicted to encode surface binding proteins that may have a functional role in adherence to host fibrinogen.



Conclusion: MLVA is a promising typing approach for molecular epidemiology studies of *S. epidermidis* due to the speed of analysis, a high discriminatory power, and an ability to produce discrete character data that can be easily exchanged among laboratories.

P1708

A pathogen or a contaminant: the emerging significance of *Kytococcus schroeteri*

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Background: 'Micrococci' are found as normal inhabitants of human skin and mucous membranes and are usually disregarded as contaminants in clinical isolates. Nevertheless, strains identified as members of the Micrococcinae suborder have been described to be increasingly involved as causative organisms in septicemia, endocarditis peritonitis, pneumonia and other severe systemic infections.

Patients and results: Following the recent description of *Kytococcus schroeteri*, four severe infections due to this pathogen were observed in different hospitals. After the first case, reporting a prosthetic valve endocarditis in a 34-year-old female without any other known diseases, a *K. schroeteri* infection associated with a prosthetic aortic valve was observed in a 38-year-old diabetic women. A further prosthetic valve infection affected the mitral valve of a 79-year-old patient. The first fatal case due to *K. schroeteri* was noted in a patient with asthma chronically treated with corticosteroids. This 71-year-old female referred to a French medical ICU for management of severe respiratory distress developed bacteremic pneumonia leading to distributive shock that progressed to multiorgan failure. In addition to phenotypical analyses, all isolates were confirmed on species level by 16S rDNA sequencing. *K. schroeteri* strains hitherto recovered were resistant to penicillin and oxacillin, but susceptible to glycopeptides, gentamicin, and rifampin. Of particular interest, rifampin in combination with other antibiotics (gentamicin, glycopeptides) was administered in all non-fatal cases.

Conclusions: Though it appears to be an organism of low virulence, the emerging number of reported cases of *K. schroeteri* infection has established the pathogenic potential of this organism. Thus, if an infection is clinically suspected, the repeated isolation of such isolates in the absence of other microorganisms should not be underestimated. Accurate identification of *Kytococci* is of importance as these species have been shown to be resistant to penicillin and -lactamase-resistant penicillins in contrast to members of other 'micrococcal' genera.

P1709

The changing spectrum of diseases causing fever of unknown origin in Turkey: a multicentre study

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Objectives: The spectrum of diseases causing Fever of Unknown Origin (FUO) not only seems to be determined by geographical factors but also appears to change over time. To update information on FUO and incorporate these new ideas, we conducted a prospective multicentre study on patients with FUO in ten Turkish University Hospitals.

Methods: The present study was undertaken between January 2003 and June 2004. The Petersdorf criteria (illness characterized by rectal temperature exceeding 38.3°C, evolving least 3 weeks, with no diagnosis after one week of inpatient investigation). were used in diagnosis FUO. Patient demographics, etiology of FUO that could be detected, clinical symptoms and signs were recorded.

Results: A total of 154 patients fulfilling the criteria of FUO were determined. Mean age was 42.95 ± 17.76 (17–75) and 56.5%

of the patients were males. A total of 154 immunocompetent patients with FUO were followed up of whom 53 (34.4%) had infections, 47 (30.5%) had non-infectious inflammatory diseases (NIID), 22 (14.2%) had malignant diseases, 8 (5.2%) had miscelleneous diseases. In 15.6% (24) of the cases, the reason for high fever could not be explained despite every effort. The most common infectious etiologies were tuberculosis [20 (37.7%)] and brucellosis [8 (15.1%)]. On the contrary, Adult Still Disease was the most common NIID [22 (46.8%)], and haematologic malignancy was the most common malignant diseases [8 (36.4%)]. For the 130 patients with diagnosis, a diagnosis was established after a median of 32.12 ± 18.53 days from the onset of fever (range, 4–90). In patients with NIID, the median duration of reaching a diagnosis (37.76 ± 23.77) was significantly longer, compared with the patients with infection (25.56 ± 12.78) (p = 0.007). The median duration of fever in the group of 130 patients in whom a diagnosis was made was 35.95 ± 32.62 days (range, 3–180). In patients with malignancy, the median duration of fever (51.38 ± 35.55) was longer, compared with the patients with infection (37.58 ± 38.97) (p = 0.052).

Conclusions: Several reports have been reported a changing pattern in the prevalence of the three main categories of the FUO in Turkey since 1985: infections, malignancies and NIIS. Our multicentre study showed that a decrease in the prevalence of infectious diseases in favor of the other two groups compared with the previous Turkish studies.

Catheter-related infections

P1710

Retrospective study to evaluate differential time to positivity for diagnosing catheter-related bloodstream infections

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An accurate diagnosis, an adequate antimicrobial therapy and a rational decision concerning catheter removal are the main steps in the management of catheter-related infections(CRI). There is no established gold standard for diagnosing CRI. A newly developed method which compares the differential time to positivity (DTP) of qualitative cultures of blood samples drawn from the catheter and a peripheral vein has been proposed to avoid unjustified removal of the catheter and potential risks associated with the placement of a new catheter.

Objective: To evaluate DTP as a method for diagnosing catheter-related bacteremias (CRB) in patients with catheterization.

Methods: Patients with suspected CRI who had blood cultures drawn simultaneously through the catheter and a peripheral vein, according to the local guidelines, from January 2003 to June 2004 at Policlinico of Modena were included in a retrospective study. The blood-culture system used was the Bactec9240 and the growth times of peripheral and catheter blood cultures were recorded and expressed in min. The criteria of definite or likely CRB were clinical and/or microbiological in patients with isolation of the same microrganism from the catheter and peripheral blood cultures.

Results: During of this preliminary study, 146 episodes occurred, 23 of which were excluded (15 for incompleteness of data and 8 for different microrganisms in each paired bottles). Finally, 123 episodes of suspected CRI were analysed: in 75 the DTP was greater than 120 min. and CRB was confirmed in 90%; in 48 the DTP was less than 120 min. and in 95% of them the bacteraemia wasn't related to the catheter. The main microrganisms involved in CRB were CONS (46.3%), *S.aureus* (20.9%), *P.aeruginosa* (13.4%);the CRB was polymicrobial in three episodes.

Conclusions: The diagnostic reliability of semi and quantitative catheter cultures is dimininuished by the fact that catheter removal and subsequent culture are often performed several days after the simultaneous blood cultures are drawn, a period during which antibiotics are often given through the central venous catheter. Hence DTP introduces a useful,simple laboratory method that can be proposed for routine clinical practice in hospital using automatic devices for detection of positive blood cultures in patients with possible CRB. The accuracy of this methods requires accurate tracking of the source of blood cultures, as well as simultaneous placement of the cultures in the automated machine.

P1711

Results of active surveillance of nosocomial central venous catheter-associated bloodstream infections in a tertiary care hospital

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Objectives: The analysis of the results of active surveillance system of nosocomial bloodstream infections associated with central venous catheters (CVC) in patients hospitalised on intensive care units and surgical wards of a tertiary care pulmonological hospital (280 beds).

Methods: The surveillance was based on a system of the Polish Society of Hospital Infection. The study comprised patients hospitalised in 2003 in two surgical wards (S-A and S-B) and two intensive care units: surgical ICU (ICU-S) and cardiopulmonary ICU (ICU-CP). The mean stay in each ward was 10.8, 12.4, 1.6 and 10.0 days, respectively.

Results: In the analysed period there were 4086 patient admissions, comprising 29805 patient-days (including 2816 central venous catheter-days). The CVC utilisation rate was 9% for the whole hospital, while in the analysed units it amounted to 51% in ICU-S, 22% in ICU-CP, 3% in S-A and 4% in S-B. The highest number of cases of CVC-associated bloodstream infections was recorded in the ICU-S (6 cases), corresponding to the highest incidence rate/1000 patient-days (2.2‰), in comparison to other wards (1.9‰ in ICU-CP, 0.1‰ in S-A and 0.2‰ in S-B). However, the highest incidence rate/100 admissions (1.9%) and the highest incidence rate/1000 CVC patient-days (8.8‰) was recorded in the ICU-CP, comparing to 0.4% and 4.2‰ in ICU-S, 0.1% and 2.3‰ in S-A and 0.2% and 4.0‰ in S-B, respectively.

Conclusions: The results show that the highest frequency of a CVC-associated bloodstream infection was in ICU-S. However, a high incidence rate of CVC-associated infections/100 admissions in ICU-CP, combined with a lower CVC utilisation rate (in comparison to ICU-S), indicate a need for improvement of compliance with infection control procedures in ICU-CP. Therefore active surveillance system allows a detailed epidemiological analysis of frequency and dynamics of different forms of nosocomial infections, as well as assessment and need for modification of infection control procedures.

P1712

Assessment of the potential infection risk associated with the Q-Syte™ needleless connector

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Objectives: To determine the potential infection risk associated with the Q-Syte™ needleless connector. To determine if multiple activations of the Q-Syte™ needleless connector increases the potential risk of infection.
Method: Fifty Q-Syte™ needleless connectors were inoculated with a suspension containing 25% (v/v) blood and 6.5 x105 colony forming units of *S. epidermidis*. Suspensions were allowed to dry and each device was subsequently decontaminated with 70% isopropyl alcohol as per standard practice. Needleless connectors were then flushed with a pre-filled sterile syringe containing 10 ml of 0.9% (v/v) sterile saline. Each flush solution was collected and the number of colony forming units present determined by standard laboratory techniques. A further 50 needleless connectors were activated up to a total of 70 times to simulate routine clinical practice. After a defined number of activations each needleless connector was inoculated with *S. epidermidis*, decontaminated and flushed with 0.9% (v/v) sterile saline as described previously. The number of colony forming units present in each flush solution was determined.
Results: None of the saline solutions flushed through Q-SyteTM needleless connectors which had been activated up to a total of 70 times, contained detectable numbers of microorganisms.
Conclusion: If appropriately disinfected, the Q-Syte™ needleless connector is a needle safe barrier to microbial contamination of flush solutions even following multiple activations. These devices may have the potential for reducing the incidence of catheter contamination, colonisation and sepsis acquired via the intraluminal route.

P1713

Evaluation of RAPD for the epidemiological typing of coagulase-negative staphylococci implicated in catheter-related bloodstream infection on a bone marrow transplant unit

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Objectives: Coagulase-negative staphylococci (CoNS) are the most common aetiological agents implicated in central venous catheter (CVC)-related bloodstream infection. Phenotypic markers have been widely used for typing CoNS however, genotypic methods such as pulsed-field gel electrophoresis (PFGE) are more discriminative. PFGE is a time-consuming method therefore epidemiological results achieved are often retrospective. In this study, the ability of a rapid random amplification of polymorphic DNA (RAPD) technique to discriminate CoNS implicated in CVC-related bloodstream infection in stem cell transplantation (SCT) patients was compared to PFGE.
Methods: Patients at the University Hospital Birmingham NHS Foundation Trust, UK who underwent SCT and were diagnosed with CVC-related bloodstream infection due to CoNS whilst on the bone marrow transplant (BMT) unit were studied. Isolates of CoNS were genotyped by PFGE and RAPD. The RAPD technique evaluated employed a single primer and simple DNA extraction method. The discriminatory power of the RAPD technique and PFGE was determined.
Results: Thirty-three SCT patients on the BMT unit were diagnosed with CVC-related bloodstream infection from which 49 pure cultures of CoNS were obtained from blood cultures. Both RAPD and PFGE were highly discriminatory (Simpson's diversity index, 0.96 and 0.99, respectively). Within the isolates obtained from the 33 patients, 20 distinct strains were identified by PFGE (using the Tenover criteria for bacterial strain typing) and 25 by RAPD. Of the 25 strains identified by RAPD, there were 9 distinct clusters of CoNS which contained isolates from multiple patients, suggesting limited nosocomial spread and not a common source. However, there was no significant association between time of inpatient stay and infection due to any one strain of CoNS.
Conclusion: Genotypic analysis of CoNS isolates demonstrated clusters of CVC-related bloodstream infection caused by common strains, suggesting nosocomial cross-transmission. However, no single strain was associated with a significant number of CVC-related bloodstream infections during the study period. The expensive, time-consuming nature of PFGE indicates that it is currently unlikely to be incorporated into the routine microbiology laboratory. By contrast, the RAPD technique allows CoNS strains to be genotyped with high discrimination within 4 hours, thus facilitating real-time epidemiological investigations.

P1714

Central venous catheter related sepsis due to *Chryseobacterium meningosepticum*: a case report

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Objective: *C. meningosepticum* is cause of severe infections in premature and newborn infants. Infections due to this opportunistic pathogen in adult immunocompetent patients are uncommon.
Methods: We describe a cvc-related sepsis in an immunocompetent elderly patient.
Results: On July 2004, a 84-year-old man was admitted to hospital for acute onset of epigastric pain and fever. He had an history of hypertension, chronic bronchitis and peptic ulcer. Physical examination revealed a severely ill patient with marked tenderness to palpation and diffuse rigidity of the abdominal wall. Abdominal radiographs showed pneumoperitoneum. Clinical diagnosis of perforated peptic ulcer was done and patient underwent gastrectomy. He was transferred in the ICU for postoperatory monitoring: he was mechanically ventilated, had a cvc and a urinary catheter. Broad-spectrum antibiotics were administered. Tracheal tube was removed in a few days and clinical conditions recovered. After 2 weeks, fever with chills occurred, WBC count was 18000/mm^3 (90% neutrophils). Blood cultures from a peripheral vein and cvc were drawn and a meropenem-based empiric therapy was started, without clinical improvement. After 2 days, blood cultures yielded Gram-negative rods. Cvc was removed and the tip cultured. The isolate was further identified as *C. meningosepticum* resistant to aztreonam, amikacin, tobramicin, netilmicin, piperacillin, piperacillin/tazobactam, ceftazidime, meropenem and susceptible to levofloxacin. Also culture of catheter tip yielded *C. meningosepticum*. Therapy was changed to intravenous levofloxacin 500 mg daily. Fever subsided after 2 days and a six-day-course therapy was completed. The patient died one month later for A. *baumannii cvc* related sepsis.
Conclusions: *C. meningosepticum* has been reported to cause pneumonia, sepsis, wound infection, peritonitis in adult patients with severe underlying diseases. In a recent series, it has been described as cause of cvc-related bacteraemia in a cancer patient. Our patient had no evident causes of immunodepression but the advanced age, the severe underlying disease, and the presence of an indwelling catheter might have played a role in the

acquisition of infection. Furthermore, this pathogen has a peculiar resistance to antibiotics usually prescribed for Gram-negative bacterial infections, such as beta-lactam agents and aminoglycosides, thus representing a clinical concern in the choice of adequate antimicrobial therapy.

Molecular bacteriology and mycology

P1715

DNA microarrays for environmental, clinical microbiology, and microbial taxonomy

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DNA microarrays have the potential to be excellent tools for environmental, clinical microbiology, and microbial taxonomy due to the capability to significantly improve identification and multilocus typing of target pathogens in a bacterial community. We have developed and evaluated several microarray-based assays for the reliable detection, discrimination, and multilocus typing of the all *Listeria* species, four *Campylobacter* species (*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*), *Bacillus anthracis*, most of the *Mycoplasma* species, and enterotoxins of *Staphilococcus* spp.; many of which are know as either outbreak related or clinically relevant pathogens for humans and animals. The assay can also detect the presence of bacterial genes responsible for drug resistance in *Staphylococcus* and *Streptococcus* spp., as well as those genes related to drug resistance in *Mycobacterium tuberculosis*. The approach used in this study included: (i) simultaneous PCR amplification of several target bacterial genes; (ii) high-yield synthesis of single-stranded RNA driven by T7 polymerase from the promoter-tagged PCR amplicons; (iii) incorporation of appropriate fluorescent dye into ssRNA; (iv) hybridization of fluorescently labeled sample with the microarray containing multiple individual oligonucleotide probes, and (v) microarray scanning and image data analysis. Microarray analysis of large numbers of bacterial samples from environmental and clinical sources demonstrated that this method enabled unambiguous detection and identification of all target pathogens. The microarray approach is also a promising application in the microbial taxonomy field due to the ability to correctly identify species by a multilocus probing. During this study we found that the occurrence of weak hemolytic or non-hemolytic isolates of *Listeria* spp. (*L. seeligeri*), and atypical hemolytic activity of typical non-hemolytic *Listeria* spp. (*L. innocua*) can result in false biochemical species differentiation of Listeria as a taxon. How can a microarray improve this? We found seven isolates that initially identified as *L. welshimeri* has discrepancies with our microarray data. All seven isolates were identified by microarray as *L. seeligeri*, but lost several genes due to a deletion in the central virulence gene cluster. Thus, our data suggests that microarray technology might be a valuable tool for the identification and accurate characterization of bacterial pathogens and microbial community in general.

P1716

Atypical non-haemolytic *Listeria seeligeri* isolated in the USA

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We found that seven Listeria isolates, initially identified as Xyl+, Rha- biotypes of *L. welshimeri* by phenotypic tests, exhibited discrepant genotypic properties using a well validated species identification oligonucleotide microarray. All seven isolates were identified with the microarray as *L. seeligeri* (L.s.) based on hybridization patterns with general iap- and hly-specific oligonucleotide probes. However, these isolates did not hybridize with special L.s hly-specific oligonucleotide probes. Based on the microarray result, these seven isolates were identified as atypical hly-negative L.s. isolates, not L. welshimeri isolates. The aberrant L.s. isolates are D-xylose fermentation positive and L-rhamnose fermentation negative (Xyl+, Rha−); non-hemolytic on BBL Columbia Agar supplemented with sheep blood, and also are negative in the CAMP test with both *S. aureus*, and *R. equi*. Since, the aberrant L.s. isolates are genetically and phenotypically hly-negative other structural alterations within the PrfA regulated virulence-gene cluster of L.s. were suspected. All genes of the PrfA cluster of L.s., located in the prs-ldh region, including orfA2, orfD, prfA, orfE, plcA, hly, orfK, mpl, actA, dplcB, plcB, orfH, orfX, orfI, orfP, orfB, and orfA genes were checked by PCR and direct sequencing for evidence of their presence in the atypical isolates. The PCR-based data and direct sequencing of prs-ldh region suggested that the organization of the genes of the virulence gene cluster of the aberrant isolates is different from that in the reference strain L.s. ATCC 35967. The PrfA clusters of the L.s. isolates are approximately three-fold shorter, due to loss of orfD, prfA, orfE, plcA, hly, orfK, mpl, actA, dplcB, plcB, orfH, orfX, and orfI. The genetic map order of the cluster genes of all the atypical L.s. isolates is: prs-orfA2-orfP-orfB-orfA-ldh. Direct sequencing showed that all of these genes are L.s. specific. DNA sequencing and phylogenetic analysis of housekeeping genes (iap, 16S rRNA gene, the16S-23S rRNA inter-genic spacer region, prs, comK, groEL, cat, sigB, recA, hsp60, and ldh) indicate a L.s. genomic background in all seven of the atypical hly-negative L.s. isolates. Thus, Xyl+, Rha− Hly+ *L. seeligeri* can only be distinguished from Xyl+, Rha− *L. welshimeri* genotypically and not phenotypically. In contrast to this case, the Rha+, Xyl+ biotype of *L. welshimeri* would not present an identification issue.

P1717

Species identification of nocardial infections in Denmark

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Objectives: To implement and validate DNA sequencing methods for species identification of Nocardia isolates submitted to the Danish Reference Laboratory for Bacterial Identification and to establish phenotypic criteria for correct identification.
Methods: 29 clinical isolates presumed to be Nocardia and 9 type strains purchased from CCUG were subjected to almost complete (>1400bp) sequencing of th 16S RNA gene and partial sequencing (app. 500 bp) of the heat shock protein gene (hsp60). Strains were tested for partially acid fast staining and resistance against lysozyme to identify the genus. Phenotypic test for species identification included equivalent growth at 35°C and

45°C, opacification of Middlebrook 7H11 agar and carbohydrate assimilation using the API 20C AUX test strip. Finally, strains were tested for susceptibility to penicillin, cefoxitine, colistine, gentamicin, erythromycin, rifampicin, fusidic acid, and vancomycin using disk diffusion test on Muller-Hinton agar.
Results: 16S sequencing and BLAST searching in GenBank correctly identified all type strains. 22 out of 29 clinical isolates belonged either to *N. farcinica* (9 isolates), *N. abscessus/asiatica* (9 isolates) or *N. cyriacigeorgica* (4 isolates). To faithfully discern between *N. abscessus* and *N.asiatica* at aleast a 1000 bp 16S RNA sequence had to be analysed. Phenotypic identification of these three groups was possible using carbohydrate assimilation tests, opacification og 7H11 agar and gorwth at 45°C. Deistinction between the groups was further aided by colony morphology. Other species could also be identified by phenotypic tests and antibiotic susceptibility patterns could further aid diagnosis. Phylogenetic analysis of hsp60 gene sequences indicated that this gene carried phylogenetic information comparable to the 16S RNA gene.
Conclusions: Three phenotypic species of Nocardia constituted more than 75% of danish clinical isolates submitted for identification. These were readily identified by 16S sequencing and could also be identified by simple phenotypic tests.

P1718

Nocardia and nocardiosis in recent years: different micro-organisms in different patients

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Background: Nocardia infections have traditionally been reported in severely immunocompromised patients. The majority of the Nocardia isolates were identified as *N. asteroides*, and cotrimoxazole prophylaxis was considered protective.
Methods: Between 1995 and 2003, there were 37 episodes of nocardiosis in our center; 10 were excluded from the study due to incomplete records (6) and colonization (4). Identification to species level was performed both by classic procedures and by hsp65 PCR-RFLP in 27 cases.
Results: 89% of the patients were men and mean age was 54 ± 59 years. The most common underlying condition was HIV infection (9 pts, 33.3%), followed by 5 (19%) immunological diseases (2 rheumatic arthritis, 1 idiopathic thrombocytopenic purpura, 1 temporal arteritis, 1 sarcoidosis), solid organ transplantation (6 pts, 22%), COPD (4 pts, 14.7%) and neoplasm (3 pts, 11%). Among all patients (14 pts, 52%) were receiving corticosteroids therapy due to different diseases and 9/27 (33.3%) were receiving prophylaxis with cotrimoxazole. The infection was disseminated in 2 cases (7%), or involved the lung (70%), skin and soft tissues (4 pts, 15%), CNS (1 pt, 4%), or caused otomastoiditis (1 pt, 4%). The species of Nocardia were as follows: *N. asteroides* VI 48%, *N. farcinica* 33%, *N. nova* 8% and *N. otitidiscaviarum* 11%. Antimicrobial resistance rates were: cotrimoxazole 15%, ceftriaxone 41% and imipenem 11%. Nocardiosis occurred while the patients were on cotrimoxazole prophylaxis in 9 cases (33.3%) and the strains isolated in those patients were susceptible (7) or resistant (2) to cotrimoxazole. Six patients died (22%), but we could not detect a higher death rate in HIV-infected patients with species other than asteroides or in cases with prior cotrimoxazole prophylaxis. The outcome of *Nocardia farcinica* infection was 3/9 (33.3%) related deaths and 6/9 (66.7%) were cured. For *Nocardia asteroides* VI the outcome was favourable in 10/13 (77%), 1/13 related death, 1/13 unknown and 1/13 non-related death.
Conclusion: Our results show important changes in *Nocardia* spp and nocardiosis in recent years. HIV infection is the most common underlying condition, new species are emerging and cotrimoxazole prophylaxis should not be relied upon as universally protective.

P1719

Detection and identification of *Ochrobactrum anthropi* strains from environmental samples using 16S rRNA-specific PCR and sequence analysis

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Objectives: There was an attempt to further characterize an *Ochrobactrum anthropi* isolate using molecular methods and specifically, sequence analysis of the 16S rRNA gene. The strain was isolated from cell culture material obtained after the application of VIRADEN (VIRus ADsorption and ENumeration) on incompletely decontaminated sewage samples for enterovirus detection. It was initially phenotypically identified by the API 20NE system, on the basis of its biochemical profile.
Methods: Genetic material was extracted from the enterovirus-positive VIRADEN material and a 16S rRNA-specific PCR was applied using universal primers for the 16S rRNA gene. The strain was successfully detected and the sequence of the largest part of the gene was obtained. Identification of the bacterial strain was then attempted using the BLAST alignment software for the comparison of its 16S rRNA-derived sequence with the respective sequences of all bacterial species and strains available in GenBank database.
Results: The results interestingly showed a 98% similarity with the 16S rRNA sequences of other *O. anthropi* strains, as well as with *O. tritici* and *O. lupini* strains. Although there is no clear consensus definition of bacterial species and genus by 16S rRNA gene sequence comparisons, it is generally believed that intra-species 16S rRNA sequence variability may not be greater than 1%. In the present study, therefore, there was a phenotypic similarity with other *O. anthropi* strains that allowed identification of the isolate, but genotypically on the basis of 16S rRNA sequence the isolate was divergent to be categorized to the same species.
Conclusion: The observed genotypic variability in the strain presented here provides further support to the fact that some wastewater treatment plants constitute reservoirs for a wide range of diverse pathogens or even novel microorganisms with possible important consequences for public health, as shown by previous studies. More 16S rRNA sequences from environmental isolates could contribute to the observed variability but, nevertheless, detection and identification of bacteria on the basis of 16S rRNA from the environment still proved to be an invaluable asset for further epidemiological surveys. This study was co-funded by 75% from the European Union and 25% from the Greek Government under the Framework of the Education & Initial Vocational Training Program – 'Archimedes'.

P1720

Enigmatic *Leuconostoc* and *Lactobacillus* infections

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Objectives: *Leuconostoc* and *Lactobacillus* are gram positive *coccobacilli* of low virulence. Human infections are commonly misdiagnosed. This report is to provide interesting diagnostic and clinical features of these organisms.
Methods: 26 clinical isolates identified as Leuconostoc by API 20 STREP (Biomérieux, Inc.) in our hospital during 1997–2004

were reevaluated by repeated API 20 STREP, API 50 CHL, conventional phenotypic assays including gas production in MRS broth and vancomycin MIC, and two genus-specific PCRs for *Leuconostoc* and *Lactobacillus* as previously described (Lett Appl Microbiol 2000;31:129–33 and FEMS Microbiol Lett 2002;214:271–5, respectively). Four ATCC strains of *Leuconostoc*, *Lactobacillus*, and *Pediococcus* were used as controls in all experiments. A review of medical records was performed only in cases with confirmed both phenotypic and PCR assays to be *Leuconostoc* or *Lactobacillus*.

Results: Only 3 isolates initially identified as *Leuconostoc* were confirmed by both manual phenotypic and PCR assays. Another two turned out to be *Lactobacillus* spp. The rest were mostly *Streptococci* or closely related bacteria. API 50 CHL identified 16 isolates as *Lactobacillus* spp., only 2 of which were confirmed by genus-specific PCR. *Lactobacillus pentosus* ATCC 8041 and *Pediococcus pentosaceus* ATCC 33316 were both identified by API 20 STREP as *Leuconostoc* and by API 50 CHL as *Lactococcus lactis ssp lactis* 1 and *Lactobacillus pentosus*, respectively. Clinical features are diverse and include both community and hospital acquired infections in normal and compromised hosts without antibiotic pressure, and with various outcomes (Table).

Organism	Specimen	C' or H*	Host status	Antibiotic pressure	Foreign body or device	Co-pathogen isolated	Treatment & outcome
Leuconostoc	Corneal discharge	C	Normal	No	Tiny piece of wood	no	Topical antibiotic recovered
Leuconostoc	Ascitic fluid	C	suspected chronic liver disease	No	No	No	Cefotaxime expired
Leuconostoc	blood	H	Stroke	No	Peripheral IV line	no	Co-amoxiclav expired
Lactobacillus	Ascitic fluid	C	HIV	No	no	*E. coli* Candida	Ciprofloxacin matendazole recovered
Lactobacillus	Duodenal content	C	Pancreatic carcinoma	No	No	No	Ciprofloxacin recovered

* = community or hospital acquired

Conclusions: These bacteria are underrecognized emerging pathogens which are frequently misidentified. Without confirmation by manual phenotypic and genotypic assays, widely-used commercial could potentially mislead clinical management and reports in medical literature.

P1721

PCR-based detection of *Klebsiella pneumoniae* in blood cultures

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Objective: *K. pneumonia* infections are a major source of morbidity and mortality among hospitalized adult and paediatric patients. They rank second as cause of gram negative sepsis in our medical centre and 45% of them are caused by extended spectrum b-lactamase producers. Early detection is a key factor in determining the outcome of *K. pneumoniae* infections and numerous attempts have therefore been made during the last years to develop more rapid and accurate detection systems. The aim of the present study was to establish rapid and reliable PCR-based assays aimed at detecting gram negative bacterial DNA and *K. pneumoniae* nucleic acid sequences in blood cultures obtained from patients with suspected sepsis.

Methods: DNA was extracted from positive blood culture bottles using the benzyl alcohol-guanidine hydrochloride method. Two sets of oligonucleotide primers were designed: the first one identifies a nucleotide sequence of the 16S rRNA gene that is specific for gram negative bacteria; the second one targets *K. pneumoniae* haemolysin gene.

Results: The sensitivity of gram negative specific PCR targeted to the 3'-end of the 16S rRNA gene and to the haemolysin gene of *K. pneumoniae* was optimized to 100fg/ml and 200pg/ml, respectively, using DNA extracted from a standard isolate of *K. pneumoniae*. The specificity of the assay was established using 100 different bacterial isolates and found to be 100%. The assay performance was assessed using 108 consecutive positive blood cultures identified as part of the routine work-up of hospitalized patients. Gram staining was performed on positive blood cultures and DNA was extracted from these cultures bottles and PCR-amplified using the assay described above. The results were compared to the final microbiological diagnosis as determined using standard methodology. 108 out of 108 positive blood culture bottles were correctly identified using the PCR based assay with 100% specificity and 100% sensitivity. The turnaround time for the molecular identification was 4 hours.

Conclusions: PCR-based detection of *K. pneumoniae* directly from blood culture bottles is rapid (4h), and highly specific (100%) and sensitive (100%). Implementation of this and similar assays targeted to common bacterial pathogens should allow for earlier treatment to be provided and for a more rationale and cost-effective use of antibiotic treatment, thus cutting down costs while at the same time preventing the emergence of resistant strains.

P1722

Diagnosis of human brucellosis by PCR assay

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Introduction: Brucellosis is a world-wide re-emerging zoonosis causing severe disease in humans with unspecific clinical signs affecting numerous organs. Therefore, clinical diagnosis is difficult to establish but effective therapy requires an early diagnosis. Laboratory diagnosis of chronic brucellosis is generally difficult because conventional methods (blood culture, serological tests) lack sensitivity and specificity. PCR has offered a new dimension in the diagnosis of *Brucella* spp.

Methods: Blood specimens were collected from 18 patients with suspected brucellosis. All patients had two or more blood cultures and a serological battery of tests, including the Rose Bengal plate agglutination test, Wright's seroagglutination, and Coombs' antibrucella test. Blood specimens were also tested by PCR technique. PCR assay consists of amplification of a 223-bp fragment from the gene coding for the synthesis of an immunogenic protein on the external membrane of *Brucella abortus* (BCSP31). All amplicons had the expected size of 223 bp. DNA exctracted from blood taken from healthy individuals did not show any amplification with the primers used. All PCRs were carried out in duplicate.

Results: The sensitivities of the serological tests range from 17% to 44.5%. Brucella was isolated in blood cultures from 3 of 18 (16.7%) cases. In all 18 cases diagnosis of Brucella was established by PCR, confirming the clinical suspicion. Post-treatment blood samples examined by PCR proved to be negative for brucellosis. The sensitivity of our PCR assay was 100%, since it correctly identified all 18 cases of brucellosis, regardless of the duration of the disease, the positivity of the blood culture, or the presence of focal forms.

Conclusions: In this study PCR has been shown to be a valuable molecular technique, higly sensitive and specific for the diagnosis of human brucellosis. Culture sampling sensitivity

is often low, depending on the disease stage, quantity of circulating bacteria and blood culture technique employed.

P1723

Rapid molecular detection of clinically relevant yeasts by PCR-based denaturing high performance liquid chromatography

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Objectives: The detection and identification of clinically relevant yeast species by cultivation and biochemical testing is costly and time consuming. Thus, we established a rapid culture-independent molecular approach allowing both sensitive detection, as well as identification of individual yeast species.
Methods: Yeast DNA was detected in total DNA samples isolated from stool and from blood cultures, respectively, by amplification of the intergenic regions between ribosomal genes. Amplicons were separated by denaturing high-performance liquid chromatography (DHPLC) with the WAVE system (Transgenomics Inc.). Cultivation and biochemical testing of yeasts was performed in our diagnostics laboratory according to standard procedures.
Results: The analysis of yeast reference strains showed that a set of 13 different species could be identified by their DHPLC peak profiles and the corresponding retention times. The applicability of the method was evaluated by detection and identification of yeasts in clinical samples taken from complex matrices such as stool and blood. The peak-profiles and retention times obtained by DHPLC corresponded with the dominant yeast species in the samples, as confirmed by sequence analysis, cultivation and biochemical testing. If the retention times of amplicons from the samples were compared to standard strains in each run, the technique was reproducible, and valid data were obtained from blood cultures as well as from stool samples.
Conclusions: Detection and identification of yeast species in clinical specimens by PCR-based DHPLC complements the conventinal techniques available for yeast diagnostics.

P1724

Culture-independent identification of fungal and bacterial species using one convenient automated platform

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Objectives: In most clinical settings, detection of bacterial or fungal species has been focused on conventional culture-based approaches. However, these traditional methods can lead to unreliable results. Broad range molecular detection, using PCR to target the ribosomal RNA gene of microorganisms from clinical specimens is an approach that can provide a much more rapid and accurate identification of organisms, especially from the so-called 'non-culturable' or slow growing organisms. Indeed, this method is only applicable to the direct diagnosis of mixed infections when the mixed rRNA gene amplication products can be separated and sequenced for confirmation. We applied this approach for separation of mixed rRNA gene amplification products prior to sequencing using the WAVE® Microbial Analysis System.
Methods: Genomic DNA was extracted individually from 15 bacterial and 19 fungal species, derived from clinically significant specimens. Broad range primers specific for either fungal (5.8S-28S) or bacterial (16S) rRNA genes were developed, and the regions of interest were amplified. The amplified PCR products from different species were either mixed in different combinations or unmixed and analysed.
Results: The WAVE® System detected *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Serratia*, *Citrobacter*, *Salmonella*, and *Proteus* species either as single or mixed species. Every individual species from a mixture of 12 bacteria can be resolved and detected simultaneously in 20 minutes. The separated and collected amplification PCR products provided clean sequences for the identification of each of the unique species. Six commonly seen candidosis causative agents – krusei, glabrata, albicans, tropicalis, parapsilosis, and lusitania – can all be resolved and detected at one time, as well as four major aspergillosis causative agents of fumigatus, niger, terreus, and flavus. Furthermore, the same system also resolved and detected *Rhizopus*, *Mucor*, *Fusarium*, *Absidia*, and *Pseudallescheria* species of either single or mixed species.
Conclusions: We conclude that the WAVE® Microbial Analysis System is a powerful single platform capable of detecting bacterial and fungal microorganisms without culturing. This technique can be applied to the monitoring of microflora from complex samples such as stool or sputum, as well as identification of the important individual species from either complex or sterile samples.

P1725

Stability of the bacterial flora composition in chronic wounds

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Objectives: Longitudinal investigation of the bacterial composition in chronic venous leg ulcers, by culturing and molecular typing.
Methods: Forty-six patients were included in the study. All had persisting ulcers for more than three months. The size and the healing of the ulcers were followed for 8 weeks or until the ulcers healed. Every second week biopsies and filter paper pads were collected. Bacteria were isolated and identified at species level by standard methods. Molecular typing was performed by RiboPrinter®Microbial Characterisation system (Qualicon).
Results: During the study 5 of the 46 ulcers healed. The remaining ulcers had a relatively stable ulcer size during the 8 weeks. More than one bacterial species was detected in close to all the samples (94.4%). None of the patients' chronic wounds were colonized by a single bacterial species. Between four and six bacterial species were detected in 50% of the ulcers. 39% of the patient ulcers were colonized with more than six species. The most common species found in the ulcers were *Staphylococcus aureus* (isolated at least once during the study period in 95.7% of the patients), *E. faecalis* (71.7%), *Pseudomonas* species (58.7%, mainly *P. aeruginosa*), coagulase-negative *staphylococci* (45.7%), *Proteus* species (41.3%), anaerobic bacteria (39.1%), *E. cloacae* (37.0%) and *E. coli* (32.6%). A comparison of the microbial findings from the same ulcer showed that one or more of the same species were re-isolated from all the samples from 41 out of 46 ulcers. In the remaining 5 ulcers one or more of the same species were present in all but one of the samples. The most common resident bacteria species were *S. aureus*, re-isolated in 32 ulcers and *P. aeruginosa* re-isolated in 17 ulcers. RiboPrint analyzes were performed on *S. aureus* and *P. aeruginosa* isolates, the results revealed that bacteria isolated from the same patient always belonged to the same ribogroup. None of the patients belonged to the same ribogroup showing that the infection was not hospital-acquired. Furthermore were ulcers

with *P. aeruginosa* found to be significant larger than ulcers without the presence of *P. aeruginosa* ($p = 0.006$). No significant correlations were found between ulcer size and other bacteria species.
Conclusion: The results showed that chronic wounds are colonized by a community-acquired resident bacteria flora, and once a bacteria species is established in the wound no re-infections occur.

P1726

EMM-typing of invasive T28 group *A streptococci*, 1995–2002, Finland

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Objectives: Surveillance of invasive group *A streptococcal* (GAS) disease in Finland is based on mandatory laboratory notifications to the National Infectious Disease Register at National Public Health Institute (KTL) and typing of corresponding GAS isolates referred to the reference laboratory at KTL. During years 1995 to 2002, serotype T28 remained amongst the most common T serotypes in isolates causing bacteremic GAS cases, together with serotypes T1 and B3264. In order to study the clonality of T28 strains, M protein genotyping (emm-sequencing) was introduced.
Methods: All blood GAS isolates referred to the Hospital Bacteriology Laboratory at KTL in 1995 to 2002 were included. The GAS strains were T serotyped and their emm genes sequenced. emm-types were compared with the CDC *Streptococcus* pyogenes emm sequence database.
Results: A total of 735 bacteremic GAS cases were referred to KTL in 1995–2002; annual numbers varied from 43 to 139. The T28 strains (a total of 229 isolates) represented 25–40% of annual GAS isolates with 14 strains in 1995 to 56 strains in 2002. The T28 strains distributed to 6 distinct emm-types: emm28 (125 isolates, 55% of all), emm77 (55 isolates, 24%) and emm53 (34 isolates, 15%), emm87 (3 isolates, 1%), emm2 (7 isolates, 3%) and emm4 (2 isolates, (1%). For three strains (1%) emm-typing is in progress. The proportion of T28/emm28 strains was highest in 1995–97 (86–44% of all), declined in 1998–2000 (26–29%) and showed an increase again in 2001–2002 (80–79%). During 1998–2000, the most prominent type was T28/emm77 (31–40% of all). T28/emm53 was uncommon during the three first study years, peaked sharply in 1998 (30% of all) and became again rare by 2001. T28/emm2 strains were only identified in 1997–2000.
Conclusion: This study shows that T-serotyping alone is not a sufficient method for epidemiological surveillance of invasive GAS disease caused by T28 isolates. Analysis of M-proteins by emm-sequencing showed that several T/M-types circulated during the eight year study period. Overall, T28/emm28 strains were amongst the most common GAS types, but they showed a cycling prevalence pattern. During periods of low T28/emm28 prevalence, other T28/emm combinations seemed to emerge and partially replace them.

P1727

Genome-based amplified fragment length polymorphism for typing of *Francisella tularensis* strains

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Francisella tularensis is facultative inracellular bacterium spread mainly in the Northern hemisphere. It causes a disease in mammals known as tularemia and due to its high level of virulence and infectivity is considered a potential biological weapon (CDC list, Category A). Tularemia is a major problem in Bulgaria since 1998 when an outbreak occurred. Every year about 40 new cases are registered and the increasing number of isolated strains raised the need for their typing.
Objectives: The objective of the study was the development of a genome based AFLP approach that detects good level of polymorphism among *F. tularensis* strains.
Methods: The pDraw32 (Acaclone) software was applied for genomic screening. 150 different restriction enzymes were tested for generating a sufficient number of fragments. HindIII, XbaI and MboI were selected as working enzymes. DNA isolated from 30 *F. tularensis* strains, 24 originating from all over the world and 6 Bulgarian isolates were submitted to digestion with XbaI/HindIII and HindIII/MboI enzyme combinations. Double stranded DNA adaptors were designed and ligated to the ends of the obtained fragments. Several PCR primer combinations were tested. The PCR products were run on an automated electrophoresis system and the patterns were submitted to cluster analysis with GelComparII (Applied-Maths) software. Dendrograms were generated using Pearson correlation and UPGMA.
Results: The AFLP approach was applied for the typing of 30 *F. tularensis* strains. The best results were obtained with HindIII/MboI restriction enzyme and Hind/Mbo(C primer combinations. In general, it was found little variability (up to 30%) among the strains which is in consistence with previously published data. Bulgarian isolates are more closely related and form a separate cluster.
Conclusion: The AFLP method proved to be a powerful technique for typing of various types of organisms. With its high discriminatory power and type ability it is capable of distinguishing very closely related strains. However, AFLP is necessary to be properly designed for a particular organism with respect to the relevant genomic characteristics. Some critical parameters (e.g. enzyme selection, primer design and concentration) have to be taken into account.

P1728

A comparison of *Listeria monocytogenes* serovar 4b isolates of clinical and food origin in Austria by automated ribotyping and pulsed-field gel electrophoresis

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Objectives: In this study, automated ribotyping and pulsed-field gel electrophoresis (PFGE) were evaluated in an epidemiological study of *Listeria monocytogenes* serotype 4b strains from patients and food samples collected in Austria. These isolates were compared with isolates from foodborne incidents which have occurred in other European countries and North America.
Methods: The *L. monocytogenes* serovar 4b isolated were comprised of isolates from listeriosis patients in Austria, isolates from food and environmental samples in Austria as well as isolates from foodborne listeriosis in other European countries and North America. Automated ribotyping was performed using the RiboPrinter microbial characterisation system according to manufacturer's instructions using EcoRI as restriction enzyme. PFGE was performed according to the standardized protocol for molecular subtyping of *L. monocytogenes* by PulseNet USA using restriction enzymes AscI and ApaI.
Results: Two PFGE subtypes dominated among human isolates, and these PFGE subtypes were also found among food

isolates. Some ribotypes contained different PFGE types, but PFGE types were also assigned to different ribotypes. The largest group of Austrian clinical isolates was of the same PFGE subtype as those isolated from foodborne outbreaks in the United Kingdom and in the USA. Another subtype of clinical isolates from Austria was indistinguishable to that obtained from isolates responsible for foodborne outbreaks in Canada, Switzerland, the USA and France.
Conclusion: Although the discriminatory power of PFGE is believed to be higher than that of ribotyping, some PFGE types included several ribotypes. Thus, combining data obtained by ribotyping and PFGE increases the likelihood of strains discrimination. Nevertheless, many of the Austrian strains remain indistinguishable from strains of foodborne outbreaks in other countries. This complies to previous results which show the highly clonal nature of the serotype 4b clonal group.

P1729

Multi-locus sequence typing of MNR loci for the typing of bacterial pathogens

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Background and Objectives: Mononucleotide repeats (MNR) are major component of the Simple Sequence Repeat (SSR) tandem genomic sequences that are highly mutable. Variation in MNR tracts may affect the bacterial phenotype, thereby allowing it to adapt to changing environments. Being highly mutable, MNR loci can be used as genomic markers for phylogenetics and typing. Here, we evaluate the potential of multi-locus sequence typing (MLST) analysis of loci harbouring MNR (MNR-MLST) for bacterial typing, and for inferring phylogenetic relationship among strains.
Methods: Our analysis was performed by the following order: (i) in silico genome-wide screen for MNR-containing sequences and selection of candidate loci (ii) characterization of the variation at the chosen MNR loci by sequencing these loci in several bacterial strains (iii) phylogenetic and statistical analyzes for,discrimination between given strains (iv) typing by allele specific oligonucleotide (ASO) hybridization.
Results: Genome-wide MNR search in published bacterial genomes showed that the majority of the SSRs (88–90%) were found to be MNRs, 4 to 9bp in length. MNRs are evenly distributed throughout the bacterial genomes with an average appearance of an MNR tract every 241 bp, 225 bp, 110 bp, and 224 bp in *E. coli* K12, *E. coli* O157:H7, *L. monocytogenes* and *V. cholerae* genomes, respectively. Among strains of each of these species, a high level of MNR polymorphism was found, however low enough to allow discrimination between strains. Sequence analysis of the MNR-containing loci, showed an average variation of 1 to 4 bp, either as polymorphism in the MNR itself or as single nucleotide polymorphisms (SNPs) in their flaking sequences. These MNR sequences were further used as probes for a custom-made allele specific oligonucleotide optic fiber microarray, dedicated to rapid high-throughput typing of bacterial strains based on their MNR-MLST signature. Amplified MNR-containing loci of *E. coli* strains that were hybridized to the array, clearly showed discrimination between all strains, and, moreover, were able to classify different O157 serotypes.
Conclusion: DNA sequences of MNR loci are a ready source of high variation content, which can be utilized for rapid strain identification. Using the MNR-MLST method, we can assign an 'identity card' to any given strain. Also, we show that MNR-MLST approach can be integrated in a high-throughput method for bacterial typing.

P1730

Bacteroides fragilis enterotoxin gene isoforms (bft 1, bft 2 and bft 3) among strains isolated in the Netherlands, France and Poland

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Bacteroides fragilis Gram-negative anaerobic rods were isolated from intestinal and extraintestinal sources: in France 53 strains, in The Netherlands 78 strains and in Poland 82 strains. The identification of bacterial strains was done on the basis of Gram staining, growth on selective BBE (Bacteroides Bile Esculine) medium, and biochemical characteristics determined by the API 20 A test (bioMérieux, France). For assessment of the presence of the enterotoxin (fragilysin) gene PCR was used. Amplification was performed with primers 404 and 407. For isoform identification PCR-RFLP was performed. PCR product was digested using restriction endonuclease Mse I. After DNA digestion we received fragments: for bft 1 263 bp and 165 bp, for bft 2 263 bp, 83 bp and 82 bp, for bft 3 211 bp, 110 bp, 82 bp and 18 bp. Among the 78 Dutch strains 12 contained the fragilysin gene (bft 1 – 8 strains, bft 2 – 1 strain, bft 3 – 1 strain and 2 strains were not identified). Between the 53 French strains 10 contained the fragilysin gene (bft 1 – 5 strains, bft 2 – 4 strains and bft 3 – 1 strain). Between 82 Polish strains 14 contained the fragilysin gene (bft 1 – 9 strains, bft 2 – 2 strains and 3 strains were not identified). In conclusion prevalence of the ETBF strains in The Netherlands, France and Poland is similar (15, 18, and 17%). Among the ETBF strains bft 1 isoform dominate (50–64%). bft 2 isoform were isolated most frequently in France (40%), in Poland only (14%) and in The Netherlands (8%). The first bft 3 isoform isolated in Europe was described by Scotto D'Abusco (2000). We found new bft 3 strains: one (isolated from ascites) among Dutch strains and one (isolated from peritoneal fluid) among French strains. We did not observe the bft 3 isoform among Polish ETBF strains.

P1731

Phenotypic and genotypic detection of 3 Mex efflux pumps in *P. aeruginosa*

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Objectives: Infections by *P. aeruginosa* are difficult to manage because of its frequent multiresistance. This multidrug resistance is often associated with active efflux by several transporters, among which MexAB-OprM, MexCD-OprJ, MexXY-OprM appear the more prevalent. Precocious detection of this mechanism of resistance by the clinical microbiologist appears worthwhile in this context. We have thus developed a combined phenotypic and genotypic methodology for the detection of these pumps.
Methods: We use 3 *P. aeruginosa* strains previously published as presenting resistance associated to 1 of these 3 Mex pumps and compared them to a wild type strain. MIC were determined by microdilution for 3 antibiotics selected for differential recognition by the 3 Mex pumps. Expression of inducible pump (MexD) was assessed by RT-PCR and overexpression of constitutive pumps (MexB, MexY), by QC-RT-PCR.
Results: RT-PCR confirmed the expression of MexD; QC-RT-PCR demonstrated overexpression of MexB (6-fold) and of MexY (8-fold). The table shows the MIC measured in the

pump involved in resistance	MIC (mg/L) – and + inhibitor					
	carbenicillin		erythromycin		gentamicin	
	-	+	-	+	-	+
Wild type	32	8	128	64	0.5	0.5
MexAB-OprM	**256**	8	256	64	0.5	0.5
MexCD-OprJ	16	8	**512**	64	0.5	0.5
MexXY-OprM	32	8	256	64	**8**	0.5

absence or in the presence of 50 mg/L Phe-Ala-beta-Naphtylamide (inhibitor of Mex transporters). Thus, overexpression of MexAB-OprM increased mainly the MIC of carbenicillin, of MexCD-OprJ, that of erythromycin, and of MexXY-OprM, that of gentamicin.

Conclusions: Resistance due to active efflux by Mex pumps can be easily detected by a simple phenotypic characterization and further confirmed by molecular biology approaches.

Antifungal susceptibility studies

P1732

Susceptibility pattern analysis to determine the in vitro susceptibility to fluconazole, voriconazole, itraconazole, and flucytosine in 1404 clinical yeast isolates (*Candida* spp.)

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Objectives: Resistance to azoles has become an emerging problem in Candida spp. In order to determine the frequency and type of resistance to different azoles, clinical yeast isolates collected in a multicentre study (2001–2002, performed by the DMykG antifungal study group) were tested and analysed using susceptibility pattern analysis (SPA).

Methods: A total of 1404 clinical isolates comprising *C. albicans* (47.6%), *C. glabrata* (23.3%), *C. tropicalis* (10.6%), *C. parapsilosis* (6.1%), and *C. krusei* (5.5%) were included. MICs were determined by microdilution according to the German reference method DIN-58940–84 in YST medium (Sifin, FRG). In order to achieve standardized results, susceptibility of each isolate to four antifungal agents (VOR, FLC, ITR, 5FC) was evaluated in a microdilution plate (Merlin Diagnostics, FRG) using one inoculum. SPA was performed for each isolate on a basis of the MICs read after 24 hours of incubation at 36°C. An isolate was classified as susceptible (S) or resistant (R) by applying the following breakpoints (R: FLC ≥ 64 mg/l, ITR ≥1 mg/l, VOR ≥4 mg/l (arbitrary), 5FC ≥ 32 mg/l). A susceptibility pattern (SP) was generated for each isolate by ranking the susceptibility class (S or R) of each antifungal agent in a specified order (FLC-VOR-ITR-5FC). The SP characterized the individual isolate and is mathematically an element of a set. Theoretically, 16 different sets of susceptibility patterns may occur here.

Results: Among 1404 isolates, only one *C. krusei* strain was found to be resistant to all four antifungals (SP: RRRR). Resistance to the three azoles (FLC-VOR-ITR) tested (SP: RRR) was found in 0.9% distributed as follows: *C. albicans*, 1% (n=7/669); *C. glabrata*, 1.2% (n=4/327), and *C. krusei*, 1.3% (n=1/77). Except one *C. krusei* isolate, all isolates were susceptible to 5FC. The SPA revealed that multi-resistant isolates were distributed as single spots in individual centers of different geographic origin. The percentage of isolates which were susceptible to all of the azoles tested varied between 95.7% (*C. albicans*) and 36.4% (*C. krusei*). Depending on the Candida species, SPs to azoles differed in a significant manner.

Conclusion: Multi-resistant Candida isolates to the antifungal agents tested are still rare, however, there is an obvious need for surveillance of resistance in yeasts. For this purpose, susceptibility pattern analysis offers a reliable tool.

P1733

Inhibitory effect of the Penicillium chrysogenum antifungal protein on different zygomycetes

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Objectives: Cases of zygomycosis (the opportunistic fungal infection caused by the members of Zygomycetes) increased dramatically over the past several years. Therefore, there is a substantial demand for new-type compounds with antifungal activity against the pathogenic species of Zygomycetes. The filamentous fungus Penicillium chrysogenum secretes a small, highly basic and cysteine-rich antifungal protein named PAF. This protein inhibits the growth of some filamentous fungi, including opportunistic human pathogens and phytopathogens. PAF induces a typical hyphal tip branching, oxidative stress and K+-efflux in the sensitive strains.

Methods: The effect of PAF on 21 fungal isolates representing 16 different genera has been investigated. *Aspergillus niger* and *A. terreus* were used as control for a PAF sensitive and a PAF insensitive strain, respectively. The effect of PAF on germination efficiency of sporangiospores was examined in different culture media. Untreated sporangiospores were plated as controls. An agar diffusion technique was used to estimate the size of inhibition of hyphal extension by PAF.

Results: The Mucor, Actinomucor, Cokeromyces, Gilbertella, Rhizopus, Saksanea and Syncephalastrum strains tested were practically insensitive to PAF independently of the culture conditions and inhibition tests applied. On the other hand, Absidia, Micromucor, Mortierella, Mycotypha, Rhizomucor, Rhizopus, Thamnostylum and Umbellopsis were sensitive to PAF. The PAF-sensitivity of some of the zygomycetes depended on the culture medium and the inhibition test selected, e.g. in the case of *M. africana*, *R. pusillus* and *U. isabellina*, the inhibitory effect was usually weaker on hyphal growth than on sporangiospore germination.

Conclusion: Among the zygomycetes considered as opportunistic human and/or animal pathogens, PAF was effective against Absidia, Mortierella, Rhizomucor and Rhizopus. These results support the assumption that PAF and similar low molecular mass basic antifungal proteins produced by filamentous fungi should be considered as promising candidates in future antifungal drug research.

P1734

Fungicidal activity of caspofungin and amphotericin B against *Candida guilliermondii* determined by time-killing studies

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Introduction: *C. guilliermondii* is isolated with a low frequency from blood culture, but treatment of infections produced by this species may present problems, specially in immunocompromised patients since a high percentage of strains have diminished susceptibility to fluconazole (MIC90, 16 mg/L) and itraconazole (MIC90, 0.5 mg/L), furthermore, a fungicidal agent is preferred for treatment of these patients. Amphotericin B and caspofungin have fungicidal activity against yeasts, however, most studies have focused on *C. albicans*.
Objective: To determine the killing kinetics of caspofungin and amphotericin B against *C. guilliermondii*.
Methods: Three strains of *C. guilliermondii* isolated from blood cultures were tested. MICs were determinate following M27-A2 method, but using an inoculum of $0.5–2.5\times10^4$ CFU/mL. Caspofungin MIC2 and MIC0 (minimum concentration of drug that produces a growth reduction 50% and 100% respect to growth control, respectively) were determinate; and for amphotericin B the MIC0 and the MFC (minimum fungicidal concentration). The MFC was obtained by transferring 0.1 mL from all clear MIC wells onto Sabouraud dextrose agar. The MFC was the lowest drug concentration that killed ≥99.9% (<5 colonies) from the starting inoculum. Time-killing studies were performed in RMPI 1640 medium (5 mL) by using an inoculum size from $1–7\times10^5$ CFU/mL. Concentrations tested were: 0.12, 0.5, 8, 2 and 32 mg/L. Aliquots were removed to determine CFUs at 0, 2, 4, 6, 12, 24 and 48h. Time-killing data were fitted to a lineal equation (log Nt = log N0 + Kt, where N = CFU, t = time, K = killing rate) to determine the time (hours) to achieve 50, 90, 99 and 99.9% (T50, T90, T99 and T99.9, respectively) of growth reductions from the starting inoculum at each concentration tested.
Results: Amphotericin B MICs were 0.06 and 0.5 mg/mL and MFCs were equal to MICs. Caspofungin MIC2 were 1 mg/L for the three strains and the MIC0 1–4 mg/L. The kinetics of amphotericin B fungicidal activity against *C. guilliermondii* was fast. With 2 mg/L, the T50 ranged was 6–35 minutes, depending on the strain, and the T99.9 between 1.5–5.9 hours. Caspofungin just produced growth inhibition, without fungicidal activity.
Conclusions: Amphotericin B and caspofungin were active against *C. guilliermondii* but only amphotericin B showed fungicidal activity.

P1735

Susceptibility testing of amphotericin B and caspofungin-resistant *Candida glabrata* isolates

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Objectives: *C. glabrata* isolates recovered from an ICU patient with invasive candidiasis, were found to be resistant to either amphotericin B (AmB), caspofungin (Caspo), or both. The aim of this study was to determine and compare the MICs by various in vitro methods.
Methods: The MIC for 4 *C. glabrata* isolates (labelled A–D) were determined by two broth microdilution methods: NCCLS (M27–A2) and EUCAST (discussion document E.dis 7.1). The MICs of AmB were also determined using the E-test. Time-kill studies were performed using both RPMI-1640 medium and Antibiotic Medium 3 (AM3). The criterion for determining MIC in the time-kill studies was the lowest concentration giving minimum 1-log reduction in cfu/mL after 24 hours.
Results: Three of the 4 isolates were clearly AmB resistant according to the E-test results with MICs ranging between 6 and >32 mg/mL. Microdilution endpoints ranged between 1 and 4 mg/mL and time-kill endpoints between 2 and 4 mg/mL. Two isolates were proven to be resistant to Caspo in the microdilution methods, a finding that was confirmed by time-kill studies. The MIC values for Caspo were found to be reduced in all methods in AM3 medium compared to RPMI1640. For both Caspo and AmB the MICs determined by the NCCLS method and the EUCAST method were all within ±1 dilutions. Table 1 shows the MICs for all four isolates.

Table 1. MIC values (μg/mL) obtained by various methods

	E-test		Broth microdilutions				Time–kill	
			RPMI		AM3		RPMI	AM3
	min	max	NCCLS	EUCAST	NCCLS	EUCAST		
AmB								
isolate A	>32	>32	1-2	1-2	2-2	4-4	4	4
isolate B	8	12	1-1	1-1	1-1	2-2	2	4
isolate C	1,5	2	0,5-0,5	0,25-0,5	0,5-0,5	0,5-0,5	2	0,5
isolate D	6	6	1-1	1-1	2-2	2-4	2	4
Caspo								
isolate A		-	0,5-0,5	0,125-0,25	<0,06	<0,06-<0,06	0,5	<0,015
isolate B		-	8-8	4->8	0,5-0,5	0,5-0,5	>32	>2
isolate C		-	1-1	0,25-1	<0,06-<0,06	<0,06-<0,06	1	<0,015
isolate D		-	>8->8	8->8	4-8	4-8	>32	-

Conclusion: The MIC values of AmB depend on the method. The E-test showed the highest, and the broth microdilutions methods the lowest values. The MIC values of Caspo depend on the medium used. Values obtained using AM3 were lower than values obtained using RPMI–1640. The NCCLS and EUCAST broth microdilution methods were equally useful in determining MICs of AmB and Caspo for *C. glabrata*.

P1736

Correlation of neo-sensitabs on three media with NCCLS reference disk diffusion and broth microdilution methods for testing the susceptibility of *Candida spp*. and *Cryptococcus neoformans* to fluconazole and voriconazole

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Objective: The purpose of this study was to compare both NCCLS reference agar disk diffusion (M44-A document) and broth microdilution (M27-A2 document) methods with the Neo-Sensitabs method (Rosco, Taastrup, Denmark) for determination of the susceptibility of 10 to 20 isolates each of *Candida spp*. (8 species) and *C. neoformans* to fluconazole and voriconazole.
Methods: NCCLS agar disk diffusion and broth microdilution methods were performed according to NCCLS standard conditions. The Neo-sensitabs method was performed according to the manufacturer's instructions on three different media: Mueller-Hinton + 2% glucose and 0.5 mg/L methylene blue [MHGM, M44 agar], RPMI-1640 [Remel, RPMI] and Shadomy [E&O laboratories, SHAD] agars), 25-mcg fluconazole and 1-mcg voriconazole tablets and the same inoculum size as for M44-A method. Plates were incubated for 24 to 72 h.
Results: The performance of Neo-sensitabs was similar to that of the M44-A disk diffusion method as demonstrated by comparison of zone diameters and M27-A MIC values. The

regression statistics by the M44-A method for fluconazole and voriconazole provided R values of 0.877 and .759, respectively. The R values for the Neo-sensitabs fluconazole tablets (24 h results for Candida spp. and 48 h for *C. neoformans*) were: .892 (MHGM), .929 (RPMI) and .874 (SHAD) and for voriconazole tablets: .790 (MHGM), .757 (RPMI), and .736 (SHAD). The overall categorical agreement (>90%) was also similar to that obtained between M44-A zone diameters and M27-A MICs; both fluconazole and voriconazole tablets were able to identify fluconazole-resistant strains (MICs >64 mg/L) as well as those with decreased susceptibility to voriconazole (MICs >2 mg/L).
Conclusions: The tablets had suitable and similar performance on the three media evaluated with both antifungal agents. Therefore, the Neo-sensitabs tablet method is a potential alternative to the M44-A method for testing the susceptibility of yeasts to fluconazole and voriconazole using the standard MHGM agar.

P1737

Candida strains with decreased susceptibility to fluconazole. Cross-resistance to other azoles and activity of other antimycotic agents

P. Gaustad, P. Sandven *(Oslo, N)*

Objective: Decreased susceptibility to azoles has been reported in Candida species and in particular in *C. glabrata*. Selection pressure due to the continuous exposure to azoles seems essential in the development of resistance in Candida spp. The aim of the study was to look for cross-resistance between antimycotics. Strains with decreased susceptibility to fluconazole were chosen and tested for resistance to other azoles and to other groups of antimycotics.
Methods: The study included 101 yeasts isolates from patients with various fungal infections. Strains with varying susceptibility to fluconazole (range MIC≥ 0.25–256 mg/l) were included: *C. albicans* (n = 35), *C. dubliniensisis* (n = 1), *C. glabrata* (n = 24), *C. guilliermondii* (n = 3), *C. krusei* (n = 10), *C. norvegensis* (n = 11), *C. parapsilosis* (n = 2), *C. tropicalis* (n = 13) and *Saccharomyces cerevisiae* (n = 2). The yeast strains were tested for susceptibility to fluconazole, itraconazole, ketoconazole, voriconazole, 5-flucytosin, amphotericin B and caspofungin using Etest. When available the NCCLS breakpoints were used (resistant (R) MIC ≥64 mg/l; itraconazole R MIC ≥1 mg/l; 5-flucytosin R MIC ≥ 32 mg/l), otherwise the breakpoints were chosen from recommendations in the literature (voriconazole R MIC ≥ 1 mg/l; ketoconazole R MIC ≥0.125 mg/l; amphotericin B R MIC ≥1; caspofungin R MIC ≥1 mg/l).
Results: All the strains were susceptible to caspofungin (MIC < 1 mg/l) and to amphotericin B (MIC < 1 mg/l) except 7 strains (MIC = 1–3 mg/l). 24% of the strains were resistant to 5 flucytosin (MIC ≥ 32 mg/l).For the azoles the results indicated cross-resistance. The 23 strains of the species *C. krusei*, *S. cerevisiae* and *C. norvegensis*, species known for resistance to fluconazole, were all resistant to ketoconazole and itraconazole (MIC≥ 1 mg/l) and had decreased susceptibility to voriconazole (MIC = 0,5 mg/l 8 strains) or were resistant (MIC≥1 mg/l 15 strains) (Table 1). In the other species included in the study decreased susceptibility to fluconazole was linked to decreased susceptibility or resistance to other azoles.
Conclusions: Strains with decreased fluconazole susceptibility frequently showed cross-resistance to other azoles. Treatment with non-azole antimycotics should be considered in serious infections caused by yeasts with decreased fluconazole susceptibility. All the isolates were fully susceptible to caspofungin, the majority to amphotericin B and 76% to 5-flucytosin.

Table 1. Cross distribution of fluconazole and voriconazole MICs

Flukonazole MIC (mg/l) \ Voriconazole MIC (mg/l)	0,008	0,016	0,032	0,064	0,125	0,25	0,5	1	2	4	8	32
0,25		1										
0,5	1	1		1								
1		1	1	3	3							
2			1	1	1							
4			1	2	8	5		1				
8				2	5	8	3					
16			1	2		3	3	2				
32						1	1			1		
64							6	3	2			
128								1				
256						1	2	1	9	6	1	5

P1738

In vitro activities of voriconazole, fluconazole, itraconazole and ketoconazole against non-albicans Candida strains

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Objective: The aim of this study was to evaluate the in vitro activity of voriconazole (VZ) compared with those of fluconazole (FZ), itraconazole (IZ) and ketoconazole (KZ) against non-albicans Candida strains.
Material and methods: All 253 non-albicans Candida species (132 *C. glabrata*, 67 *C. parapsilosis*, 40 *C. tropicalis* and 14 *C. krusei*) were recovered from clinical samples isolated between June 2002 and September 2004. The minimal inhibitory concentrations (MICs) were determined by Sensititre YeastOne colorimetric method in accordance with the manufacturer's instructions. *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 200956 were used as control strains.
Results: Table I shows the MIC 90 to FZ, IZ, KZ and VZ of the 253 non-albicans Candida species. VZ demonstrated excellent activity in all studied species. All of the *C. krusei* isolates tested were susceptible to VZ (MIC ≤ 1 mg/L). All of the isolates of *C. glabrata* susceptible-dose-dependent (MIC from 16 to 32 mg/L) to FZ (78 strains) showed a MIC to VZ ≤ 1 mg/L. Among the FZ resistant *C. glabrata* isolates (38 strains), all but one were also resistant to IZ (RR phenotype). The MIC to VZ was ≥2 mg/L in 24 out of the 37 (65%) *C. glabrata* with RR phenotype.

	CMI 90 (mg/L).			
Species (n)	Fluconazole	Itraconazole	Ketoconazole	Voriconazole
C. glabrata (132)	256	≥16	8	2
C. parapsilosis (65)	8	0.25	0.25	0.25
C. tropicalis (40)	8	0.5	0.125	0.25
C. krusei (14)	128	0.5	1	0.5

Conclusions: Voriconazole showed good activity against all Candida non-albicans strains, including *C. krusei*. Cross-resistance was observed at the strains of *C. glabrata* with RR phenotype.

P1739

In vitro activity of voriconazole and fluconazole against Candida species: a report from the ARTEMIS global surveillance program

D.L. Gibbs, D.J. Sheehan *on behalf of the ARTEMIS Global Antifungal Study Group*

Objective: To compare the susceptibility of 140,767 yeasts collected worldwide over a six-year period to voriconazole

and fluconazole by disk diffusion and analysed by the BIOMIC Vision System.

Methods: Fluconazole disk susceptibility test results were collected from 127 investigators in 39 countries from 1997 through 2003. Data was collected on voriconazole from 2001–3. All investigators tested yeast isolates by NCCLS M44-A disk method. Test plates were read and results recorded with the BIOMIC® Vision System. Species, drug, zone diameter, susceptibility category, MIC, and Quality Control results were collected quarterly via email. Duplicate (same: patient, species, and sensitive-resistant biotype profile during any 7-day period) and uncontrolled test results were not analysed. MICs were automatically determined through the BIOMIC Vision System from the agar gradient around each disk calibrated with MICs by NCCLS M27-A.

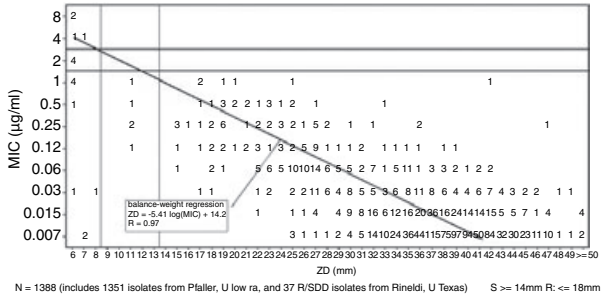
Results: Fluconazole and voriconazole disk diffusion test results were obtained on 140,767 and 79,343 yeast isolates, respectively. The cumulative susceptibility of >79,000 Candida spp tested from 2001–2003 showed approximately 1.5 log lower MIC values for voriconazole versus fluconazole. The 10 most common Candida species showed less resistance to voriconazole than to fluconazole. *C. krusei* and *C. rugosa* showed the greatest difference with >50% resistance to fluconazole and <2% to voriconazole. All species were more susceptible to voriconazole than fluconazole assuming proposed voriconazole interpretive breakpoints of 1 (susceptible) and 4 mcg/ml (resistant).

Voriconazole MICs and Interpretation by Organism (1-2-4*)

Organism	%S	%SDD	%R	MIC 50	MIC 90	N
Candida albicans	99.0	0.4	0.7	0.02	0.09	47,584
Candida glabrata	78.6	15 6	5.8	0.36	2.08	8,719
Candida tropicalis	93.3	4.3	2.4	0.11	0.86	5,643
Candida parapsilosis	98.2	1.0	0.8	0.02	0.16	5,233
Candida species	95.3	2.0	2.8	0.03	0.41	4,094
Candida krusel	92.5	5.8	1.7	0.24	0.95	1,996
Cryptococcus neoformans	98.3	0.6	1.1	0.01	0.14	1,266
Other Yeast	95.2	1.9	2.9	0.04	0.41	1,103
Candida guilliermondii	95.1	3.2	1.7	0.09	0.60	633
Candida lusitaniae	98.0	0.4	1.6	0.01	0.12	445
Saccharomyces cerevisiae	96.8	1.2	2.0	0.03	0.24	401
Candida rugosa	73.6	25.1	1.3	0.34	1.81	394
Candida kefyr	99.4	0.3	0.3	0.01	0.06	331
Trichosporon sp.	97.3	1.9	0.8	0.06	0.60	264
Candida famata	94.5	3.8	1.7	0.04	0.72	238
Candida inconspicua	94.6	2.7	2.7	0.14	0.72	186
Candida norvegensis	98.9		1.1	0.11	0.54	91
Rhodotorula sp.	51.7	15.7	32.6	0.86	>4.5	89
Trich. beigelii / cutaneum	84.6	6.4	9.0	0.08	2.88	78
Crypto neo var. neoformans	98.5		1.5	0.08	0.34	67
Candida dubllniensis	100.0			0.01	0.05	63
Blastoschizomyces capitatus	94.5	3.6	1.8	0.09	0.95	55
Candida lipolytica	80.8	19.2		0.11	1.25	52

115 Sites in 35 Countries. All Specimen Types, All Locations in Hospital.
Breakpoints based on MIC of 1/2/4 = S/SDD/R.

The Correlation of Broth Microdilution and Disk Diffusion Test Results with Non-glabrata Yeast Species and Voriconazole



N = 1388 (includes 1351 isolates from Pfaller, U low ra, and 37 R/SDD isolates from Rineldi, U Texas) S >= 14mm R: <= 18mm

Conclusion: Susceptibility of Candida spp (collected worldwide) to voriconazole and fluconazole have changed minimally over the study period. Both agents show stable susceptibility patterns under the selection pressure of increased hospital usage. Voriconazole appears to be a promising new agent for the treatment of Candida spp based on its extended spectrum in vitro activity.

P1740

In vitro activity of voriconazole and fluconazole vs >8000 *C. glabrata* isolates

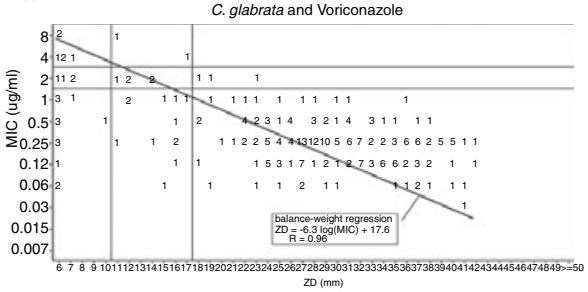
D.J. Sheehan, D.L. Gibbs *on behalf of the ARTEMIS Global Antifungal Study Group*

Objective: To compare the activity of voriconazole and fluconazole against >8000 isolates of *C. glabrata* determined by disk diffusion and analysed by the BIOMIC Vision System.

Methods: 115 investigators in 35 countries participated in the ARTEMIS global surveillance program from 2001 through 2003. *C. glabrata* isolates were tested by the NCCLS standard M44-A disk diffusion method. Test plates were read and results recorded with the BIOMIC® Image Analysis System. Species, drug, zone diameter, susceptibility category, MIC, and Quality Control results were collected quarterly via email. Duplicate (same: patient, species, and sensitive-resistant biotype profile during any 7 day period) and uncontrolled results were not analysed. MICs were automatically determined through BIOMIC Image Analysis System by balance weight regression from the agar gradient around each disk calibrated with MICs by NCCLS M27-A.

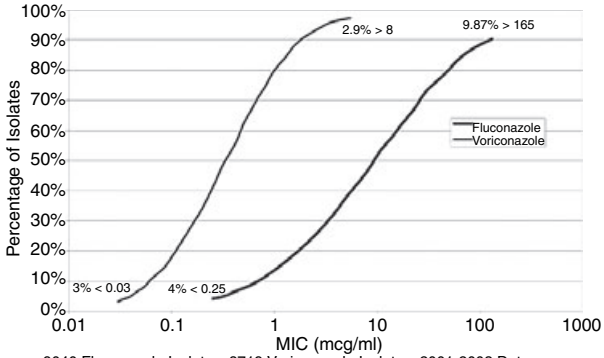
Results: Voriconazole and fluconazole disk diffusion test results were obtained on 8719 and 9040 *Candida glabrata* isolates, respectively. Voriconazole demonstrated approximately 1.5 log lower MICs than fluconazole. MIC 50 and 90 values for voriconazole were 0.36 and 2 mcg/ml, respectively versus 10 and 128 mcg/ml for fluconazole. Assuming interpretive breakpoints of 1 (susceptible), 2 (susceptible-dose dependent) and 4 mcg/ml (resistant) for voriconazole, 5.8% of isolates were resistant to voriconazole, versus 16.6% resistant to fluconazole at

Appendix 1b, the Correlation of Broth Microdilution and Disk Diffusion Test Results with *C. glabrata* and Voriconazole



N = 262 (includes 235 isolates from Pfaller, U low ra, and 47 R/SDD isolates from Rinaldi, U Texas) S >= 18mm R: <= 10mm

Cumulative Susceptibility of *C. glabrata* to Fluconazole and Voriconazole



9040 Fluconazole Isolates, 8719 Voriconazole Isplates, 2001-2003 Data

≥64 mcg/ml. *C. glabrata* isolates were plotted separately from other species which resulted in a high correlation (R = 0.96) between disk diffusion and reference broth dilution MIC.
Conclusion: This investigation demonstrated that 94.2% of 8719 *Candida glabrata* isolates were susceptible (S + S-DD) to voriconazole. Based on in vitro data, voriconazole is a promising new agent for treating *C. glabrata*. Disk diffusion testing correlated well with the reference broth dilution MIC.

P1741

Potential for a natural anticandidal combination comprising garlic, cinnamon and *Lactobacillus acidophilus*

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Objectives: Increasingly, Candida spp. are developing resistance to standard antimicrobial therapies and recurrent candidal infections are becoming a major problem. In order to express its pathogenicity, a yeast cell must attach to epithelial tissue (particularly oral and vaginal tissues). In this study, the efficacy of garlic and cinnamon, two plant materials with anticandidal activity, but no known resistance susceptibility, were investigated. The ability of these materials individually, and in combination with *Lactobacillus acidophilus*, to inhibit attachment of *C. albicans* to human buccal epithelial cells (BEC) was studied.
Methods: The in vitro attachment of the yeast to the BEC of 18 healthy volunteers (14 females, 4 males, mean age 33.3 ± 2.8 years) was assessed using a modification of Kimura and Pearsall (1978). The minimum inhibitory concentration (MIC) of garlic and cinnamon powders with *C. albicans* and *L. acidophilus* were evaluated using a broth bioassay procedure. Electron microscopy was used to visualise structural damage to the yeast in the presence of garlic.
Results: The MIC for garlic with *C. albicans* is 2–3 mg/ml and with *L. acidophilus* is 17.5 mg/ml. The MIC for cinnamon with *C. albicans* is 1–2 mg/ml and with *L. acidophilus* is 4 mg/ml. Candidal attachment to the BEC was inhibited with greatest effects observed at 5 mg/ml garlic or 5–10 mg/ml cinnamon or in the presence of $1x10^8$ CFU/ml *L. acidophilus*. Structural damage to the yeast cells occurred at 1.5 mg/ml garlic with severe disruption of the cellular integrity at 20 mg/ml garlic. When garlic and *L. acidophilus* were added to the BEC together, a synergistic effect on the inhibition of candidal attachment occurred but if cinnamon was combined with *L. acidophilus* (which had been shown to have an inhibitory effect on the growth of this bacteria) the opposite effect was observed.
Conclusion: From these in vitro studies it appears that the combination of *L. acidophilus* and garlic should provide a preventative/treatment regime to address the growing problem with candidal infections. The next stage will involve setting up a randomised, double blind, placebo controlled trial with a product comprising the combination of *L. acidophilus* and garlic.

P1742

The effect of subinhibitory concentrations of antifungal agents on variation of cell surface hydrophobicity and biofilm formation with relation to genotype of *Candida albicans* and *Candida dubliniensis*

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Objectives: This work studied the variation of cell surface hydrophobicity (CSH), the biofilm formation and their relationship to genotype in clinical isolates of *C. albicans* and *C. dubliniensis*. Next objective was aimed at the effect of sub inhibitory concentrations of the conventional azole derivates fluconazole and itraconazole, the new azole voriconazole, polyene amphotericin B and the experimental antifungal agent 6-amino-2-n-pentylthiobenzothiazole (APB) on both CSH and biofilm formation.
Methods: For this study, 50 *C. albicans* and *C. dubliniensis* clinical isolates from immunocompromised patients were collected. Genotyping of Candida isolates was performed by PCR assay using set primer pair: Ca-INT-L and Ca-INT-R. The CSH was measured by biphasic separation method in YNB medium/octane overlay. Biofilm formation was quantified as the ability to adhere to polystyrene plastic surfaces and the formation of biofilm was measured by the XTT reduction assay. The MIC95 values for tested antifungal agents were established by broth microdilution method according to NCCLS M27-A reference method and these values were used to calculate sub inhibitory concentrations in subsequent experiments.
Results: All *C. albicans* isolates were separated into three genotype groups: genotype A (12), genotype B (13), genotype C (12). Thirteen isolates represented genotype D (*C. dubliniensis*). In the XTT assay, the mean A490 values for the 50 strains were 0.359 ± 0.130, for the genotype A 0.42 ± 0.154, for the genotype B 0.262 ± 0.072, for the genotype C 0.281167± 0.059 and for genotype D 0,.471231 ± 0.07. The relative hydrophobicity of cells was established: 11.48% for the genotype A, 18.32% for the genotype B, 18.44% for the genotype C and 71.55% for genotype D. Although, no significant difference was detected among biofilm formation in genotype A and genotype D, the CSH calculated for genotype D was 6.2-fold highest in comparison with genotype A. The difference between genotype B and C was determined neither in biofilm formation nor in CSH. The influence of sub inhibitory concentrations of tested antifungal agents on CSH and biofilm formation was observed as well. Moreover, sub inhibitory concentration of fluconazole decreased the ability to form biofilm, but increased the CSH in fluconazole-resistant isolates.
Conclusion: Results suggested the association between genotype and biofilm formation, as well as confirmed the effect of antifungal agents on both biofilm and CSH.

P1743

Can we accurately assess antifungal cidality in vitro? Cidality studies of voriconazole and caspofungin against *Candida* species

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Objectives: A perception exists among many that antifungal agents exhibit either fungicidal or fungistatic activity. For example, caspofungin (CAS) is perceived to exhibit fungicidal activity and voriconazole (VOR) fungistatic activity against Candida spp. However, standard methods to accurately assess these phenomena are currently lacking. In this study, we employed the suggested standard method of Canton et al. (Diag Microbiol Infect Dis 2003) to determine the fungicidal activity of an antifungal agent against Candida species.
Methods: MIC testing was performed according to the standard NCCLS M27-A2 method except that the inoculum size was increased to 104 colony-forming units (CFU)/ml. The total content (200 microliters) of each clear well in the broth microdilution assay was subcultured onto two potato dextrose agar plates (100 microliters onto each). To minimize antifungal carryover, the 100 microliter aliquots were allowed to soak into the agar before streaking for isolation. Plates were incubated for

48 hours at 35°C. Fungicidal activity was defined as > 99.9% reduction in the number of CFU/ml. Ten *Candida albicans* and eighty non-albicans isolates, including *C. dubliniensis* (3), *C. glabrata* (20), *C. tropicalis* (20), *C. krusei* (20), *C. lusitaniae* (7), and *C. parapsilosis* (10) were tested.
Results: As expected, VOR exhibited fungistatic activity and CAS fungicidal activity against all *C. albicans* isolates. However, VOR demonstrated cidal activity against 10% of the non-albicans strains tested while CAS was cidal against only 81% of these same non-albicans isolates. Of note, VOR was cidal versus 40% of *C. parapsilosis* isolates tested while CAS exhibited fungistatic activity against 20% of these strains.
Conclusion: Using a method that limits drug carryover when assessing cidality, we found that CAS lacked complete cidality against non-albicans spp and especially, *C. parapsilosis*. This finding may provide an explanation why CAS MICs are elevated for this particular Candida species. Importantly, until methods are standardized to accurately assess the cidal nature of an antifungal agent, we should be wary of preconceived antifungal characterizations.

P1744

Susceptibility of itraconazole and voriconazole using flow cytometry

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A. Gonçalves-Rodrigues *(Porto, P)*

The need of antifungals susceptibility tests is now a reality to aid empiric selection of antimicrobials, specially on candidemia, deep infections or on isolates from recurrent mucosal disease. NCCLS methods are standardized and reproductive but labor intensive, time consuming and takes at least 48 hours to give results. Flow cytometry analysis, has been repeatedly used by our team to evaluate susceptibility of Candida to different drugs, with considerable advantages (1).
Objective: To optimize conditions to study the susceptibility to voriconazole and itraconazole.
Methods: Ten Candida strains (9 clinical isolates and one ATCC type strain) with different susceptibility patterns were studied. Itraconazole was a kind gift by Jansen Pharmaceutic and voriconazole by Pfizer. Minimal Inhibitory Concentrations (MIC) were determined using the NCCLS protocol M27-A. For flow cytometry analysis 106 cells/ml were incubated with MIC values, MIC/2 and 2xMIC in PBS (Sigma) during 1, 2 and 3 hours. The yeasts were washed and stained both with 0.5 μM of FUN-1 (Molecular Probes) an indicator of metabolic activity and with 1 μg/ml of propidium iodide (PI; Sigma) a marker of cell death. The cell suspension was analysed on a flow cytometer (Beckman Coulter XL-MCL): the morphology (scattergram) and the intensity of fluorescence of the stained cells were evaluated.
Results: No obvious morphologic cell changes were observed under the described experimental conditions. FUN-1 staining increased on susceptible strains, after 1 hour incubation with MIC or with higher concentrations, meaning a metabolic impairment; resistant strains, at MIC concentrations, didn't present any increase on the staining. PI was unable to stain the cells, even at the highest concentrations of the drug and following the longest incubation periods.
Conclusions: Flow cytometry analysis and FUN-1 staining allowed the determination of the susceptibility profile to itraconazole and voriconazole, soon after 1 hour of incubation, contrasting with the 48 hours needed on classical methods. PI was not a good marker to study the effect of these azoles, which is in accordance with the fungistatic mechanism of action. (1) Pina-Vaz C, et al. J Med Microbiol 49: 831–840, 2000

P1745

Antifungal resistance and production of virulence factors in *Candida* spp. isolated in Italy during the period 2002–2004 from paediatric and adult patients

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A. Cavallero, M.C. Ossi, G.C. Schito *(Genoa, Milan, I)*

Objectives: 1 - to assess the rates of antifungal resistance and of phospholipase and proteinase production in Candida spp. recently isolated form clinical specimens in adult and paediatric patients and 2 - to establish whether a correlation exists between virulence factors, resistance, site of infection and age of patients.
Methods: 505 Candida spp. including 337 *C. albicans* , 68 *C. glabrata*, 40 *C. parapsilosis*, 34 *C. tropicalis*, 11 *C. krusei* and 15 other species have been studied. Susceptibility to fluconazole, amphotericinB and flucytosine was determined by the broth dilution method (M27-A2, NCCLS). Strains producing proteinases and phospholipases were identified using plates containing media supplemented with BSA and egg yolk respectively.
Results: Susceptibility to fluconazole, amphotericinB and flucytosine was more common among *C. albicans* (97.6%, 99.1%, 99.4% respectively) than among non-albicans species (83.3%, 85.7%., 92.2.%). Production of proteinases and phospholipases was significantly more common among *C. albicans* (91.1% and 92.6% respectively) than among non-albicans strains (38.1% and 35.1%). There were no important differences in the patterns of resistance and production of exoenzymes when grouping the strains according to the patient's age (resistance from 95.4 to 99.1% in 348 adults vs 93.1–98.7% in 157 children and synthesis of exoenzymes from 71.5–79.5 vs 77.4–81.8). Strains from vaginal swabs were slightly more resistant to fluconazole (3.2%) than isolates from other specimens (urine, respiratory secretions, blood and wound/skin/soft tissues: 0.8–1.9%). Concerning production of exoenzymes, no significant differences were observed among *C. albicans* isolates from different specimens, while isolates of *C. glabrata* originating from blood produced more frequently proteinases (23.1%) and less frequently phospholipases (23.1%) than isolates obtained from urine, vaginal swabs and wound/skin/soft tissues (13.6%, 6.7%, 0% and 59.1%, 53.3%, 50% respectively). Similar results were obtained for *C. tropicalis*.
Conclusions: *C.albicans* and non-albicans isolates originated from paediatric and adult patients possess similar characteristics in terms of antifungal susceptibility and exoenzyme production. However, rates of antifungal resistance and synthesis of virulence factors are different among different Candida species and may vary among strains belonging to the same species depending on the site of infection.

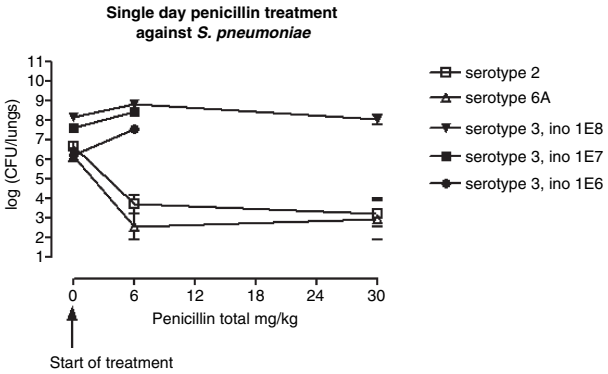
Pathogenesis of respiratory tract and other infections

P1746

Reduced effect of penicillin treatment against a penicillin susceptible *Streptococcus pneumoniae* serotype 3 as compared to serotypes 2 and 6A in a murine pneumonia model

R.L. Fischer, N. Frimodt-Møller *(Copenhagen, DK)*

Objectives: The effect of a single day treatment with penicillin was tested against three penicillin susceptible *Streptococcus pneumoniae* strains with different serotypes in immunocompetent mice.
Methods: Pneumonia was induced in outbred, immunocompetent mice by inoculation of a *S. pneumoniae* intranasally. Three penicillin susceptible strains were tested; D39: serotype 2, 68034: serotype 3 and strain 6A from Iceland. In addition, three different 68034 inoculi of 1e8, 1e7 and 1e6 cfu/ml, respectively, were tested in the pneumonia model to investigate the correlation between inoculum size and effect of penicillin. The mice were treated with penicillin BID 3 or 15 mg/kg at 24 and 30 hours after inoculation. Colony counts from lung homogenates were determined at start of treatment and 24 hours after start of treatment. MIC's were determined by Etest, and in vitro time-kill curves were performed for 1, 2, 4 and 8 times MIC according to the NCCLS guideline.
Results: All 3 strains had a penicillin MIC of 0.016–0.032 μg/mL. The in vivo results showed a 3–4 log reduction in cfu/lungs for the *S. pneumoniae* strains serotype 2 and 6A after treatment with 6–30 mg penicillin/kg, compared to colony counts at start of treatment (see Fig). The opposite effect was observed for the *S. pneumoniae* serotype 3 strain, as a one log increase in growth was detected after treatment with 6 mg penicillin/kg compared to colony counts at start of treatment. The lack of effect after the 6 mg/kg penicillin treatment was observed for all three tested serotype 3 inoculi (see Fig). A static effect on serotype 3 was observed for the higher penicillin dose of 30 mg/kg. The in vitro time-kill curves, however, showed equal non-concentration dependent killing of all three strains.



Conclusion: The effect of penicillin on the penicillin susceptible *S. pneumoniae* serotype 3 strain was greatly reduced compared to two other tested serotypes. No difference in penicillin activity was observed for the three strains in vitro. Serotype 3 has a huge polysaccharide capsule giving it a typical mucoid appearance of the colonies on blood agar, which from the present data apparently impedes the effect of penicillin in vivo.

P1747

Interaction between penicillin and erythromycin treatment on *Streptococcus pneumoniae* in a murine pneumonia model

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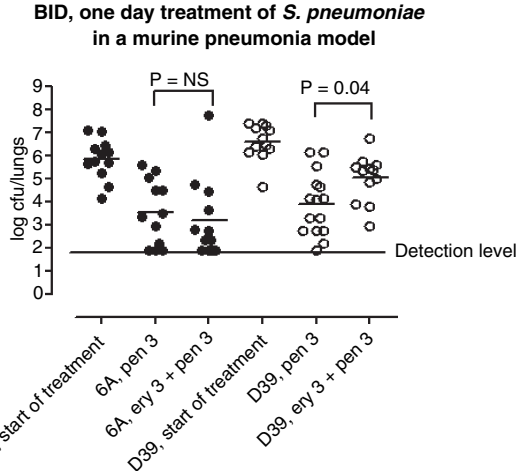
Objectives: The antimicrobial response after treatment with penicillin (P) and erythromycin (E) in combination was investigated against *S. pneumoniae* in a murine pneumonia model.
Methods: Pneumonia was induced in outbred, immunocompetent mice by inoculation of a *S. pneumoniae* intranasally. Two strains were used, D39 (serotype 2) and serotype 6A from Iceland, both P and E susceptible. All mice were treated BID in groups of 6 mice at 24 and 30 hours after inoculation. The following five BID dose regimens were tested: P 3 mg/kg, E 3 or 13 mg/kg, P 3 mg/kg in combination with E 3 mg/kg and finally P 3 mg/kg in combination with E 13 mg/kg. In addition, the combination of the two compounds was either dosed simultaneous or P was given a half hour later than the E treatment. Colony counts from lung homogenates were determined at start of treatment and 24 hours after start of treatment. Time-kill curves with P and E alone and in combination against the two strains were performed in addition.
Results: In vitro antagonism was shown for the combination of P and E, i.e. same killing effect as for E alone but less so than for P alone. Not only P, but also E had a cidal effect on both strains in vivo when tested individually. The E mean log reduction in colony counts compared to start of treatment was 1.6 for BID 3 mg/kg and 3 for BID 13 mg/kg, similar for both strains. The P and E combination did not increase the effect on the tested pneumococci compared to the effect of P alone (see Fig). The colony counts for strain 6A after P treatment was not significantly different to the treatment with E 3 mg/kg (P 3 mg/kg, whereas, E had an antagonistic effect on P for the D39 strain (P = 0.04, Mann–Whitney test). No difference in effect was observed between dosing simultaneous and using half hour separation of the two compounds.



Conclusion: Combination treatment with a beta-lactam and a macrolide is commonly recommended for empiric treatment of community acquired pneumonia. Testing such a combination in the experimental mouse pneumonia model showed no additive effect on the pneumococci, and a significant antagonistic effect was observed for one strain. We recommend that the combination of P and E should not be administered if a pneumococcal etiology is suspected.

P1748

Efficacy of moxifloxacin significantly more effective than levofloxacin against a first-step ParC mutant of *Streptococcus pneumoniae* in a murine model of pneumonia

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Objective: Fluoroquinolone (FQ) resistance in *Streptococcus pneumoniae* (SPN) occurs by stepwise selection of mutations in genes encoding type II topoisomerases. Clinical isolates with single-step ParC mutations are increasing & their subsequent exposure to a less active FQ could render them resistant. In a bid to reduce numbers of resistant bacteria the use of more potent agents like MOX to treat pneumonia has been advocated. We tested this by comparing moxifloxacin (MOX) & levofloxacin (LEV) efficacy against pneumonia caused by a ParC mutant in mice.
Methods: Pneumonia in Swiss Webster mice was induced by endotracheal inoculation with a susceptible (LEV MIC 2 mg/L; MOX MIC 0.06 mg/L) serotype 3 SPN that harbours an D83Y substitution in ParC. At 24 h, surface-temperature (ST) was measured for assessment of disease severity prior to treatment. We have shown that moderately (MOD)-ill mice have a ST of ≥32°C & 6–$\log_{10}$ CFU in their lungs. By comparison, severely (SEV)-ill (>30°C but <32°C) & gravely-ill (≤30°C) mice have 7- & 8-$\log_{10}$CFU, respectively. Drug was administered subcutaneously to all mice (50 mg/kg q12) at 24 h & for up to 5 days. Since we have shown death to be imminent at a ST ≤30°C, mice were euthanized during treatment if their ST dropped to 30°C or less & continued to drop over the next 24 h. Bacterial counts from lungs of all mice were determined using an automated spiral plating system. Resistant isolates (LEV MIC ≥4 μg/ml) recovered from mice were detected similarly on a LEV concentration gradient & characterized for resistance mechanisms.
Results: Among MOD-ill mice, 21 of 23 (91%) survived MOX treatment & 5 of 37 (14%) survived LEV treatment ($P < 0.001$). Among SEV-ill mice, 7 of 7 (100%) survived MOX & 1 of 4 (25%) survived LEV ($P = 0.02$). Among MOD-ill mice, bacterial counts in 3 of 23 (13%) mice treated with MOX & 36 of 37 (97%) treated with LEV were noted ($P < 0.0001$). Among SEV-ill mice, none treated with MOX but all treated with LEV had counts (mean, 7.3$\log_{10}$CFU) ($P = 0.003$). Resistant isolates (LEV MIC ≥4 mg/L) were recovered from 11 of 41 mice treated with LEV but only 1 of 30 mice treated with MOX.
Conclusions: MOX is significantly more effective than LEV for treatment of pneumonia caused by a ParC mutant. Its ability to minimize selection of high-level FQ resistance compared with LEV further supports the notion that a more potent FQ should be used as a means to reduce FQ resistance among SPN.

P1749

Effect of porins and AmpC betalactamases in the treatment of experimental pneumonia due to *Klebsiella pneumoniae*

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Objectives: Enterobacterial strains with decreased outer membrane permeability in combination with plasmid-mediated class C betalactamases (such AmpC) show an increased in vitro levels of resistance to betalactams, including carbapenems. The aim of this study was to evaluate the in vivo efficacy of human regimens of imipenem (IMP), meropenem (MEM) and cefepime (FEP) in the experimental pneumonia due to AmpC producing *Klebsiella pneumoniae* (Kp) and one of its in vitro-porin-deficient mutant by using an animal model with immunocompetent rats.
Methods: Strain Kp12 is a derivative of Kp52145 (clinical isolate) containing an AmpC betalactamase, which contains the plasmid coding for the ampC gene. Kp12dp is a Kp12 porin deficient strain. Each strain used to induce pneumonia was studied separately. A total of 162 animals (81 animals for each strain group) were intratracheally inoculated with 1.3E + 06 CFU of Kp12 or 1E + 07 CFU of Kp12dp to reproduce pneumonia. In turn, each group was divided in three subgroups according to the antibiotic used. Moreover, 9 extra control rats were used for each strain. After 3 days of treatment rats were sacrificed and their lungs were analysed. A reduction in bacterial counts measured in Log CFU/g of lung was used as the parameter of efficacy. The nonparametric Kruskal–Wallis and Mann–Whitney statistical tests were used.
Results: MICs obtained for Kp12 and Kp12dp were the following: IMP, 1.5 and 16 μg/ml, MEM, 0.03 and 8 μg/ml, and FEP, 0.25 and 2 μg/ml respectively. Mean Log CFU/g of lung for Kp12 were: 8.0 for controls, 1.8 IMP, 1.7 MEM and 2.0 FEP. The corresponding results for Kp12dp were: 9.6 for controls, 2.9 IMP, 2.4 MEM and 2.7 FEP. All therapeutic groups showed significant statistical differences in comparison with controls ($p < 0.01$). In terms of efficacy, comparison within the three antibiotics tested showed MEM slightly better bacterial counts than IMP, but only with Kp12dp strain ($p < 0.05$).
Conclusion: IMP, MEM and FEP showed in vivo efficacy in decreasing bacterial count lungs in Kp AmpC producing strains regardless of the presence of porins. However with Kp12dp, MEM seems to be more active than IMP.

P1750

Effect of dehydroepiandrosterone in an experimental pneumonia model caused by *Klebsiella pneumoniae*

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Objectives: A relationship has been referred between dehydroepiandrosterone (DHEA) levels and survival in sepsis in human beings, and in a murine model of cecal ligation and puncture DHEA administration results in an increase of survival. The aim of this study was to evaluate the effect of administration of DHEA in a severe experimental model of pneumonia of *Klebsiella pneumoniae*.
Methods: First, a toxicity study was made in 3 groups of uninfected male C57BL/6 mice (16–20 g) using different subcutaneous doses of DHEA (30, 15, and 7.5 mg/kg/24 h, during 48 h), with mortality rates of 90%, 33%, and 0%, respectively; then, a dose of 7.5 mg/kg was used in the in the pneumonia model. An experimental pneumonia model in male C57BL/6 mice was developed, using the modified method of Esposito and Pennington (Rodríguez-Hernandez MJ et al. JAC 2000); an intratracheal inoculum size of 8 $\log_{10}$ CFU/ml was used. The animals were grouped in CON (controls, no treatment) and DHEA (7.5 mg/kg/24h) during 48 h. The results are expressed as percentages in qualitative variables and as mean ± SD of log CFU/g of tissues. Statistical analysis: Chi-square and Fisher test, ANOVA, and the t-student test were performed; a $P < 0.05$ was considered significant.
Results: The mortality rates (92% and 100%), the bacterial concentration in lungs (10.6 ± 0.9 and 10.1 ± 0.8), liver (6.8 ± 1.7 and 6.3 ± 1.2), spleen (6.8 ± 1.5 and 5.9 ± 0.8), and the sterile

blood cultures (8% and 17%) were not different between CON and DHEA groups, respectively.
Conclusion: In the model of experimental pneumonia caused by *K. pneumoniae* the administration of DHEA did not protect from mortality nor reduce the bacterial concentration in tissues.

P1751

Efficacy of rifampin, imipenem, and sulbactam in monotherapy and combination, in the experimental pneumonia caused by *panresistant Acinetobacter baumannii*

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Objectives: There is not efficacious treatment for infections caused by panresistant *A. baumannii*. The aim of this study was to evaluate the efficacy of rifampin (RF), imipenem (IMP), and sulbactam (SB), in monotherapy and in combination, in an experimental murine pneumonia model caused by panresistant *A. baumannii.*
Methods: In vitro studies: MIC and MBC were determined in two different panresistant clinical strains. The bactericidal activity and the synergy (time-kill curves) of the combinations were evaluated in these strains. Cmax in animal serum of each antimicrobial was also determined. In vivo studies: Both in vitro analysed strains were used. An experimental pneumonia model in C57BL/6 mice with an intratracheal inoculum of approximately 8 $\log_{10}$ CFU/ml was used. The animals were grouped in CON (controls, no treatment), IMP (120 mg/kg/d), SB (240 mg/kg/d), RF (100 mg/kg/d), IMP+SB, IMP+RF, and SB + RF, receiving treatment during 72 h. The mortality rate, the bacterial clearance from lungs, and the sterilization of blood cultures were analysed. Statistical analysis: Chi-square and Fisher test, ANOVA, and post-hoc Tukey and Dunnett tests.
Results: MIC/MBC (mg/l): strain A: IMP 128/>256, SB >256/>256, RF 128/>128, and colistin (COL) 32/>32; strain B: IMP 256/>256, SB >256/>256, RF 128/>128, and COL 32/>32. Cmax (mg/l) were: 16.9, 81.5, and 13.39 for IMP, SB, and RF, respectively. RF and SB were bactericidal against two and one strains, respectively. According to time-kill curves, synergy was found using the following combinations: SB+RF and IMP+RF against the two strains. RF, IMP+RF, and SB+RF decreased the mortality respect to CON ($p < 0.05$) in both strains; in the strain A, RF and IMP(RF were better than IMP+SB ($p < 0.05$). In regard with the bacterial clearance from lungs, RF and the all combinations cleared the lungs respect to CON ($p < 0.05$) in both strains. Respect to sterilization of blood cultures, RF and the three combinations improved the results of CON ($p < 0.05$).
Conclusions: In vitro and in vivo, RF alone or associated to SB or IMP were effective against panresistant *A. baumannii*. The association of imipenem plus sulbactam was also effective.

P1752

Effects of flavonoids quercetin and luteolin on acute *Chlamydia pneumoniae* infection in a mouse model

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Objectives: Dietary flavonoids, found in almost all foods of plant origin, are receiving increasing attention as potential protectors against a variety of human diseases. These compounds are shown to prevent lipid peroxidation and inhibit LPS-induced production of cytokines and NO both in vitro and in animal models. Antimicrobial properties of the flavonoids are well known and we have found earlier that some of the compounds are strongly chlamydiacidal in cell cultures. Our aim was to find out if two common flavonoids, quercetin and luteolin, would have an effect on acute *C. pneumoniae* (Cpn) infection in vivo in mice.
Methods: C57BL/6J mice were given quercetin and luteolin intraperitoneally once daily for three days prior to inoculation. The treatment was continued for ten days post infection (p.i). Three groups of 8 mice, inoculated intranasally with Cpn isolate K7, were treated either with 20 mg/kg quercetin, 2 mg/kg luteolin or given diluent (infected controls). Fourth group was inoculated with SPG instead of Cpn (uninfected controls). Samples were taken on days 3, 6, 10, 13 and 20 p.i. Lung tissue was analysed for the presence of viable Cpn by culture and Cpn DNA by quantitative PCR. Lung inflammation was analysed from haematoxylin eosin-stained paraffin sections. Expression levels of cyclooxygenases (COX-1, COX-2) and nitric oxide synthases (eNOS, iNOS) in the lung tissue were determined by quantitative RT-PCR from different set of samples collected at 4 days p.i.
Results: Quercetin significantly increased the presence of infectious chlamydia in the lung tissue on day 6 p.i. compared to infected control mice (468 000 IFU vs. 57 000 IFU, respectively). The number of infectious chlamydia in the luteolin-treated group was lower than in the control group at every time point. Significant reduction in the lung mononuclear cell infiltrates as a response to infection was seen in luteolin-treated mice as well. Infection had no statistically significant effect on NOS and COX expression levels but luteolin suppressed both eNOS and COX-1 levels and quercetin increased iNOS levels when compared to infected controls.
Conclusions: Luteolin treatment was found to have significant attenuating effects both on the presence of chlamydia and on the inflammatory response in vivo. Further studies are needed to clarify the surprising worsening effect of quercetin, which might be due to the higher treatment dose compared to luteolin or inappropriate administration route.

P1753

Effect of the delay of antibiotic treatment on the therapeutic outcome of an experimental acute otitis media (AOM) caused by an erythromycin-susceptible *Streptococcus pneumoniae*

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Objective: To evaluate the effect of delayed administration of erythromycin in the course of an experimental acute otitis media (AOM) in gerbils caused by an erythromycin- susceptible *Streptococcus pneumoniae.*
Methods: The organism was inoculated by transbullar challenge in the middle ear (ME) and antibiotic treatment (2.5, 5 and 50 mg/kg) was administered at various times thereafter. Treated and control animals were studied longitudinally for the presence of otorrhea, changes in weight, otoscopical aspects, and composition of ME samples. Bacterial counts in ME washing fluids were also determined.
Results: The bacteriological efficacy of erythromycin administered in a single dose at different intervals after bacterial inoculation was as follows: A further experiment was carried out administering the antibiotic at standard and high doses in one and three shots administered in early and delayed regimen obtaining the following results: Table 2 Erythromycin was able to significantly diminish the number of organisms in the ME if the drug is administered early, even in a single dose. However the delayed administration of the antibiotic was associated with bacteriological failure even when the drug was given at a higher and repetitive dose.

Table 1.

Dose (mg/kg)	Time (h) from challenge to treatment	% of culture-positive ME samples	Mean bacterial count (log_{10}CFU/20 micro L)
Control	-	100	3.18 ± 0.55
2.5	2	50	0.97 ± 0.67
	5	60	2.55 ± 1.96
	18	100	3.25 ± 0.66
	21	100	3.27 ± 0.94
5	2	37.5	0.97 ± 0.59
	5	56.2	1.52 ± 0.98
	18	100	2.78 ± 0.79
	21	100	3.04 ± 0.67

Table 2.

Dose (mg/kg)	Time (h) from challenge to treatment	% of culture-positive ME samples	Mean bacterial count (log_{10}CFU/20microL)
Control	-	100	3.44 ± 0.44
5	2	37.5	0.97 ± 0.59
	2,5,8	0	<0.6
	21	100	3.04 ± 0.67
	21,24,27	93.7	2. 67 ± 0.79
50	21,24,27	75	1. 84 ± 0.84

Conclusion: The delayed erythromycin treatment in an experimental AOM caused by an erythromycin-susceptible *S.pneumoniae* strain is associated with bacteriological failure as also has been described with amoxicillin.

P1754

Clinical analysis of anti neutrophil cytoplasmic antibody against bactericidal/permeability increasing protein (BPI-ANCA) in chronic *Pseudomonas aeruginosa* lung infections

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Objectives: It is well known that persistently colonization of Mucoid *Pseudomonas aeruginosa* on lung in patients with diffuse panbronchiolitis (DPB), bronchiectasis (BE) and Cystic fibrosis (CF) induce immunological modification to the host side. Our previous basic studies have indicated that the phagocytic activity of neutrophil was significantly inhibited by anti neutrophil cytoplasmic antibody against bactericidal/permeability increasing protein (BPI-ANCA) in a dose dependent manner in vitro ($P < 0.01$), and in clinical studies, high titres of serum BPI-ANCA played an important role in the clinical course of disease such in patients with DPB, BE and CF. However, the influence of BPI-ANCA on the clinical picture and prognosis of in patients of these disease has not been clarified in detail. In this time, we are going to make discussion with the clinical role of such auto antibody in chronic *P. aeruginosa* lung infections.
Materials and Methods: Sixteen patients of DPB and 34 patients with BE are sampled. Relationship between serum BPI-ANCA titres and clinical symptoms, respiratory function, chest X-ray findings, and bacteria detected were evaluated. Serum BPI-ANCA was tested with ELISA testing kit (BPI IgG kit, GENESIS diagnosis, Cambridge, UK).
Results: Serum BPI-ANCA titre was 1) correlated with the severity of clinical symptoms, in patients with chronic *P. aeruginosa* lung infections ($P < 0.01$), 2) decreased with the improvement of the clinical picture ($P < 0.05$), 3) significantly higher in patients with in patients with far-advanced lesions on chest X-rays than with milder lesions ($P < 0.05$) and in patients with reduced pulmonary function ($P < 0.05$), 4) significantly higher in the patients with poor prognosis ($P < 0.05$).
Conclusion: The above results suggests that BPI-ANCA, an autoimmune factor, appears during the course of chronic *P. aeruginosa* lung infections, and that this autoimmune factor may make chronic lung infections more intractable, by inhibiting the phagocytic activity of neutrophil for *P. aeruginosa*.

P1755

Prevalence of the internalisation-associated PRTFf1gene in a bacterial population of *Streptococcus pyogenes* isolated from patients with acute pharyngotonsillitis in Greece before and after antibiotic therapy

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Objectives: The finding that some isolates of *Streptococcus pyogenes* can enter respiratory epithelial cells by means of fibronectin –binding proteins, becoming in this way inaccessible to antibiotics unable to reach high intracellular concentrations, such as penicillins, has been proposed as an attractive explanation for treatment failure conferring a selective advantage to these organisms. Purpose of this study was to determine the prevalence of the internalization-associated prtf1 gene, that codes the fibronectin–binding protein, in 100 isolates of *Streptococcus pyogenes* obtained from Greek patients with acute pharyngotonsillitis and to investigate the correlation between the presence of this gene with the β-lactam therapy failure.
Methods: A hundred of *Streptococcus pyogenes* isolates were collected during 2001–2004 in Central Greece from pharyngeal swabs of patients presenting with acute pharyngotonsillitis. β-lactam therapy (penicillin, ampicillin, amoxicillin and clavulanic acid, cefaclor, cefixime, cefuroxime axetil, and ceftibuten) was administered to all patients and cultures were obtained before and after therapy. Thirty patients remained carriers of *S. pyogenes* after therapy. In order to avoid a new re-colonization, isolates (before and after therapy) from each of these patients were compared by PFGE,. The presence of prtf1 was detected by PCR.
Results: All isolates recovered from patients, that remained positive after therapy, carried the prtf1-gene(100%), while, only ten of the 70 patients, that were successfully treated by β-lactam antibiotics, were prtf1-positive (14%).
Conclusions: The isolation of prtf1-positive *S. pyogenes* was significantly higher in patients that remained carriers after therapy than in patients successfully treated (p: 0.0001). We believe it reasonable to hypothesize that the capacity of some *S. pyogenes* isolates to resist to β-lactam antibiotic therapy is related in part to prtf1-mediated enhanced colonization. Additional studies are under to test this idea.

P1756

Effect of three different treatment methods on probing pocket depth in the management of chronic periodontitis

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Objectives: The objective of the present study was to evaluate the effects of three modalities of non surgical periodontal therapy on probing pocket depth (PD)in a group of patients with chronic periodontitis.
Methods: The study sample was selected from the patients attending the Periodontal Screening clinic at the Faculty of Dental Sciences. Each subject was assigned to a severity category (S1 ≥ 3mm -≤ 5mm, S2 ≥ 5mm - ≤ 7mm, or S3 ≥ 7mm) depending on the maximum PD in any site at baseline. The subjects in each severity category were then randomly assigned into three treatment groups; T1 = oral health instruction (OHI)+ scaling and root planing (SRP), T2 = OHI + SRP+0.2% Chlorhexidine mouthwash (CHX) for two weeks, or T3 = OHI + SRP+ antibi-

otic therapy +Amoxycilin+Metranidazole combination for one week). PD was recorded for all teeth at six sites at baseline and 6 weeks after treatment. Florida Probe-FP32 was used for recording of data. The differences in probing pocket depth before and after treatment was assessed. One way ANOVA was used to compare the differences in PD between the three treatment categories in each severity group. If ANOVA revealed a significant difference($p < 0.05$), Scheffe's test was performed to ascertain where the differences occurred.

Results: In all severity categories, ANOVA revealed significant differences in the mean PD in the three treatment groups. The mean reduction in PD in the S1 category after T1, T2 and T3 treatments were 1.47 mm, 1.45 mm and 1.69 mm respectively. In S2 category they were 2.50 mm, 3.10 mm and 3.21 mm whereas in S3 category the figures were 3.92mm, 4.32 mm and 7.02 mm respectively. In the severity category-S1 there was a significant difference between T2 and T3 treatment groups ($p < 0.05$). However the actual differences in reduction of PD was marginal (0.22 mm). In severity category-S2 there was a significant differences between treatment groups T1 and T2 and also between T1 and T3 ($p < 0.005$ and $p < 0.001$ respectively).

Conclusion: The use of systemic antibiotics as an adjunct to oral health instruction, scaling and root planing has proven to be significantly beneficial over treatments carried out using oral health instruction, scaling and root planing with or without Chlorhexidine in the treatment of periodontal disease sites with probing pocket depths exceeding 7 mm.

P1757

Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity

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Chronic periodontitis (CP) is characterised by the imbalance of indigenous microflora and resulting in the overgrowth of putative periodontal pathogens. Although lactobacilli play an important role in the maintenance of health by stimulating natural immunity and contributing to the balance of microflora, their role in CP is less clear.

Objectives: To identify *Lactobacillus* species isolated from oral cavity in periodontally healthy subjects and in patients with CP, and to characterize their antimicrobial activity against oral pathogens.

Methods: The saliva and two subgingival sites of 12 CP and 11 healthy subjects were cultured for lactobacilli. Species were identified using rapid amplified ribosomal DNA restriction analysis. Antimicrobial activity against *Streptococcus* mutans (Sm), *Actinobacillus actinomycetemcomitans* (Aa) and *Candida albicans* (Ca) was assessed using deferred antagonism method (115 Lactobacillus strains tested), and against *Porphyromonas gingivalis* (Pg) and Prevotella intermedia (Pi) using a streak line procedure (63 Lactobacillus strains tested).

Results: A total of 115 strains (48 from CP and 67 from healthy patients) of lactobacilli were identified as *L. acidophilus*, *L. crispatus*, *L. delbrueckii*, *L. gasseri*, *L. salivarius*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, *L. fermentum* and *L. oris*. *L. fermentum* (52%) and *L. plantarum* (48%) were the most prevalent. In comparison to healthy subjects, in CP patients obligately homofermentative lactobacilli, particularly *L. gasseri*, were less prevalent (73% vs 17% for homofermentatives, $p = 0.012$, and 64% vs 8% for *L. gasseri*, $p = 0.009$). 69% of tested lactobacilli inhibited Sm, 88% Aa, 82% Pg and 65% Pi but none inhibited Ca. The strongest antimicrobial activity was associated with *L. paracasei*, *L. plantarum*, *L. rhamnosus* and *L. salivarius*. Strains from CP patients expressed stronger antimicrobial activity against Sm than strains from healthy persons (mean growth-free zone 2.3 vs. 1.2 mm; $p < 0.001$).Conclusions: the composition of oral lactoflora between healthy subjects and patients with CP differs by the higher prevalence of homofermentative lactobacilli, particularly *L. gasseri*, in former group. The antimicrobial properties of oral lactobacilli are related to their fermentation type and species. These findings indicate that lactobacilli may play a crucial role in the maintenance of microecological balance in oral cavity.

P1758

Identification of *Streptococcus mutans* bacteriocins in children with and without dental caries

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Streptococcus mutans is the main microorganism implied in dental caries. Different researches suggest that bacteriocins produced by this bacterium may be very important in the ability to exclude or preempt *S.* mutans establishment in the oral cavity.

Objectives: The aim of this study was to identify *S.* mutans strains which produce bacteriocins.

Methods: Thirty three *S.* mutans strains, previously isolated in patients with and without dental caries, were taken for the bacteriocin detection. The 33 strains were biotypified with the Api-ZYM (bioMérieux; Marcy-ltoile, France) enzimatic system. In order to determine the bacteriocins production, the strains were inoculated onto brain heart infusion agar (BHI). After a 24-hour CO_2 incubation, the plates were overlaid with 5 ml of 0.75% BHI agar containing 0.5 ml of an overnight BHI culture of the indicator strain. After an additional CO_2 overnight incubation at 37°C the width of the inhibition zone was measured.

Results: In the 33 strains of *S. mutans* 10 biotypes were found. The most frequent biotypes were the 10, 15 and the 11 with 9, 8 and 4 strains, respectively. Eight (24%) of the 33 evaluated strains produced bacteriocins, 6 of these strains came from patients with dental caries and the other two from patients with no caries. The 8 strains which produced bacteriocins presented 5 different biotypes: 3 strains with biotype 10, 2 strains with biotype 14, and the last three ones were biotypes 11, 15, and 17, respectively.

Conclusion: The obtained results in this study are in agreement with the information reported in other works done with *S.* mutans. In the 33 strains of *S.* mutans evaluated, 8 strains (24%) which produced bacteriocins with 5 different biotypes were identified, which after accomplishing other requirements have a great option to be used in oral infections control in which *S.* mutans is involved.

P1759

Bacterial electroporation using an endox endodontic system derived instrument

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Objectives: Endox® Endodontic System is used for the treatment of bacterial infections in the root canal by application of a high frequency alternating current (HFAC). The generated electromagnetic field (which lasts 140 msec) results bactericidal. The aim of this study was to investigate the mechanism of action of this instrument. It was hypothesised that this apparatus produces pores on the cell membrane leading to bacterial death. In order to demonstrated this assumption some parameters were adjusted, in particular frequency, voltage and time-pulse.

Methods: The instrument was setted as follows: frequency 300 kHz, power 600 kV, time-pulse 60 msec. Electroporation was verified using E. coli K-12 strains C600 carrying the amikacin (AK) resistant pBP517(gyrA+) non conjugative plasmid as donor and C600 rifampicin (RIF) and nalidixic acid (NAL) resistant as recipient. Cells were prepared for electroporation according to standard method. Donor (0.1 ml) and recipient (0.1 ml) strains were mixed in 1.8 ml of saline solution and 0.2 ml of mixture were exposed to the electromagnetic field. Untreated coltures were used as controls. After incubation the recombinants were counted on selective plates (RIF and AK) and then tested for NAL susceptibility.
Results: In a first experiment 60 recombinants were obtained, they also resulted NAL susceptible. Plasmid stability was verified growing bacteria in non selective broth for 48 hours. The culture was then plated in rich medium. The singles colonies obtained were tested for AK, RIF and NAL susceptibility. The results showed that among 60 recombinants, 15 clones maintained the plasmid while the others revealed that original phenotypes of the recipient C600 strain.
Conclusion: The present findings indicated that non conjugative plasmid pBP517 can be transferred from a donor to a recipient via an exposure to the electromagnetic field generated by Endox. The recombinants resulted stable in 15/60 isolates. The mechanism of plasmid transfer is reminiscent with that occurs in electroporation. The great difference between the setting instrument used for clinic treatment and that used in this study is the time-pulse: probably a time-pulse of 140 msec produces pores formation for too long time so the bacterial cells are killed for osmotic-lysis, while a time-pulse of 60 msec produces pores-formation for a time that allows the electroporation process. Further studies are in progress to confirm the present result.

P1760

Oral linezolid as an adequate therapy of experimental endocarditis by methicillin-resistant *Staphylococcus aureus*

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Objective: Linezolid is a new antimicrobial agent with excellent oral bioavailability and well documented in vitro activity against gram positive cocci when resistance to other anti-staphylococcal agents is present. Little is known on its potential use in endocarditis. The efficacy of oral linezolid on experimental endocarditis by MRSA was studied.
Methods: Left sided endocarditis was induced by the insertion of a catheter in the aortic valves of 19 white male rabbits. Twenty-four hours after catheter insertion, animals were challenged by a $7\log_{10}$ inoculum of an MRSA clinical isolate, with MIC of linezolid equal to 4 μg/ml. Eleven animals were administered linezolid at a dose of 75mg/kg q8h per os for five days. Eight served as controls. Blood samples were drawn 16, 32, 48, 72, 96 and 120h post therapy, immediately before drug administration, for the determination of trough levels. At the end of treatment animals were sacrificed and valves were quantitavely cultured. Concentration of linezolid was determined by an HPLC method with UV detection.
Results: Mean ± survival was 133.27 ± 10.23 hours and 66.00 ± 13.75 hours animals treated with linezolid and controls respectively. Mean $\log_{10}$ (±SE) bacterial counts of valves was 3.9 ± 0.86 CFU/g and 7.93 ± 1.21 CFU/g (p 0.019) for animals treated with linezolid and controls respectively. Mean ± SE trough linezolid concentrations were 5.22 ± 1.96 mg/l at 16 h, 5.52 ± 1.57 mg/l at 32 h, 8.29 ± 1.62 mg/ml at 48 h, 17.94 ± 8.65 mg/l at 72 h, 27.58 ± 8.67 mg/l at 96 h and 8.78 ± 1.36 mg/l at the end of therapy.
Conclusion: Therapy of experimental endocarditis by MRSA with oral linezolid results in increased survival and in lower bacterial counts of valve vegetations. Trough blood concentrations of linezolid are above the MIC of the MRSA isolate even reaching 7 times the MIC at the 4th day of therapy. The results are promising for the therapy of serious staphylococcal infections when resistance to other antistaphylococcal agents is present.

P1761

Assessment of *Staphylococcus aureus* pathogenicity using the nematode *Caenorhabditis elegans*

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Several bacterial pathogens, both Gram-positive and Gram-negative, kill the nematode *Caenorhabditis elegans* when supplied as a food source, and a variety of bacterial virulence factors, known to play a role in mouse-models, have been shown also to play a role in nematode pathogenesis. The Gram-positive bacterium *Staphylococcus aureus* is an opportunistic human pathogen, which is implicated in a wide range of diseases including septicaemia, meningitis and toxic-shock syndrome.
Objectives: With the aim of investigating the virulence of various *S. aureus* genes, we have used *C. elegans* as a model.
Methods: Mutants in *S. aureus* genes involved in the bacterial stress-response were tested in the killing-assay. To follow the fate of the bacteria upon ingestion, we used strains of *S. aureus* that expresses the green fluorescent protein (GFP) which is visible in the transparent nematode. We analysed both the ability of the bacteria to kill the nematode and the effect of the bacteria on nematode propagation, but also how well the bacteria adhere to the intestine.
Results and Conclusions: A difference in virulence was observed when the wild-type bacteria were compared with various mutants, which confirms the importance of the stress genes on virulence. In addition, a difference on nematode propagation was seen when *C. elegans* where feed the wild-type- and mutant-strains.

P1762

Whole genome expression profiling of intracellular *Staphylococcus aureus* in human lung epithelial cells

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S. aureus is classically considered as an extracellular pathogen, although recent reports from several laboratories, including our, suggest that it can invade and persist intracellularly in a variety of non-professional (non-myeloid) phagocytes. The intracellular environment is thought to constitute a protected niche against immunological defenses or pharmacologic treatments. Intracellular survival might also explain the frequent relapses observed in osteomyelitis, foreign body infections, and poorly successful attempts to eradicate nasal carriage. In gene expression profile studies of host-pathogen models, pathogen RNA is often present in low abundance and constitutes a major limiting factor for global gene expression analysis. We successfully developed a procedure to stabilize and amplify bacterial RNA. Using a

custom-designed *S. aureus* microarray, we monitored *S. aureus* gene expression following host cell internalisation. A549 human lung epithelial cells were infected with *S. aureus* strain 6850 and bacterial RNA was extracted at different time points. A panel of control experiments revealed no significant changes in bacterial gene expression following host cell contact, exposure to secreted cellular products in the culture medium, or when internalization was blocked by cytochalasin D. In contrast, we detected significant alterations in bacterial gene expression at 2 and 6 hours post-infection. At 2 hours post-infection, we observed a substantial down-regulation of genes involved in general metabolism. A significant number of regulatory proteins, virulence factors, components of mobile genetic elements, and genes involved in iron metabolism and defence against oxidative stress were upregulated. At six hours post-infection, we observed clear evidence of metabolic restart that was consistent with bacterial survival and adaptation to the intracellular growth. We conclude that *S.aureus* rapidly undergoes changes in its genetic program upon contact with the intracellular environment and that global gene expression analysis improves our understanding of events contributing to intracellular pathogen survival. Finally, this study has revealed strategies that might lead to new therapeutic targets.

P1763

Persistence of *Staphylococcus aureus* capsular polysaccharides during infections in a passively-immunised mouse challenge model

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Objectives: *Staphylococcus aureus* (SA) infection isolates possess capsular polysaccharides (CP) that help the organism evade immunological mechanisms of clearance by the host. Our goal was to evaluate the role of CP in protecting bacteria from being killed during in vitro opsonophagocytosis and the persistence of CP in an in vivo challenge model in the presence of CP-specific antibodies.
Methods: A Type 5 SA strain was cultured to log and stationary phase to yield both partially capsulated and fully capsulated forms, respectively. Bacterial counts were measured at time 0 and 1 hour after incubation in a whole blood killing assay in the presence or absence of CP-specific IgG. To determine the persistence of CP during infection, a mouse survival model (a passively-immunized mouse challenge model) was used. Mice were passively immunized with CP-specific IgG prepared from StaphVAX (a SA-CP conjugate vaccine) immunized subjects, challenged 48 hours later with capsulated Type 5 SA and then bled 24 hours later. Blood samples were evaluated for bacterial counts and for persistence of capsulation by a colony lift/blotting technique.
Results: Upon incubation of partially capsulated form of a Type 5 SA strain for 1 hour in freshly drawn whole blood >90% of the bacteria were killed in the absence or presence of CP-specific antibodies. When fully capsulated bacteria was used <10% of the organism was killed after 1 hour incubation in the absence of CP-specific antibody. Only when CP-specific antibody was added a >90% reduction in bacterial counts was observed. Sequential transfer of the SA challenge through animals 5 times showed consistently that 100% of the colonies retrieved from bacteremic animals were capsulated after each passage. None of the lifted colonies showed a loss of capsulation through the fifth transfer.
Conclusions: Our data show that CP promotes survival of SA in the presence of complement and phagocytes in the blood. CP-specific antibodies are required for clearance of those organisms and that immunotherapy mediated by CP-specific antibody did not constitute a selective pressure towards partially capsulated SA. This indicates that protective immunity is conferred by CP-specific antibody, which is essential for combating infections caused by SA.

P1764

Characterisation of haem-deficient mutants in *Staphylococcus aureus* displaying the small colony variant phenotype using a full-genome DNA-microarray

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Background: *Staphylococcus aureus* small colony variants (SCVs) are found in patients with persistent, antibiotic-resistant, and recurrent infections. The SCVs from clinical infections are commonly auxotrophic for menadione and hemin, which are used for the formation of menaquinone and cytochromes, respectively, and are thus important components of the electron transport chain. The alterations in electron transport in such SCVs result in multiple phenotypic changes, including slow growth, increased MIC's to several antibiotics, and reduced alpha-toxin production.
Method: In order to analyze the SCV phenotype, a *S. aureus* full-genome DNA-microarray was used for transcriptional profiling of heme-deficient mutants in comparison to their parent strains. This microarray is based on the recently published genome sequence of *S. aureus* N315. Copy-DNA (c-DNA) was generated by reverse transcription from total bacterial RNA harvested from cells at different stages of growth and was used in competitive hybridization experiments with fluorescently labeled c-DNAs.
Results: Monitoring the relative expression levels of the heme-deficient mutants mimicking the *S. aureus* SCV phenotype, about 60 out of 2334 annotated genes were found to be up-regulated compared to those of the parent strains. The most prominent genes up-regulated encode for enzymes involved in energy metabolism (e.g. L-lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and triose phosphate isomerase). Furthermore, genes encoding enzymes of the arginine metabolic pathway were found to be up-regulated in the mutants generated by interrupting the electron transport.
Conclusions: Applying a *S. aureus* full-genome DNA-microarray to site-directed mutants displaying the SCV phenotype, it was found that genes involved in glycolytic and related as well as in fermentation pathways were up-regulated. These transcriptional data are in agreement with data obtained by biochemical studies and proteomic approaches.

P1765

An animal model of staphylococcal graft infection without mortality

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Objective: To constitute a valid and reproducible graft infection model without mortality in Wistar rats.
Method: In the first group of the study, 2×1 cm^2 polypropilen grafts were incubated in Brain Heart Broth containing slime positive *S. epidermidis* 2×10^8 cfu /ml for 24 hours at 37°C. Incubated grafts, were then washed with physiologic saline. Under general anaesthesia, these grafts were placed subfascially to the groins of rats. In the second group naive polypropilen

grafts were placed to the groins of rats. Before closing the fascia and skin 1 ml of slime positive *S.epidermidis* (3×10^9 cfu/ml) containing sample were placed inside of the wounds. At the end of day 8, rats were killed; wounds were assessed with macroscopic evaluation and microscopic cultures in both groups.
Results: In the first group of the study, wound infection rate was 100%. No mortality occurred in this group. In one blood culture slime positive *S. epidermidis* was cultivated. In the second group, graft infection rate was only 20%. No mortality was seen in this group.
Conclusion: Incubation of polypropilen grafts in Brain Heart Broth containing slime positive *S. epidermidis* for 24 hours in pre application period enhances occurrence of graft infection without generating mortality. We propose to use this reproducible and valid animal model of staphylococcal graft infection in future studies.

P1766

Fusidic acid impregnation inhibits adherence of *S. epidermidis* to peripheric venous catheters

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Objective: To evaluate the effect of impregnation of fusidic acid on adherence of slime poisitive *S. epidermidis* to peripheral catheters in an in vitro model.
Methods: Segments of 0.5 cm of uncoated catheters were cut. Both internal and external surfaces of these catheters were coated with fusidic acid dispersed in polylactic acid/dichloromethane solution. Fusidic acid release experiments were performed under unstirred conditions at pH 7.4 as a physiological buffer. Fusidic acid-impregnated catheters were added to the release medium. At predecided intervals, samples were withdrawn and the release of fusidic acid in the buffer solution was followed by UV spectrophotometer at 250 nm. The cumulative fusidic acid released into the medium from catheters was measured as a function of time. Fusidic acid impregnated and naive cahteters were incubated in Brain Heart Broth containing 104 cfu/ml slime positive *S. epidermidis*. At intervals of 12, 24, 48, 72 hours catheters were washed and vortexed for two minutes in 1 ml of physiologic saline. 100 microliter of samples of vortexed material and Brain Heart Broth were incubated in blood agar. 24 hours later colony numbers were assessed.
Results: Growths at 12, 24, 48, 72 hours were, 104, 105, 105, 105 colony/ml, and 10, 0, 0, 0 colony/ml, in naive catheters and impregnated catheters, respectively. No bacteria was cultivated from the Brain Heart Broth containing fusidic acid impregnated catheters 24 hours afterward.
Conclusion: Adherence of slime positive *S. epidermidis* to catheter in in vitro conditions was restricted with fusidic acid impregnation.

P1767

Impregnation of polypropilen graft with gold-palladium decreases staphylococcal graft infection in vivo and in vitro

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Objective: To evaluate the local anti staphylococcal activity of gold and gold-palladium in an established in vitro and in vivo model.
Method: In vitro: Naive, gold and gold-palladium coated polypropilen grafts of 2×1 cm^2 were incubated in physiologic saline buffered, 0.5 McFarland slime positive *S. epidermidis*. At intervals of 6, 12, 24, 48, 72 hours grafts were washed with saline and vortexed for two minutes in 2 ml of physiologic saline. 100 μ litres of samples of vortexed material were incubated in blood agar. 24 hours later colony numbers were assessed. In vivo: Naïve polypropylene, gold coated and gold and palladium coated grafts were incubated with slime positive *S. epidermidis*. 24 hours later grafts were inserted subfascially to the groins of Wistar rats under general anesthesia. 8 days after inoculation of grafts, rats were killed and macroscopic and microbiologic examinations were performed.
Results: In vitro: The least bacterial growth was detected in the samples obtained from gold-palladium coated grafts, where as the highest rate of growth was found in samples of naive grafts. In vivo: 8 days after inoculation, in naive polypropylene group, all groin incisions were infected with *staphylococcus*. The graft infection rate was 100%. This finding was shown by microbiologic cultures and macroscopic examination of the wounds. No mortality occurred in this group. In palladium- gold impregnated group wound infection rate was 0%, where as this figure was 30% for gold plated group.
Conclusion: Impregnation of polypropylene graft with gold-palladium reduces the wound infection rate in vivo and in vitro.

P1768

There is no correlation between the minimal inhibitory concentration of teicoplanin against *Staphylococcus epidermidis* and efficacy in the mouse sepsis/peritonitis model

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Objectives: The predictive value of teicoplanin Minimal Inhibitory Concentration (MIC) for clinical efficacy in the treatment of *Staphylococcus epidermidis* infections has been questioned. We aimed to assess the relation between the MIC and experimental in vivo efficacy of teicoplanin against *Staphylococcus epidermidis*.
Methods: We selected 7 clinical strains of *Staphylococcus epidermidis* with MICs of teicoplanin ranging from 0.25 to 32 mg/L. Two ciprofloxacin-resistant mutants (strains 15657 and A1894) were respectively obtained from wild-type strains 7453 and 7318. MICs were determined by agar dilution. Cyclophosphamide pre-treated female 20-gram Swiss mice were inoculated intraperitoneally with 2 10^7 CFU. Antibiotics (teicoplanin or ciprofloxacin, used as a control antibiotic) were administered subcutaneously at increasing concentrations immediately and 4 h after inoculation. Median effective doses (ED50) were calculated from mortality at day 6. Correlation was tested between $\log_2$ transformed MIC and ED50.
Results: MICs and ED50s are reported in the table. There was a significant positive correlation between $\log_2$ MIC and ED50 for ciprofloxacin ($r^2 = 0.79$, $p = 0.005$), but not for teicoplanin ($r^2 = 0.35$, $p = 0.18$).

Strain	Ciprofloxacin MIC (mg/L)	Ciprofloxacin ED50 (mg/kg)	Teicoplanin MIC (mg/L)	Teicoplanin ED50 (mg/kg)
7453	0.125	14.5	0.25	45.6
7318	0.125	2.3	0.5	2.3
7026	1	18	4	23.2
7418	2	>50	32	4.8
7371	4	>50	16	1.9
15657	8	>50	Nd	nd
A1894	8	>50	Nd	nd
5331	32	>50	4	5.3
5056	Nd	nd	4	1.5

Conclusion: Our data suggest a lack of correlation between MIC of teicoplanin and in vivo activity in the mouse sepsis/peritonitis model. Further experimental and clinical studies are needed to determine the impact of elevated MICs of teicoplanin against *Staphylococcus epidermidis*.

Adverse drug reactions, probiotics

P1769

Impact of adverse drug events on resource consumption: amoxicillin-clavulanate vs gemifloxacin

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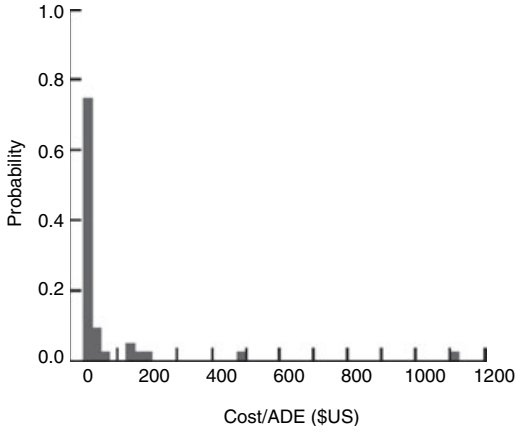
Background: Morbidity and mortality associated with ADEs cost > $136 billion annually in the US (Arch Intern Med 1995; 155:1949). Major contributors to these costs are increased hospital admissions and lengths of stay, medical procedures, and medicinal therapy related to the ADE. Despite these observations, specific cost data associated with a given ADE category are limited.
Methods: Data, including the nature and body system of the ADE, clinical actions, and associated medical procedures and therapy, were obtained from a double-blind Phase 3 clinical trial comparing AC to GEM for the treatment of community-acquired pneumonia. Only those ADEs with probable or suspected associations with study drug and requiring treatment were evaluated. Medical procedure fees and drug costs, based on the 2004 US Medicare database and Red Book, respectively, were used to construct specific ADE cost ($US) distributions, which were then compared across body systems and between study drugs within a body system.
Results: Of the 320 patients who received at least 1 dose of study drug, 246 had 1 or more ADEs (totaling 712 ADEs). Of these, 43 ADEs from 34 patients were associated with study drug and required a medical procedure (AC, 2; GEM, 4) and/or medicinal therapy (AC, 29; GEM, 12) (AC, 29/336; GEM, 14/376; p = 0.006). None required prolonged hospitalization. ADEs commonly involved the gastrointestinal (GI) tract (44%) and skin (19%). Across all ADEs, the median cost/event was $8.80 (range 0.24–1077.09) (Figure). For GI ADEs, the median cost/event for AC was $3.04 (range 0.24–24.30) and for GEM was $3.43 (range 0.88–454.26) (NS). For skin ADEs, the median cost/event for AC was $12.63 (range 2.00–178.74) and for GEM was $4.92 (range 1.51–7.62) (p = 0.1). Assuming a standard course of AC or GEM costs $65/patient, ADEs added 12% ($8.01) to antibiotic treatment costs.



Conclusions: A greater proportion of ADEs associated with drug and requiring treatment were observed for AC than GEM. While no difference existed in treatment costs of GI ADEs between agents, a trend toward lower median treatment cost for skin reactions was apparent for GEM. Analysis of larger databases will allow for better resolution of specific ADE cost distributions. Given that the cost of treating ADEs is an important economic determinant, such data will allow for improved pharmacoeconomic models for health maintenance organizations, governmental agencies, formulary-decision makers, and clinicians.

P1770

Fluoroquinolone-associated anaphylaxis in spontaneous adverse drug reports

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Objectives: Anaphylaxis has been reported associated with the intake of fluoroquinolone (FQ) antibiotics. According to pathophysiology such reactions may be immune-mediated (anaphylactic) or result from direct stimulation of effector cells (anaphylactoid). Both mechanisms produce the same clinical picture of anaphylaxis, however, anaphylactoid reactions may occur after first intake since no sensitization phase is necessary. In Germany, numerous cases of suspected ADRs are reported spontaneously to the Federal Institute for Drugs and Medical Devices and registered in a large ADR data base. The aim of the study was to analyze all cases of FQ-associated anaphylaxis contained in the BfArM database with respect to previous exposition, time to onset and other determinants.
Methods: All FQ-associated cases of anaphylaxis, anaphylactic shock, anaphylactic/anaphylactoid reaction reported to the BfArM between 1993 and 2004 were identified and assessed with regard to correctness of diagnosis and causal relation. Further analyses were performed in defined subgroups.
Results: 172 cases reporting the aforementioned terms were identified. In 152 cases correctness of diagnosis and causal relation was considered at least as possible and further analyses were restricted to this subgroup. Administration of moxifloxacin was reported in 75/152 cases (49%), and this figure did not seem to be matched by a comparable high number of exposed patients. Levo-, cipro-, and ofloxacin accounted for 25 (16%), 21 (14%) and 17 (11%) of the 152 cases, respectively. Intake of other FQs was reported in 14/152 cases (9%). Occurence of the ADR after the first dose or within the first three days was reported in 63/152 cases (41%), but no information on pre-exposure with this or any other FQ was provided with these reports. In 20/152 cases (13%) the reaction occurred within the first three days and it was stated, that the respective FQ has never been taken before, although previous administration of a different FQ was not explicitly excluded. In 1/152 cases (0.7%) it was stated explicitly, that no FQ has ever been taken before.
Conclusions: Anaphylaxis appears to be an ADR of the class of FQs. Time to onset is compatible with an underlying anaphylactoid mechanism in a relevant number of cases. Differences in reported frequencies constitute a signal for the true differences and should be further investigated.

P1771

Amoxicillin and Co-amoxiclav (amoxicillin + clavulanic acid): differences in adverse drug reactions type and seriousness extrapolated from an Italian inter-regional database

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Objectives: Amoxicillin (4.0 DDD/1000 inhabitants/die) and co-amoxiclav (5.3 DDD/1000 inhabitants/die) are antibiotic drugs largely prescribed for the same indications in Italian medical practice (year 2003). The co-amoxiclav is also the fifth drug for public health spending and alone represents the 71% on the total amount of expenses for penicillin class. Aim of the present study was to investigate adverse events caused by them and reported through the Italian spontaneous reporting system.
Methods: The Pharmacovigilance Inter-regional Group (GIF) database contains 31.000 reports of adverse drug reactions (ADRs), related to ~25,000,000 inhabitants (43% of Italian population), collected from 1988 to now. From this database, amoxicillin and co-amoxiclav reports were extrapolated. Particularly, ADRs seriousness was estimated, comparing adverse reactions reported for both antibiotics.
Results: The number of reports relating to amoxicillin and co-amoxiclav is almost the same (986 vs 923, respectively). In the ADRs reported, the mean age of patients is similar (amoxicillin: 42.3 ± 22.9; co-amociclav: 42.8 ± 22.8). Co-amoxiclav caused haematologic, hepatic and gastroenteric ADRs more often than amoxicillin (2.3% vs 0.9%, 3.6% vs 0.6%, 13.7% vs 7.8%, respectively). Serious haematologic and hepatic ADRs were significantly higher following co-amoxclav than following amoxicillin (2.2% vs 0.9%, 2.5% vs 0.4%, respectively). Examples of difference between co-amoxiclav and amoxicillin are the following: hepatitis (15:1), cholestatic hepatitis (7:2), jaundice (4:10), purpura (13:5) and Stevens-Johnson Syndrome (10:2).
Conclusion: These data indicate a different safety profile between amoxicillin and co-amoxi-clav. The latter was responsible for more serious adverse reactions. Thus, in therapeutic choice, this difference should be borne in mind.

P1772

The genotoxic effect of ribavirin in the patients with Crimean-Congo haemorrhagic fever

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Objectives: Ribavirin (RBV) is a synthetic purine nucleoside analogue with a broad spectrum. RBV is used to therapy of viral infections because it prevents replication of a large variety of viruses. It has a lot of adverse effect such as haematologyc toxicity, mitochondrial toxicity, liver toxicity and teratogenicity. Since it has not been studied enough, the genotoxicity of RBV has not been known exactly. We aimed to seek the genotoxicity of RBV.
Methods: Three patients with Crimean-Congo hemorrhagic fever diagnosed clinically, laboratory and microbiologically were included in this study. The diagnosis was confirmed by ELISA. RBV was used for therapy at high dose (after 2000 mg initially dose, 1000 mg per 6 hours at first 4 days, and 500 mg per 6 hours 6 days). In these patient, we studied the mutagenic effects of RBV with 2 methods; the MN assay and the SCE method. In the 9th day of the therapy and after one month following the therapy, 2 ml perypheral blood was taken from these patients and two different blood culture for each patient carried out for 3 days. The first culture used to SCE studies and the second culture used to micronuclei studies. For SCE studies; 1ml perypheral blood and 5-BromodeoxyUridine were added to 15 ml falcon tupe including 5 ml culture medium. After 72 hours, the cultures were stopped and harvested. And then SCE method was applied. For each patient, 20 metaphase sample was examined and SCE rate was detected. For MN study, 1 ml perypheral blood was added to 15 ml falcon tupe including 5 ml culture medium. After 44 hours, cytochalasine-B was added to the cultures and then the cultures was harvested at 72nd hour. For each patient, 1000 binucleate cells were examined to establish the frequency of micronuclei.Results: The results of this study is shown as table.
Conclusion: We used two different methods to indicate the in vivo genotoxicity of RBV. In all patients, the SCE rates at 9th day of therapy were higher than the rates after one month following therapy. In the same way, the MN amounts increased at 9th day of therapy but they were regress to normal levels after one month. The results of this two studies were appeared the in vivo toxicity of RBV. Although the number of patient was inadequate, the presence of in vivo genotoxicity was supported by concordance between the results of MN method and of SCE method.

P1773

Safety of probiotics – a surveillance study of lactobacilli induced bacteraemia

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Objectives: To determine the incidence of bacteraemia caused by lactobacilli in Stockholm, Sweden, between January 1998 and March 2004, and to identify the possible presence of the probiotic strains most commonly used in Sweden.
Methods: Strains of lactobacilli isolated at the two microbiological laboratories of Karolinska University Hospital in Huddinge and Solna, Stockholm, Sweden have been analysed. All isolates were checked initially for growth on Rogosa- and MRS-agar with and without vancomycin (20 mg/l). The plates were incubated for 72 hours at 37°C in anaerobic jars. Isolates growing on Rogosa- and MRS-agar were further characterized biochemically by API 50 CHL test kit. Strains of lactobacilli belonging to the groups *Lactobacillus paracasei*, *Lactobacillus acidophilus* or *Lactobacillus rhamnosus* according to the API test were further analysed with molecular methodologies (randomly amplified polymorphic DNA, RAPD-PCR or species-specific PCR followed by pulsed-field gel electrophoresis, PFGE) to ascertain eventual similarities with the probiotic strains *L. paracasei* subsp. paracasei F19, *L. acidophilus* NCFB 1748 and *L. rhamnosus* GG (ATCC 53103). To secure an unbiased identification of the strains, positive and negative controls were randomly included in the sample series.
Results: *Lactobacilli* were isolated in blood samples from 74 patients during the period January 1998 to March 2004. The incidence remained at the same level during the period and patients with lactobacilli induced bacteraemia constituted <1% of the total numbers of patients with bacteraemia. Isolates from 59 of the patients were available for identification. The majority of strains belonged to *L. paracasei* ssp. *paracasei* (n = 14), *L. rhamnosus* (n = 12), *L. plantarum* (n = 8) and *L. acidophilus* (n = 5). *L. paracasei subsp. paracasei* F19 and *L. acidophilus* NCFB 1748 were not identified in any of the samples. The identification of possible *L. rhamnosus* GG strains is under investigation.
Conclusion: The introduction of probiotic strains in food is Sweden has not led to an increased incidence of lactobacilli induced bacteraemia in Stockholm.

P1774

Effects of oral *Biffidobacterium lactis* on the faecal flora and the growth of preterm infants

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Objectives: The aim of this study was to evaluate the effect of *Biffidobacterium lactis* (BL) supplementation on the intestinal microflora balance of preterm infants and its contribution to maintaining health.
Methods: In a prospective randomized study 35 healthy infants of less than 2080 g birth weight, with gestational age 28–35 weeks were included. Eighteen infants received a preterm milk formula to which BL was added (group A), whereas the other 17 infants were fed an unsupplemented formula (controls, group B). *Feacal flora* was examined three times a) before treatment, b) on day 7 and c) on day 21 after the onset of enteral feeding, for quantitative aerobic and anaerobic cultures. BL tolerance and growth rates were also evaluated.
Results: *Enterobacteria* and *enterococci* were the first genera to appear on stools after birth. Both persisted as the predominant organisms in the feacal flora of both groups. An increase in species number was noted in infants of group A by day 7 and became significant by day 21, being mainly the result of increased Gram (+) and anaerobic species. More specificly, by the third week, infants in this group attained significantly higher mean log colony forming units (CFU) for bifidobacteria and lactobacilli compared to group B (9.7 ± 0.402 vs 8.9 ± 1.152 ($P < 0.05$) and 9.6 ± 0.413 vs 8.5 ± 0.901 ($P < 0.05$) respectively). In addition, the number of staphylococci in stools was significantly lower in BL group compared to controls (42 ± 1.658 vs 8.1 ± 1.493) ($P < 0.05$). No yeasts were found in the BL group, whereas in group B *candida* spp. were recovered at a rate of 11.7% of infants studied. There was no remarkable difference in weekly weight, length and arm circumference gain. Head circumference was significantly higher in infants treated with BL formula than controls by day 21(mean ± SD, 33 ± 1.6 vs 32.5 ± 1.3 $P < 0.05$). Moreover, BL was well tolerated in all infants included.
Conclusions: A BL supplemented milk formula appeared to affect neonatal intestinal colonization patterns, increasing anaerobic flora and decreasing staphylococci and yeasts. It was also well tolerated in preterm infants.

Antibiotic-resistant community-acquired pathogens – II

P1775

Antimicrobial susceptibility of bacterial enteric pathogens isolated in humans from 2002 to 2004 in the Province of South-Kivu, Democratic Republic of Congo

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Objectives: Diarrheal diseases represent a major health problem in Africa where they account for 15% of all deaths in children. However, little data's are available on the antibiotic resistance of bacterial enteric pathogens in African countries. To develop guidelines for the management of gastroenteritis in the province of South-Kivu, Democratic Republic of Congo, a provincial survey was conducted to determine the antimicrobial susceptibility patterns of enteric pathogens isolated between 2002 and 2004.
Methods: During the study period, all bacterial enteric pathogens isolated from blood or stool specimens received at the Provincial Public Health Reference laboratory were identified using conventional laboratory methods. Antimicrobial susceptibility tests were performed by disk diffusion method following the recommendations of the NCCLS. The antimicrobial drugs tested were ampicillin (AM), ceftriaxone (CTX), chloramphenicol (CL), ciprofloxacin (CI), co-trimoxazole (SXT), tetracycline (TC), erythromycin (EM) and amikacin (AK).
Results: Among the 182 bacterial enteric pathogens studied, *Salmonella* spp. (53.3%) were the most commonly identified, followed by *Vibrio cholerae* (20.9%), *Campylobacter* spp. (20.3%), and *Shigella* spp. (5.5%). Eighty-nine per cent of salmonellae isolates were multidrug resistant with the following proportion of resistant strains to: AMP (92.5%), CL (94.0%), SXT (93.8%), and TC (60.0%). Resistance to CI was uncommon in *Salmonella* spp. (1.2%) but detected in 16.2% of *Campylobacter*. All *Campylobacter* strains were susceptible to EM but 21.6% and 5% of them were resistant to AM and TC respectively. Most of *Vibrio cholerae* isolates were resistant to CL and SXT but sensitive to AM, CI, TC and AK. Resistance to CI, AK and CTX was not detected in *Shigella* isolates but all of them were resistant to SXT.
Conclusions: Due to the high prevalence of multidrug resistance, third generation cephalosporins or fluoroquinolones are now the drug of choice to treat severe *Salmonella* infection in the province of South-Kivu. Quinolones should be used with caution as empirical therapy for dysentery because of the increasing incidence of ciprofloxacin resistance in *Campylobacter*. Erythromycin remains the agent of choice for campylobacter infection.

P1776

Surveillance of antibacterial resistance in major pathogens of community-acquired respiratory tract infections in Moscow, Russia, 2004

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Objectives: *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* a are key pathogens of community-acquired respiratory tract infections. The objective of the study was to determine the prevalence of resistance against commonly used antibiotics.
Methods: From January to November 2004 clinically significant isolates of respiratory pathogens from 8 hospital laboratories were sent to a central laboratory for re-identification and susceptibility testing using the microdilution method according to NCCLS recommendations. Antibiotics tested were: penicillin G (Pen), amoxicillin (Amx), amoxicillin/clavulanic acid (Amc), ampicillin (Amp), cefotaxime (Ctx), cefuroxime (Cef), erythromycin (Ery), clindamycin (Cli), levofloxacin (Lev), ciprofloxacin, trimethoprim/sulfamethoxazole (Stx), tetracycline (Tet). The ermA, ermB and mefA genes were amplified using specific primers. Production of beta-lactamase was detected by nitrocefin.

Results: Susceptibility rates of *S. pneumoniae* (n = 225) were as follows: Pen 89.5%, Amx 100%, Ctx 100%, Ery 94.8%, Cli 95.6%, Lev 100%, Tet 80.3%, and Stx 65.5%. Among 6 high-level Pen-resistant strains all were Ery and Tet-resistant, and 4 were Stx-resistant. In 9 of 11 Ery-resistant strains ermB and mefA genes were detected simultaneously, in one strain only mefA was detected and in one – only ermB. 23F and 19F serotypes were prevalent among resistant strains. *H. influenzae* isolates (n = 155) showed the following susceptibility rates: Amp 95.6%, Amk 100%, Ctx 100%, Cip 100%, Tet 99.4% and Stx 69.8%. Beta-lactamase production was detected in 2 of 5 Amp-resistant isolates. One isolate demonstrated Cip MIC = 1.0 mg/l. Susceptibility rates of *S. pyogenes* (n = 89) were as follows: Pen 100% Ery 93.3%, Cli 98.9%, Tet 44.9% and Lev 100%. One isolate demonstrated Lev MIC = 2.0 mg/l.
Conclusions: With the exception of Tet and Stx the prevalence of resistance among major respiratory pathogens in Moscow remains relatively low in comparison with other parts of the world.

P1777

Community-acquired pneumococcal pneumonia in Germany: first results of the CAPNETZ study

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Introduction: *Streptococcus pneumoniae* is considered to be one of the major causative agents of community acquired pneumonia (CAP) infections. CAPNETZ was conducted in 2003 to collect and analyze all possible clinical and microbiological data of patients with CAP. These are the first data on the pneumococcal pneumonia patients showing the resistance and clonal relatedness of the pneumococcal isolates using MLST.
Methods: Isolates of *S. pneumoniae* were sent to the NRCS in Aachen-Germany for confirmation of species diagnosis by optochin testing, bile solubility and for serotyping by the Neufeld Quellung reaction. MICs of penicillin G (PEN), cefotaxime (CEF), erythromycin A (ERY), clindamycin (CLI), tetracycline (TET), telithromycin (TELI), and ciprofloxacin (CIP) were determined using the microdilution method according to the latest NCCLS guidelines. All included strains of *S. pneumoniae* were analysed for clonal relatedness using the multilocus sequence typing methodology. Isolates resistant to macrolides, tetracycline and fluoroquinolones were checked for mechanisms of resistance and resistance phenotypes.
Results: Between June 2003 and August 2004, 75 isolates were included in the study. Resistance to antibiotics was as follows: PEN (6.7%), CEF (0%), ERY (33.3%), CLI (9.3%), TET (10.7%), TELI (0%), and CIP (0%). The predominant serotypes were: 14 (20%), 3 (13.3%), 19F (9.3%) and 11A (6.7%). 19 macrolide resistant isolates were M-phenotypes and possessed the efflux mechanism of resistance (mefE) and 6 were cMLSB with ermB. Among macrolide resistant isolates, MLST 9 (England macrolide R clone) was the most prevalent (60%) and was directly followed by the Taiwan resistant clone MLST 242 (4%). The British sensitive serotype 3 clone (ST 180) was the predominant MLST-clone among sensitive pneumococcal isolates (10.7%).
Conclusions: Macrolide resistance is increasing drastically in Germany and the most prevalent clone is the England clone ST 9.

P1778

A comparison of trends in antibiotic resistance in *Staphylococcus aureus* and *Streptococcus pneumoniae* in the UK

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Objectives: To compare the trends in methicillin resistance in *S. aureus* and reduced susceptibility to penicillin and erythromycin in *S. pneumoniae* in the UK between 1999 and 2004, using the UK EARSS (European Antimicrobial Resistance Surveillance System) dataset.
Methods: The UK has participated in EARSS since it began in 1999. Under the scheme, a network of sentinel laboratories across the country sends information on all *S. aureus* bloodstream infections and *S. pneumoniae* isolates from blood or cerebrospinal fluid to the Health Protection Agency Centre for Infections. Data collected include the age and sex of the patient, hospital department and antimicrobial susceptibilities of the isolate. Laboratories may also include clinical information.
Results: The percentage of *S. aureus* isolates reported as resistant to methicillin increased from 33.2% in 1999 to 44.9% in the first quarter of 2004, an average increase of 1.96% per annum (p = 0.047, 95% CI 0.05–3.88%). The UK has one of the highest proportions of methicillin resistant *S. aureus* (MRSA) in Europe, ranking among the top five countries participating in EARSS between 1999 and 2004. By contrast, the percentage of *S. pneumoniae* isolates reported as non-susceptible (either resistant or intermediate) to penicillin decreased from 7.1% in 1999 to 3.9% in the first quarter of 2004, a significant average annual decrease of 0.61% (p = 0.012, 95% CI −(1.00% to −0.22%). For each of the years 1999–2004, the UK percentage of penicillin non-susceptible *S. pneumoniae* (PNSP) was ranked in the lower half among countries participating in EARSS. There was no trend in the percentage of *S. pneumoniae* isolates reported as resistant to erythromycin (p = 0.53). In 1999, 14.8% of isolates were reported as resistant to erythromycin with little fluctuation in the following years (15.2% resistant in the first quarter of 2004).
Conclusion: In the UK very different trends have been seen in two important antibiotic-resistant pathogens, MRSA and PNSP, since 1999. While the percentage of *S. aureus* isolates resistant to methicillin has increased, the percentage of PNSP has decreased, with the proportion of erythromycin-resistant pneumococci remaining stable. These trends may be related to differential antibiotic prescribing between hospital and community environments. The differences between these contrasting trends merit further epidemiological investigation and analysis.

P1779

Evaluation of tet genes distribution in bacteria isolated from milk samples of ovine herds

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Objectives: The use of tetracycline, a broad-spectrum antimicrobial for animals and human therapy, can induce antimicrobial resistance (AR) in bacteria. AR genes, carried by mobile elements, e.g. plasmids, transposons or integrons (Robert et al., 2003), can be transmitted among different species and genera of bacteria. Our work is an evaluation of the distribution of tetracycline resistance genes in strains of *Staphylococcus aureus*, Coagulase Negative Staphylococci (CNS), *Streptococcus uberis* and *Escherichia coli* found in milk samples of sheep belonging to extensive breeding where Somatic Cell Count was >1,000,000.

Methods: 115 *S. aureus*, 146 CNS, 58 *S. uberis* and 10 *E. coli*, identified by biochemical miniaturized system (API System) and also coagulase test for *staphylococci*, have been screened for tetracycline resistance by Kirby-Bauer method, according to NCCLS Documents. Since several genes for tetracycline resistance had already been detected (Chopra et al., 2001), resistant strains were checked by PCR to evaluate the presence of the following resistance genes: tetM, tetK, tetO and tetL in Gram positive bacteria and tetA, tetB, tetC e tetE in *E. coli*, by using primers proposed by Ng et al. (2001)

Results: 5 strains of *S. aureus* out of 115 (4.3%), 18 strains of CNS out of 146 (12.3%), 4 strains of *S. uberis* out of 58 (6.9%), 3 strains of *E. coli* out of 10, were resistant to tetracycline. All the *staphylococcus* strains harboured the tetK gene (100%) while tetL, tetO and tet M determinants were not detected; 3 strains of *S. uberis* were positive for tetM gene and 1 strain harboured both tetK and tetO; no one streptococcus harboured the tetL. In *E. coli* resistance genes detected were tetA (2 strains out of 3) and tetB (1 strain out of 3). tetC and tetE were not detected.

GENUS (N°)	GENE
CNS (18)	*tet*K
S. aureus (5)	*tet*K
S. uberis (3)	*tet*M
S. uberis (1)	*tet*K, *tet*O
E. coli (2)	*tet*A
E. coli (1)	*tet*B

Conclusion: The tetK is the most representative plasmid harbouring gene in our *Staphylococcus* strains. It has been found more in CNS than in *S. aureus*, suggesting that environmental microbes, causing subclinical mastitis, may transfer easily resistance genes more than microbes causing acute diseases. Antibiotics in extensive sheep breeding are utilized in acute disease only, other than in intensive breeding; it may explain the lower diffusion of chromosomal resistance genes, as tetM.

P1780

Establishment of a network of laboratories participating in the European Antimicrobial Resistance Surveillance Scheme in Northern Ireland

J. McCarroll *on behalf of Northern Ireland EARSS participating laboratories*

In 2002, the Department of Health, Social Services & Public Safety for Northern Ireland (DHSSPSNI) published its Antimicrobial Resistance Action Plan (AMRAP). This document outlined a three-year action plan with practical application over a range of pertinent areas, including surveillance. Recommendation 6.6 stated that all Northern Ireland (NI) clinical laboratories should be invited by CDSC (NI) to participate in the European Antimicrobial Resistance Surveillance Scheme (EARSS).

Objectives: To recruit NI laboratories to EARSS, through promotion of the benefits of participation. To agree, in conjunction with key stakeholders, a minimum reporting dataset and establish reporting arrangements. To develop, in conjunction with key stakeholders, appropriate feedback material. To devise a means of data collection, storage and analysis which would facilitate all other objectives.

Methods: Consultant microbiologists were approached in writing, regarding the possible participation of their laboratories in EARSS. They were given a copy of the EARSS newsletter and Annual Report, provided by EARSS management team. Scientists were identified as contacts for data collection issues in each laboratory. The core dataset was agreed with each laboratory, and a Microsoft Access database was developed to allow transfer, storage and analyses of data. Feedback arrangements are currently being developed with individual laboratories, to suit their specific needs.

Results: All laboratories have agreed to join EARSS. To date, six of nine (67%) laboratories are regularly reporting data to CDSC (NI). It is anticipated that the remaining three laboratories will have commenced reporting by January 2005. Most laboratories report an enhanced dataset which permits more detailed analyses to be performed and utilised at ward level. Data is collected at NI level before being included in the United Kingdom database for submission to EARSS.

Conclusions: Contribution to EARSS has led to a greater level of discussion and harmonisation amongst microbiologists, and has had a generally positive impact on Northern Ireland laboratories. Regional management of the data in this way has lead to a greater sense of ownership at local level, and had permitted adaptation of outputs to suit local needs. Collection of an enhanced dataset has allowed health Trusts to provide clinicians with locally relevant, clinically-focused information which can be used to effect change and improve practice.

P1781

Frequency and susceptibility to antimicrobial agents of the *Proteus* genus strains isolated in chronic middle ear disease

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Objectives: To evaluate the frequency of the species of *Proteus* isolated in the chronic middle ear disease and their susceptibility to the antimicrobial agents.

Methods: From June 1997 to February 1998, all the patients addressed to our laboratory for chronic middle ear were questioned and a sample taken by a physician microbiologist. The specimen was already seeded on different specific medium. The strains isolated were identified with the biochemical galleries (API 20E –Biomerieux) and their susceptibility to the antimicrobial agents was evaluated by the disk diffusion method according to the NCCLS.

Results: 130 patients have been addressed to our laboratory during this period and 140 specimens analysed. The sex ratio was: 1.18 (96/58). The infection rates were 84.61% (110/130 patients). 111 specimens out of 140 were positive (79%). In 5 specimens we isolated more then 3 bacterial species so they have been done again. 125 strains were isolated 81 out of 125 strains (64.8%) were represented by the most frequent which were: *Pseudomonas aeruginosa* 46(56.79%) *Proteus*: 22(27.16%) and *Staphylococcus aureus* 13(16%). These strains have been isolated either alone or associated: *Pseudomonas aeruginosa* alone: 74%, associated to another species:35%.*Proteus* alone: 65% associated:35%. *Proteus* was associated to *Pseudomonas aeruginosa* in 5 cases out of 7, once to *Klebsiella pneumonia* and once to Alkali genes. Among the 22 strains of the *Proteus* Genus, 20 belonged to the species *mirabilis*, 1 to *vulgaris* and 1 to *penneri*. The susceptibility test of the *Proteus mirabilis* strains showed that: 20% of the strains were sensitive to all antimicrobial agents tested. 35% were resistant to Ampicillin, 25% to Piperacillin, 20% to Cefalotine and 10% to chloramphenicol. Moreover 100% of *Proteus mirabilis* strains were sensitive to Cefotaxim, Gentamycin, Amikacin and Pefloxacin, 70% to amoxicillin/clavulanic acid, 75% to Cefoxitin and 95% to Cotrimoxazol.

Conclusion: The *Proteus* Genus and especially the species *mirabilis* are frequently isolated in the chronic middle ear disease. It occupies the second place just behind *Pseudomonas aeruginosa*, with which he is often associated. Besides *Proteus*

mirabilis remains relatively susceptible to the majority of the antimicrobial agents tested.

P1782

Determination of ciprofloxacin resistance in non-typhoidal Salmonellae in Scotland: 1993–2004

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Objectives: To determine the levels of resistance to ciprofloxacin among non-typhoidal salmonellae in Scotland.
Methods: E-test strips were used to determine the minimum inhibitory concentrations (MICs) of ciprofloxacin for nalidixic acid resistant salmonellae isolated from human, veterinary and environmental sources between 1993 and 2004.
Results: Of 911 strains of *Salmonella* tested, 24 were resistant to ciprofloxacin at the NCCLS breakpoint of 4 mg/L. A further 14 were resistant at the SSRL breakpoint of 0.5 mg/L, while 784 isolates had reduced susceptibility with MICs of greater than or equal to 0.125 mg/L and less than to 0.5 mg/L.
Conclusions: These results show that very few non-typhoidal strains of *Salmonella* in Scotland are resistant at the current recommended breakpoint of 4 mg/L. The majority of strains examined however, do show reduced susceptibility at 0.125 mg/L. This confirms the necessity for continuing antimicrobial surveillance of resistance to this important antibiotic.

P1783

Combined resistance to penicillin and gentamicin in community-acquired bacteraemia in Copenhagen County, 1991–2000

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Objectives: For several years, a combination of penicillin and gentamicin has been our recommendation for the initial treatment of community-acquired septicemia with an unknown focus, supplemented with metronidazole if an abdominal or genital focus is suspected. We sought to verify if this regime is still valid according to resistance patterns and mortality over a recent period of ten years.
Methods: From results registered in our laboratory database (ADBakt), we analysed 6476 cases of community-acquired bacteraemia (CAB), excluding contaminants, during Jan 1, 1991 to Dec 31, 2000 from the County of Copenhagen (three hospitals, 2400 beds) for resistance patterns and clinical information (initial antibiotic treatment given, follow-up treatment and patient survival).
Results: Resistance to both penicillin and gentamicin were found in 239 isolates, of which 191 were anaerobes sensitive to metronidazol, and 48 isolates from 46 patients were facultative or aerobic bacteria [Enterobacteriaceae (23), enterococci (8), *Staphylococcus aureus* (1), coagulase-negative staphylococci (10), *Pseudomonas* spp (2), *Stenotrophomonas maltophilia* (1), *Streptococcus pneumoniae* (1), *Mycobacterium fortuitum* (1), and *Haemophilus influenzae* (1)]. There was no increase in the occurrence of microorganisms with resistance to both penicillin and gentamicin over the years. Most notably, the incidence of gentamicin-resistant Enterobacteriaceae remained low (0–5/year). Forty-two of the 46 patients survived, 31 receiving irrelevant or no antibiotic treatment until diagnosis. Treatment according to our recommendations was relevant in 99% of cases if strict adherence to the guidelines, also with respect to the addition of metronidazole, is presumed.
Conclusion: The incidence of CAB with aerobic and facultative bacteria resistant to both penicillin and gentamicin remains low in our county. Our recommendations for the initial antibiotic treatment of community-acquired sepsis will remain unchanged.

P1784

Macrolide resistance associated with chronic azithromycin use in cystic fibrosis

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Objectives: To study the effects of chronic azithromycin use on change over time in macrolide susceptibility of *S. aureus* and *Haemophilus* spp in CF patients.
Methods:
Setting: Erasmus MC - Sophia Children's Hospital. Materials: All sputum cultures from CF-patients, obtained at routine visits and at time of pulmonary exacerbations, cultured between January 1999 and March 2004. The study period was divided in octiles of 240 days. *S. aureus* and *Haemophilus* were cultured on selective media. Antibiotic susceptibility was tested routinely. A random set of *S. aureus* and *Haemophilus* strains from non-CF patients, cultured during the same period, was used as controls. Azitromycin use in CF started in January 1999 and increased gradually. In March 2004 one-third of patients was on maintenance therapy. Cultures were classified as from non-azitro (NA), azitro (AZ), or post-azitro (PA) user. Cultures were classified AZ until one month after azitro was stopped.
Results: During the study period 2522 sputum cultures were received from 155 patients (mean 16.3, range 1–111 cultures). In 699 (27.7%) of these cultures we grew *S. aureus* (713 isolates) and in 524 (20.8%) *Haemophilus* spp (537 isolates). Erythromycin resistant *S. aureus* was found in 2.8% of NA, 8.7% of AZ, and 15.4% of PA cultures. Among all strains resistance increased from 8% (octile 1) to 52% (octile 8). Clarithromycin resistant *Haemophilus* spp were found in 2.4% of NA, 2.4% of AZ, and 7.7% of PA cultures. Clarithromycin resistance increased from 5% of the strains (octile 1) to 40% (octile 8). Both of 2140 non-CF *S. aureus* and of 3015 non-CF *Haemophilus* control strains 8% was resistant. No increase in resistance was observed in these strains. Not only in AZ strains, but also in NA strains resistance increased significantly over time (Chi square for trend $p < 0.0001$ for both *S. aureus* and *Haemophilus*).
Conclusion: Over a 4-year period we noticed a 6–7 fold increase in macrolide resistance in CF pulmonary pathogens, associated with chronic use of azithromycin. Increasing resistance in strains from patients not using azitro could only partly be explained by former use.

P1785

Trends in antimicrobial resistance among *Escherichia coli* in Europe reported via the European Antimicrobial Resistance Surveillance System (EARSS)

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Objectives: The prevalence of fluoroquinolone resistant *Escherichia coli* has been increasing in Europe in recent years. This trend might have led to the use of newer antimicrobial drugs and thus, increasing resistance to e.g. third generation cephalosporins and possibly, multiple drug resistance. We explored trends of antimicrobial resistance among invasive *E. coli* isolates reported

through the European Antimicrobial Resistance Surveillance System (EARSS), focusing on resistance to third generation cephalosporins and on multiple drug resistance.
Methods: Participating laboratories carry out routine antimicrobial susceptibility testing for invasive *E. coli* isolates. Data are collected at national level and forwarded to the EARSS database. Between 2001 and 2003, 502 laboratories from 25 countries reported susceptibility results for 58,061 *E. coli* isolates. Test results were available for aminopenicillins (92% of the isolates), 3rd generation cephalosporins (95%), fluoroquinolones (91%) and aminoglycosides (97%). Multiple drug resistance was defined as resistance to at least 3 drugs. Trends in resistance were calculated using the Cochran Armitage test (two-sided $p < 0.05$).
Results: From 2001 to 2003, 7 countries (Austria, Bulgaria, Spain, Finland, Croatia, the Netherlands and Portugal) witnessed a significant increase in resistance to third generation cephalosporins, whereas resistance decreased in Czech Republic and Slovakia. Eighty-one per cent of the isolates was tested for all 4 antibiotics included in the EARSS protocol, of which 50% were susceptible to all antibiotics. Multiple drug resistance occurred in 4% of these isolates and increased in most countries. Statistically significant increases were reported from Bulgaria, Finland, Hungary, the Netherlands and Sweden. Fluoroquinolone resistance continued to increase in 15 countries and reached statistical significance in 7 (Austria, Bulgaria, Czech Republic, Germany, Spain, Hungary and Sweden). No country showed a significant decrease in resistance proportion.
Conclusions: *E. coli* is rapidly becoming a 'difficult-to-treat' organism in many countries. Next to common resistance to aminopenicillins, resistance rates to fluoroquinolones and to third generation cephalosporins are increasing. Furthermore, co-resistance to several drugs apparently becomes more common in many European countries, including countries with low resistance rates.

P1786

Multi-resistance in *E. coli* – an underestimated therapeutic problem? Epidemiological results from the GENARS Project

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Objectives: *E. coli* strains are naturally sensitive for most clinically used antibiotics, except for those used only for infections with gram positive bacteria. Statistics on bacterial resistance usually focus only on the percentage of resistance of a species to single drugs. In these statistics resistance to ampicillin, tetracyclin and cotrimoxazole is usually high but resistance to 3rd generation cephalosporins, aminoglycosides and even fluorinated quinolones are usually rather low, suggesting that *E. coli* does not cause therapeutic problems due to the development of resistance. However, multi-resistant strains become more and more common, causing severe therapeutic problems. Therefore we wanted to analyze the datapool of the GENARS project for the incidence of multi-resistant *E. coli* strains.
Methods: Analysis was based on first isolates of *E. coli* from six laboratories, collected from January 2002 to June 2004. Minimal inhibitory concentrations (MICs) were determined by broth microdilution method for ceftazidime (CAZ), cefotaxime (CTX), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MER) and piperacillin (PIP). Resistance patterns were evaluated by using breakpoints according to DIN, grouping resistant and intermediate as non-susceptible; multi-drug resistance was defined as non-susceptibility to at least four of the six agents.
Results: A total of 13,544 isolates was analysed. 45.5% of these isolates were non-susceptible to at least one agent. The ranking of the five most frequent resistance patterns was: PIP (26.7%), PIP/CIP (6.0%), GEN (3.2%), PIP/CIP/GEN (3.0%) and PIP/GEN (2.6%). 0.9% of the strains were classified as multi-resistant; 3 strains were resistant to all six antibiotics. There were no significant differences in multi-drug resistance rates neither between ward types nor between the centers involved.
Conclusions: Multi-resistant, untreatable *E. coli* strains are still not too common. In the hospitals under investigation, no outbreaks with multi-resistant strains could be observed. These strains seem to be developed by individual patients with severe underlying diseases. However it cannot be excluded, that such strains become a commensal of more patients in their gut flora and then can be selected during any antibiotic treatment. Therefore these strains need significant attention in order to prevent their spread.

P1787

Sustained in vitro activity of telithromycin in Germany: the PROTEKT study (Years 1–4)

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Objectives: PROTEKT – a global, longitudinal, international surveillance programme established in 1999 to study the antimicrobial susceptibility of common bacterial pathogens associated with community-acquired respiratory tract infections (RTIs) – has now completed its fourth year. This analysis of PROTEKT Year (Y) 1 to 4 data from Germany was undertaken to assess the susceptibility of key community-acquired RTI isolates to the ketolide antibacterial, telithromycin, which was first introduced in Germany in October 2001.
Methods: Minimum inhibitory concentrations (MICs) of community-acquired RTI isolates collected within Germany as part of the PROTEKT study over 4 consecutive respiratory seasons (Y1: 1999–2000; Y2: 2000–2001; Y3: 2001–2002; Y4: 2002–2003) were determined centrally using the NCCLS broth microdilution method and interpreted using NCCLS breakpoints.
Results: In total, 2139 isolates of *Streptococcus pneumoniae* (Y1: 325; Y2: 693; Y3: 623; Y4: 498), 1711 isolates of *Haemophilus influenzae* (Y1: 284; Y2: 508; Y3: 512; Y4: 407) and 432 isolates of *Streptococcus pyogenes* (Y1: 87; Y2: 119; Y3: 122; Y4: 104) were collected from a total of 11 centres in Germany (7 centres in Y1; 11 in Y2 and Y3; 10 centres in Y4). Over the 4 years of the study, (99% of pathogens were susceptible to telithromycin (Y1: 99.7%; Y2: 99.7%; Y3: 100%; Y4: 99.6%). The mode MIC for telithromycin against *S. pneumoniae* was 0.008–0.015 mg/L, while the MIC90 ranged between 0.03 mg/L and 0.12 mg/L; in total, 99.95% (2138/2139) of isolates were susceptible to telithromycin (MIC ≤ 1 mg/L). The mode MIC and MIC90 for telithromycin against *H. influenzae* were 1 and 2 mg/L, respectively, for all 4 respiratory seasons. The mode MIC for telithromycin against *S. pyogenes* was 0.015 mg/L for all 4 years, with MIC90 values of 0.015–0.12 mg/L.
Conclusions: The ketolide telithromycin is highly active in vitro against isolates of *S. pneumoniae*, *H. influenzae* and *S. pyogenes* collected from 11 centres in Germany as part of the PROTEKT study. Since its introduction in Germany in the third respiratory season of the PROTEKT study (October 2001), the in vitro activity of telithromycin against community-acquired RTI pathogens appears unchanged.

P1788

Fluoroquinolone resistance in invasive *Escherichia coli* in Europe is related to age and gender

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Objectives: For years fluoroquinolones (FQs) have constituted effective treatment of invasive *Escherichia coli* infections. *E. coli* is the most common cause of both hospital and community-acquired urinary tract infections, and the most frequent cause of gram negative bacteraemia. However, in the data reported to the European Antimicrobial Resistance Surveillance System (EARSS), FQ resistance increases in all parts of Europe.
Methods: EARSS has collected routine FQ susceptibility results of primary invasive *E. coli* isolates from blood or cerebrospinal fluid (CSF) since 1999. In the present study we related FQ resistance to gender and age (chi-square test, 2-sided, $p < 0.05$). The patients were divided into the following age groups: <1 year, 1–14, 15–44, 45–64, and ≥65 years.
Results: For the period 2001–2003, FQ susceptibility test data was reported for 51,001 *E. coli* isolates from 500 laboratories in 25 European countries. The overall proportion of FQ resistance was 11%, and was significantly higher for men (13% of 22,515 *E. coli* isolates) than for women (10% of 28,486 *E. coli* isolates). No significant differences were found between men and women under the age of 15. The age groups <1 (3%), and 1–14 years (7%) had a significant lower proportion of FQ resistance compared to the older patients (11%).
Conclusion: The significant lower proportions of FQ resistance in patients under the age of 15 fits the limited use of FQ in this age group. The significantly higher proportion of FQ resistance in men could be related to the higher likelihood of men being infected with hospital strains, as women are more prone to ascending urinary tract infections originating from the more sensitive *E. coli* strains from normal gut flora.

P1789

Increasing prevalence of beta-lactam resistant *Haemophilus influenzae* in Japan: in vitro activity of telithromycin and beta-lactam antimicrobials over 4 years

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Objectives: There has been a steady increase in the prevalence of beta-lactam-resistant *Haemophilus influenzae* strains worldwide. This increase is of particular concern in Japan, where beta-lactams are commonly used for the empiric treatment of community-acquired respiratory tract infections (CARTIs). This analysis — part of the PROTEKT global surveillance programme — assesses the in vitro activity of the ketolide, telithromycin (TEL), against beta-lactamase-positive (BL+) and BL-nonproducing ampicillin-resistant/intermediate (BLNAR/I) *H. influenzae* strains isolated from patients in Japan.
Methods: *H. influenzae* isolates were collected over 4 years (between 1999 [Year (Y) 1] and 2003 [Y4]) from adult and paediatric patients with CARTIs at 12 centres in Japan (6 in Y1; 12 in Y2–Y4). Isolates were tested for BL production (by the chromagenic nitrocefin method) and for nonsusceptibility to ampicillin (AMP; MIC ≥2 mg/L). MICs for a panel of antibacterials were determined centrally using the NCCLS broth microdilution method and interpreted using NCCLS breakpoints.
Results: A total of 1833 *H. influenzae* isolates were collected over 4 years (Y1: 281; Y2: 442; Y3: 546; Y4: 564). The proportion of BL+ isolates was 8.5%, 8.8%, 9.7% and 5.5% for Y1, 2, 3 and 4, respectively. From Y1–Y4, isolates that were BLNAI (MIC 2 mg/L) increased from 3.5% to 16.0%, and those that were BLNAR (MIC ≥ 4 mg/L) increased from 0.4% to 2.5%. The MIC50 for amoxicillin–clavulanate (AMC) against all isolates increased from 0.5 mg/L in Y1 to 1 mg/L in Y4. Overall, 0.7% of all *H. influenzae* isolates were resistant to AMC in Y1 (MIC ≥ 8 mg/L), increasing to 1.8% in Y4. Non-susceptibility to cefdinir increased from 8.2% in Y1 to 20.4% in Y4. TEL showed sustained in vitro activity against *H. influenzae* (MIC50 = 1 mg/L for BL+ isolates) over the 4 years. Overall susceptibility for all isolates was >99%, regardless of BL status.
Conclusions: The proportion of *H. influenzae* isolates from patients in Japan that were BLNAI, BLNAR or BL+ (and therefore co-resistant to a number of other beta-lactams, including AMC and cephalosporins) increased from ~12% in Y1 to 25% in Y4, indicating that the empiric use of beta-lactams to treat CARTIs in Japan may be compromised. TEL demonstrated sustained in vitro activity against *H. influenzae*, including BL+ strains (>99% of isolates susceptible), and may be an option for empiric treatment in areas with an increasing prevalence of BLNAR isolates.

P1790

TARGET Surveillance, a part of the LIBRA initiative: comparative activity of moxifloxacin against respiratory pathogens isolated during 2003

I. Morrissey, M. McKeon, K. Dowling, A. Colclough, L. Viljoen, T. Veltman *(London, UK)*

Objectives: To assess the antibiotic (ABX) susceptibility of *H. influenzae* (HI) & *S. pneumoniae* (SP) isolates causing community-acquired respiratory-tract infections (CARTI) in 2003.
Methods: 51 centres in 7 countries submitted 2925 HI and 3233 SP. MICs for penicillin G (PEN, SP only), ampicillin (AMP, HI only), amoxycillin–clavulanate (AMX/C), azithromycin (AZI), ceftriaxone (CTX), levofloxacin (LEV), gatifloxacin (GATI) & moxifloxacin (MXF) were determined by microbroth dilution.
Results: All HI were susceptible to AMX/C, CTX, LEV, GATI & MXF. Beta-lactamase prevalence in HI was: France (32.8%), USA (30.6%), Mexico (21.6%), Spain (15.2%), Germany (8.6%), Italy (6.6%), & South Africa (7.8%). Fourteen AZI non-susceptible HI were also found (0.5%). SP resistance (number of centres, isolates per country) is shown below:

		% res						
	PDD subset (N)	PEN	AZI	AMX/C	CTX	LEV	GATI	MXF
-	Tot pop (3233)	22.1	34 0	5 0	1.8	1.3	1.1	0 3
Age	0-1 years (424)	**38.4**	**49.8**	**12.5**	**4.5**	*0.2*	*0.2*	0
	2-5 years (317)	**30.0**	**40.7**	**8.5**	**4.4**	*0.3*	0	0
	6-17 years (224)	(21.0)	*28.6*	6.7	(2.7)	(0.9)	(0.9)	(0.5)
	18-64 years (1359)	*18.0*	(30.6)	*2.9*	(0.8)	(1.8)	(1.5)	(0.3)
	>64 years (884)	*17.8*	(30.2)	*2.8*	(0.9)	(1.4)	(1.2)	(0.5)
Source	Ear (330)	**30.0**	**43.0**	**10.6**	(3.6)	(1.2)	(1.2)	(0.3)
	Sinus aspirate (176)	**30.0**	**44.9**	**9.7**	(2.8)	(0.6)	(0.6)	0
Infection	Acute otitis media (306)	**28.8**	**44.8**	**10.5**	NS	NS	NS	NS
	Chronic ottis media (63)	**36.5**	(36.5)	**12.7**	**4.8**	0	0	0
	Acute sinusitis (217)	(21.2)	(35.5)	(5 1)	(3.2)	0	0	0
	Acute tonsillitis (119)	(20.2)	(31.1)	(8.0)	(4.0)	(0.8)	0	0

Conclusion: Full ABX susceptibility was seen with HI except AMP due to beta-lactamase. However, universal susceptibility to AZI is no longer guaranteed. With SP, AZI and/or PEN resistance was high in many countries. Relatively high AMX/C & CTX resistance was also found in South Africa. SP from Germany had very low ABX resistance but resistance to AZI was observed. Although fluoroquinolone resistance was low, there was evidence of a gap emerging between LEV or GATI and MXF. This was most

prominent in Italy where LEV & GATI resistance was highest. MXF is therefore an important option for the treatment of CARTI especially where resistance to other ABX is high.

P1791

TARGET Surveillance, a part of the LIBRA initiative: the comparative activity of ciprofloxacin against urinary tract pathogens isolated during 2003

I. Morrissey, K. Dowling, A. Colclough, M. McKeon, L. Viljoen, T. Veltman *(London, UK)*

Objectives: To assess the prevalence of species and antibiotic (ABX) susceptibility of pathogens causing outpatient (out) & inpatient (in) urinary tract infections (UTIs) worldwide.
Methods: 41 centres in 7 countries submitted 6203 isolates (2518 in and 3677 out): France (6 centres, 922 isolates), Germany (5, 707), Italy (12, 1051), Mexico (3, 143), South Africa (2, 146), Spain (3, 502) & USA (20, 2732). These were re-identified & MICs for common oral and parenteral ABX were determined using NCCLS broth microdilution assays.
Results: The most common species associated with UTIs (where N > 100) was *Escherichia coli* (3743 isolates, 60.3%), followed by *Klebsiella pneumoniae* (508, 8.2%), *Proteus mirabilis* (400, 6.4%), *Enterococcus faecalis* (395, 6.4%), *Pseudomonas aeruginosa* (250, 4.0%), *Enterobacter cloacae* (144, 2.3%) & *Klebsiella oxytoca* (104, 1.7%). Generally lower antibacterial susceptibility was found with inpatients compared with outpatients. Lower susceptibility was also found to many AMs in Mexico, South Africa & Italy compared with other countries. The most significant difference was overall TSX susceptibility that reduced to 29.4% & 34.9% in Mexico & South Africa respectively. Susceptibilities (in vs out) for orally available ABX were: ciprofloxacin (CIP, 79.8% vs 85.6%), ampicillin (43.9% vs 50.0%), amoxycillin–clavulanate (59.0% vs 65.4%), nitrofurantoin (72.4% vs 78.6%), & trimethoprim/sulphamethoxazole (TMP/SMX, 65.1% vs 68.8%). Susceptibilities for parenteral administration only ABX were: piperacillin/tazobactam (82.2% vs 85.4%), gentamicin (82.8% vs 86.8%), ceftriaxone (80.1% vs 88.1%), ceftazidime (84.6% vs 88.4%) & imipenem (89.6% vs 91.9%).
Conclusion: CIP was the most active orally available ABX against the total population of pathogens associated with UTIs from 7 countries worldwide. CIP also showed good activity compared to parenteral only ABX. This is important because CIP is available in parenteral as well as oral formulation, thus avoiding any oral-switch concerns that may be associated with parenteral only antibacterial agents.

P1792

TARGET Surveillance, a part of the LIBRA initiative: the effect of patient demography on the antibiotic susceptibility of *Streptococcus pneumoniae* from community-acquired respiratory-tract infections

I. Morrissey, A. Colclough, K. Dowling, M. McKeon, L. Viljoen, T. Veltman *(London, UK)*

Objectives: To assess antibiotic (ABX) resistance (res) in *S. pneumoniae* (SP) collected in 2003 based on patient demographic data (PDD).
Methods: 512 centres in 7 countries submitted 3233 SP. Penicillin G (PEN), amoxycillin–clavulanate (AMX/C), azithromycin (AZI), ceftriaxone (CTX), levofloxacin (LEV), gatifloxacin (GATI) and moxifloxacin (MXF) MICs were determined. Specimen source, infection type, age and gender PDD were collected and res compared based on 95% confidence intervals.
Results: Most PDD subset data was not statistically different (NS) to that of the total SP population (Tot pop). Results are shown below (bold > Tot pop; italic > Tot pop; parenthesis = NS). Age had the most significant effect on ABX res. PEN, AZI, AMX/C and CTX res was highest in children <6 years old. AMX/C res was also raised in children 6–17 years old but AZI res was NS. Res to PEN or AMX/C decreased with adult patients. PEN, AZI and AMX/C res was > the Tot pop in ear specimens and acute otitis media (both associated with age) and sinus aspirate (not associated with age). Res to PEN, AMX/C or CTX, but not AZI, increased in chronic otitis media infection (associated with age). In contrast, fluoroquinolone (FQ) res, esp. MXF, was very low in the Tot pop and even lower or zero in children <6 years old, sinus source/infection, chronic otitis media or tonsillitis. None of these PDD data was linked to country of origin.

		% res						
	PDD subset (N)	PEN	AZI	AMX/C	CTX	LEV	GATI	MXF
-	Tot pop (3233)	22.1	34 0	5 0	1.8	1.3	1.1	0 3
Age	0-1 years (424)	**38.4**	**49.8**	**12.5**	**4.5**	*0.2*	*0.2*	0
	2-5 years (317)	**30.0**	**40.7**	**8.5**	**4.4**	*0.3*	0	0
	6-17 years (224)	(21.0)	*28.6*	*6.7*	(2.7)	(0.9)	(0.9)	(0.5)
	18-64 years (1359)	*18.0*	(30.6)	*2.9*	(0.8)	(1.8)	(1.5)	(0.3)
	>64 years (884)	*17.8*	(30.2)	*2.8*	(0.9)	(1.4)	(1.2)	(0.5)
Source	Ear (330)	**30.0**	**43.0**	**10.6**	(3.6)	(1.2)	(1.2)	(0.3)
	Sinus aspirate (176)	**30.0**	**44.9**	**9.7**	(2.8)	(0.6)	(0.6)	0
Infection	Acute otitis media (306)	**28.8**	**44.8**	**10.5**	NS	NS	NS	NS
	Chronic ottis media (63)	**36.5**	(36.5)	**12.7**	**4.8**	0	0	0
	Acute sinusitis (217)	(21.2)	(35.5)	(5 1)	(3.2)	0	0	0
	Acute tonsillitis (119)	(20.2)	(31.1)	(8.0)	(4.0)	(0.8)	0	0

Conclusion: Some patient subsets such as children <6 years or all sinusitis patients (i.e. sinus aspirate source) have a greater chance of harbouring PEN, AZI or AMX/C res SP than the Tot pop. This was not the case with MXF or other FQ. This should be taken into account when empirical ABX therapy is being considered.

P1793

Antimicrobial susceptibility among invasive Gram-negative bacteria in the UK and Ireland: the BSAC Bacteraemia Resistance Surveillance Programme 2003

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Objective: To monitor prevalence of established forms of resistance, detect emerging forms, and assess the activity of newer antimicrobial agents.
Methods: Bacteraemia isolates were collected from 25 laboratories in the UK and Ireland in 2003, excluding duplicates isolated within one week. MICs were determined centrally using the BSAC agar dilution method and interpreted by BSAC criteria. Isolates with MICs on or above the susceptibility breakpoint for ceftazidime (CAZ) or cefotaxime (CTX) were tested for ESBLs by clavulanate synergy tests (potentiation of 8-fold or more) with CAZ, CTX and cefepime. Results were compared with those from similar surveillance in 2001 and 2002.
Results: Table 1 shows resistance rates to important antimicrobials (excluding tetracyclines and glycylcyclines, for which breakpoints are not currently defined). There were no statistically significant trends over the three-year period. The one imipenem-resistant *Enterobacter* (MIC > 16 mg/L) produced a novel KPC enzyme, KPC-4. Among *Enterobacter* spp., 32% were CTX-resistant without producing ESBLs and were presumed to be AmpC producers. Multi-resistant isolates with CTX-M

Table 1: Percentage of isolates resistant at MIC level shown (mg/L)

resistance	*E. coli* N=248	*Klebsiella* N=245	*Enterobacter* N=217	*P. mirabilis* N=186	resistance	*P. aeruginosa* N=205
CIP >1	10.5	7.3	9.2	8.1	CIP >4	8.8
GEN >1	5.2	7.3	11.5	5.4	GEN >4	4.9
AMX >16	58.9	97.1	Amp C	29.0		
AMC >16	6.5	3.3	Amp C	1.1	TZP >16	4.4
CAZ >2	3.2	10.2	37.3	0.0	CAZ >8	1.5
CTX >1	2.0	9.0	38.2	0.0		
ESBL +	2.8	9.8	6.0	0.0		
IPM >4	0.0	0.0	0.5	0.0	IPM >4	5.9

CIP cipro floxacin GEN gentamicin, AMX amoxicillin, AMC amoxicillin-clavulanate. CAZ ceftazidime, CTX cofotasume, ESBL - extended spectrum beta-lactamase, IPM imipenem.

ESBLs were noted first in this surveillance in 2002 and persisted in 2003; all remained susceptible to imipenem and ertapenem, and were inhibited by tigecycline at or below 2 mg/L.

Conclusion: Although there was no clear increase in prevalence of resistance in these major Gram-negative agents of bacteraemia, beta-lactamases have continued to evolve and spread, and are often associated with multi-resistance. Imipenem, ertapenem and tigecycline retained good activity against ESBL-producers.

Pathogenesis of infections

P1794

magA, a previously assigned virulence gene of *Klebsiella pneumoniae*, is a part of the capsular polysaccharide gene cluster of serotype K1

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Objectives: *K. pneumoniae* is a common pathogen associated with nosocomial infections such as bacteraemia and pneumonia. In recent years, an emerging community-acquired disease with a high mortality rate has arisen. More than 900 episodes of bacteremic liver infections and associated complications due to *K. pneumoniae* have been reported in Taiwan during the last decade. The pathogenic mechanism of this infectious disease is not well understood. A virulence gene, magA (mucoviscosity-associated gene), has recently been identified, but not functionally characterized, in invasive strains from Taiwan. Furthermore, a high prevalence of serotype K1 in clinical cases has been reported. The purpose of this study was to investigate the prevalence of magA in different clinical isolates of *K. pneumoniae*.

Methods: 499 isolates of *K. pneumoniae* were screened for the presence of magA using a colony-blot hybridization technique. Isolates included test strains of all serotypes as well as isolates of different clinical origin. Results were compared to capsular serotype previously determined by countercurrent immunoelectrophoresis. A part of the CPS gene cluster of a K1 test strain was amplified by PCR using primers flanking conserved regions of the CPS operon along with primers matching magA.

Results: 40 strains were positive in hybridization, all of them belonging to the K1 serotype. One strain of K1 serotype was negative in hybridization but the presence of magA in this remaining strain was confirmed by PCR. No strains of serotype other than K1 possessed magA. Amplification products through PCR of the CPS gene cluster of serotype K1 demonstrated the location of magA to be in this region.

Conclusions: The results of this study showed that the proposed virulence gene, magA, is restricted to capsular serotype K1 of *K. pneumoniae* and is situated in the capsular polysaccharide gene cluster. Therefore, this gene might not contribute to virulence itself but its presence indicating a strain of serotype K1, which is more likely to be virulent in certain clinical cases. Thus, hybridization of magA could serve as a stable diagnostic method in clinical and epidemiological research.

P1795

Identification of genes conferring cytotoxicity in *Serratia marcescens*

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Objectives: To identify genes associated with cytotoxicity in *Serratia marcescens*.

Methods: Random insertion mutagenesis by an EZ::TN<R6-Kagmmaori/KAN-2>Tnp Transposome system was used to generate isogenic insertional derivatives of a bacteremic isolate of *S. marcescens* NB36, which has previously been shown cytotoxic to MDCK cells. Recombinant plasmids were constructed by self-ligation of BamH I- or Sac I-digested genomic DNA of non-cytotoxic insertional mutants and then transformed into the TransforMax EC100D pir-116 electrocompetent *Escherichia coli*. The transposon-containing plasmid was rescue-cloned and sequenced. The genes associated with cytotoxicity were phenotypically examined by the production of gluconic acid among 40 isolates of *S. marcescens* using a reverse-phase, ion-pairing high-performance liquid chromatography (HPLC) method.

Results: A non-cytotoxic mutant was firstly obtained during the screening of 1784 insertional clones obtained by random mutagenesis. Sequence analysis of a 7814-bp DNA fragment showed four open reading frames with high homology to pyrroloquinoline quinone biosynthesis protein F (PQQF), cytosine/purine/uracil/thiamine/allantoin permease family protein, aldehyde dehydrogenase, and glucose-6-phosphate dehydrogenase, respectively. A partial gene homologous to the pyrroloquinoline quinone biosynthesis protein E gene was found 6-bp upstream of the pqqF. Both PQQ genes were cotranscribed at the same direction, while the remaining three genes at the opposite direction. The transposon insertion site was on pqqF and close to the end of the gene. Because PQQ acts as the cofactor of glucose-6-phosphate dehydrogenase to form a holoenzyme essential for the periplasmic oxidation of glucose to gluconic acid, it was hypothesized that the transposon-inserted pqqF gene may lose its function and the production of gluconic acid may be reduced and thereby decreased the cytotoxicity. Through HPLC analysis, the production of gluconic acid was confirmed in 29 cytotoxic isolates of *S. marcescens*, while another 11 non-cytotoxic isolates produced no gluconic acid. The insertional mutant did not produce gluconic acid, whereas the parent strain, NB36, did.

Conclusions: PQQ genes are associated with the cytotoxicity of *S. marcescens*, and the cytotoxicity may be due to the production of gluconic acid. Restoration of the function of pqqF by complementation experiments is ongoing to verify these findings.

P1796

In vitro effect of subMICs of antibiotics on the adherence ability and morphology of *Pseudomonas aeruginosa* strains isolated from patients with urinary tract infection

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Objectives: The ability of *Pseudomonas aeruginosa* to adhere to the surface of mucous membranes of the human body is considered the initial step in its colonization and subsequent

infection. The aim of this study was to examine the influence of subminimal inhibitory concentrations (subMICs) of ceftazidime and ciprofloxacin on the morphology and adherence ability of wild-type *P. aeruginosa* strains to Buffalo green monkey kidney (BGMK) cell line.
Methods: A total of 14 strains isolated from urine of patients with urinary tract infection (UTI) were used. Bacterial adherence to the BGMK cell line was tested before and after treatment with antibiotics and detected by means of an immunofluorescence staining. Comparisons were made between the morphology before and that after exposure of strains to 1/2, 1/4, 1/8, 1/16 and 1/32 MIC of antibiotics, as well as between the number of bacteria attached to the BGMK cells before and the number after their exposure to the same concentrations of antibiotics.
Results: Significant dose dependent reduction of *P. aeruginosa* adherence ability was observed, wich correlated with the alterations in bacterial cell morphology. After exposure of strains to subMICs of antibiotics, normal shapes and filaments were noted. The greatest filamentation and the greatest loos of adherence ability occurred at 1/2 MIC of ceftazidime. The lenght of filaments decreased as the concentration of this antibiotic was being reduced, and at the concentration of 1/32 MIC normal bacterial morphology was detected. Treatment with subMICs of ciprofloxacin resulted in shorter filaments. Using the paired t test to compare the number of adhered bacteria before and after exposure to subMICs of antibiotics, a statistically significant difference was determined after exposure of strains to 1/2, 1/4, 1/8 and 1/16 MIC of ceftazidime, and to 1/2, 1/4 and 1/8 of ciprofloxacin.
Conclusion: The results of this study have shown that the adherence capacity of *P. aeruginosa* strains isolated from urine of patients with UTI was significantly decreased after exposure to subMICs of ceftazidime and ciprofloxacin. SubMICs of antibiotics affected not only the adherence ability of bacteria, but also their morphology.

P1797

In vivo selection of resistant subpopulations from rats infected with high inocula of *Pseudomonas aeruginosa* and treated with fluoroquinolones

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Objective: Mutant prevention concentration (MPC) defines antimicrobial (AM) drug concentration threshold that would require an organism to simultaneous possess 2 resistance mutations for growth in the presence of drug. To date, MPC measurements have been determined in vitro and we report the selection of resistant subpopulations – from strains determined to be susceptible to FQs by minimal inhibitory concentration (MIC) testing – following infection in rats and treatment with FQs.
Methods: Sprague Dawley white rats (250–300 grams) were infected with PA following implant of PA saturated foreign objects. Pre- and post-infection MICs and MPCs were determined utilizing 100,000 cfu/ml or 10 billion organisms by microbroth or agar dilution respectively. Isolates were tested following recovery from infected rats that did not receive AM therapy and those treated with ciprofloxacin (Cpx)or levofloxacin (Lfx) based on dosages utilized in humans (400 mg tid IV, 750 mg OD respectively).
Results: PA strains with MICs to Cpx and Lfx of 0.25 and 0.5 μg/ml respectively were used to infect rats (n = 22) without drug and following sacrifice (48–72 hr), recovered PA strains had MICs equal to pre-infections values for 33–35 strains and 2 strains had MICs one dilution higher to each drug. For infected and treated rats (n = 38–19 Cpx, 19 Lfx), organism recovered had MICs to Cpx 2–4 μg/ml and for Lfx 8–16 μg/ml. Corresponding MPC values were also elevated from pre-infection values. Pre-treatment and recovered PA strains were the same based on pulsed field gel electrophoresis analysis.
Conclusion: Exposure of high bacterial PA inoculums to drug in vivo resulted in recovery of organisms with elevated MICs when compared to pre-infection values. MIC values were lower for Cpx than for Lfx. This data shows dosing to prevent selection of resistant subpopulations is necessary and this could slow the rates at which resistance occurs and possibly prevent therapeutic failure.

P1798

The effect of hyperbaric oxygen treatment on the in vitro and in vivo activity of *Borrelia burgdorferi*

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Objectives: To evaluate the use of hyperbaric oxygen treatment (hbot) for its ability to inhibit the growth of *Borrelia burgdorferi* (Bb) in vitro, and in vivo, in a murine model of Lyme disease.
Methods: Several North American tick-derived and recently obtained patient isolates of Bb were studied for their sensitivity to hbot. To test for in vitro susceptibility, one million Bb were cultured in 0.2 ml of BSK growth medium using small snap-cap test tubes. With the tubes loosely capped, these cultures were then exposed, daily for one continuous hour (for 2 consecutive days), to pure, filtered oxygen pressurized to 2–3 times normal atmospheric conditions. This was achieved using a specially constructed, miniaturized cylindrical chamber (length = 25 cm; diameter = 17 cm), equipped to accept any pressurized gas mixture through one portal opening. After the final HBOT was given, all culture tubes were snapped shut. Matching control tubes received no HBOT. All cultures were incubated at 33 C for 2–3 days and were then examined microscopically for live, motile Bb at the end of the incubation period. For the in vivo studies, separate groups of C3H or CD1 mice were infected intradermally with 100.000 Bb. Two to 4 weeks later, one group of infected mice received a 1.0–1.5 hour(s) of continuous hbo exposure, for 2 consecutive or alternating days. The treated mice, along with untreated controls, were sacrificed one day after the last hbot, and extract cultures of their urinary bladders were prepared in BSK culture media. All cultures were monitored periodically for the presence of motile Bb, using darkfield or phase-contrast microscopy.
Results: Following hbot, it was found 14 of 17 different strains of Bb had their growth inhibited by 33–94%, while there was little or no growth inhibition of 3 Bb strains. For the Bb-infectivity experiments in hbot-treated mice, it was found that borrelial spirochetes grew out in only 20% of the matching extract cultures, whereas live Borrelia were recoverable from 90% of the extract cultures prepared from the matching set of infected control mice not given and hbot.
Conclusions: These data show that hbot exerts a selective antiborrelial effect which suggest that it might possibly be considered as a clinically useful form of adjunct therapy in the treatment of Lyme disease.

P1799

Monocyte apoptosis in experimental sepsis by multidrug-resistant species

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Objectives: There is little evidence regarding monocyte apoptosis during the course of sepsis. This study aims to investigate

changes in cytokine release by peripheral blood monocytes and their correlation to monocyte apoptosis.
Methods: Acute pyelonephritis was induced in 20 New Zealand rabbits after ligation of the right pyelo-ureteral junction; all were inoculated of an 8log10 cfu/ml inoculum in the renal pelvis, 10 of a multidrug-resistant clinical isolate of *Pseudomonas aeruginosa* and 10 of a multidrug-resistant isolate of *Klebsiella pneumonia*. Blood was sampled for estimation of tumor necrosis factor-alpha (TNFa) and for isolation of monocytes. Mononuclear cells were isolated after centrifugation over Ficoll and incubated for one hour at 37°C under 5% CO2 in RPMI with 10% FBS and 2 mM glutamine. Non-adherent cells were removed and after trypsinization monocytes were lyzed. TNF-alpha was estimated by a bioassay on L929 fibrosarcoma cell line; caspase-3 activity was estimated in the cytosolic extract by a kinetic chromogenic assay.
Results: Mean ± SE values for serum TNF-alpha at 4 and 24 hours after challenge by *P.aeruginosa* were 37.97 ± 28.07, and 16.19 ± 10.62 pg/ml respectively. At the same intervals, mean values for caspase-3 activity were 6472.74 ± 4850.7 and 1129.48 ± 863.35 pmol/min.10000cells respectively. Mean ± SE values for serum TNF-alpha at 4 and 24 hours after challenge by *K. pneumoniae* were 253.97 ± 170.15, and 83.77 ± 62.30 pg/ml respectively. At the same intervals, mean values for caspase-3 activity were 8.95 ± 4.40 and 10.47 ± 5.87 pmol/min.10000 cells respectively.
Conclusion: Experimental acute pyelonephritis by multidrug-resistant *Pseudomonas aeruginosa* is accompanied by enhanced activity of monocyte caspase-3 over the first hours following bacterial challenge revealing an early initiation of the apoptotic process. Contrary findings are noted for *K. pneumoniae*. Results merit clinical relevance.

P1800

Critical assessment of factors affecting adhesion properties of lactic acid bacteria to human cell lines

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Objectives: To critically evaluate factors affecting adhesion of lactic acid bacteria to three human cell lines; Caco-2, HT-29MTX and GIRARDI heart cells.
Methods: A total of 64 [23 *faecal* (F), 19 clinical (C) and 22 probiotic (P)] *lactobacilli* and 17 [5 F, 6 C and 6 P] bifidobacteria were tested for their adhesion to Caco-2 (CC2L, CC2B) and HT-29MTX (MTXL, MTXB) cells and a total of 24 [5 F, 8 endocarditis (E) and 11 P] lactobacilli were tested using GIRARDI heart cells (GIR). Two negative (NC1, NC2) and one positive control (PC) strain were used in each assay. Adherent bacteria were counted by plating serial dilutions on MRS. All assays were performed in triplicate. Adhesion was expressed as the percentage of bacteria recovered after adhesion relative to the number of bacteria added. Statistical analysis was performed using one-way ANOVA, Tukey's test ($P < 0.05$ was considered statistically significant) and Pearson's correlations.
Results: Adhesion results of the same strain varied when done in triplicate. A longer plate incubation time resulted in significantly better adhesion of PC for CC2L. The number of weeks of postconfluency of the cells did not significantly affect adhesion. Automatic plating produced significantly higher adhesion for MTXL, while a significantly higher adhesion was only noticed for PC for CC2L. A significantly higher adhesion in relation to the incubation time (23–25 h) for PC was seen for CC2L, CC2B and MTXB. The researcher (R) performing the experiments also affected assay results. R3 generated significantly higher adhesion values compared to R1 and R2, while R2 generated significantly lower adhesion values for PC. Experiments performed on later dates generated significantly higher and lower adhesion for PC and NC, respectively, probably due to increased experience of the researcher, however; significantly higher adhesion for NC1 and NC2 generated for MTXL might be correlated to automated plating. Adhesion of PC to HT-29MTX was significantly improved by *B. longum* and to Caco-2 by both *B. longum* and *L. sakei*. Also, *L. gasseri* significantly increased the adhesion of NC1 to HT-29MTX.
Conclusions: Many confounding factors affect adhesion of bacteria to human cell lines. These results question the relevance of adhesion studies widely reported in the literature. The effect of variables should be validated on cell culture test systems before setting up experiments.

P1801

Influence of subinhibitory concentrations of metronidazole, vancomycin, clindamycin and linezolid on toxin production in *Clostridium difficile*

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Objective: *Clostridium difficile* is the major cause of hospital-acquired infectious diarrhoea. Several antimicrobials are known to induce and promote *C. difficile*-associated diarrhoea (CDAD). The impact of metronidazole (MET), vancomycin (VAN), clindamycin (CLI) and linezolid (LIN) on toxin-production in *C. difficile* was investigated in this study. Metronidzole and vancomycin are first line therapy drugs of CDAD. Clindamycin is strongly associated with the induction of CDAD. Linezolid is a new antimicrobial exhibiting activity mainly against gram-positive bacteria. A previous study showed enhanced and earlier toxin detection in the presence of vancomycin and metronidazole. This study intends to differentiate between toxin release and enhanced production.
Methods: *C. difficile* strain VPI10463 and three strains recovered from patients were tested for their susceptibility to MET, VAN, CLI and LIN using Etest. Toxigenicity was determined by PCR. Growth curves with and without subinhibitory concentrations of MET, VAN, CLI and LIN ($0.5 \times$ MIC) were determined for the *C. difficile* isolates. Growth was measured spectrophotometrically and by counting of plated cells. Toxin production was detected with ELISA (toxin A) and cytotoxicity assay (toxin B) from culture supernatant and from sonicated cell pellet also. Using real-time PCR mRNA for toxin A and B gene sequences was measured. Toxin production and mRNA copies were calculated in relation to the number of bacterial cells.
Results: The four strains showed very different growth and level of toxin production in the absence of antibiotics. Metronidazole and vancomycin stimulated toxin production in three of the four strains tested. Clindamycin did not stimulate and in some cases inhibited toxin production in all strains. Linezolid had variable effects on toxin A and B production in the four strains.Conclusion: The finding that our established drugs of choice for CDAD, MET and VAN, are able to enhance toxin-production in *C. difficile* indicates the nessicity to renew the discussion of the pathogenesis of severe CDAD, pseudomembranoese colitis and toxic megacolon, where MET and VAN cannot always change the clinical course. Animal studies are needed to confirm the in vitro results.

P1802

In vivo toxin expression is increased by proton pump inhibitor (PPI) in the dominant strain of the 2003–4 Montreal *Clostridium difficile* epidemic

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The recently publicized massive outbreak of *C. difficile* associated disease (CDAD) in Montreal is largely clonal in nature, with a single, binary-toxin (CDT) positive strain accounting for a major proportion of clinical cases, with attendant morbidity and mortality. A recent epidemiologic study showed that concurrent use of proton pump inhibitor (PPI) was associated with a 2.1–2.7 fold increased risk of infection. The Calgary region experienced a large ermB + clonal outbreak in 2000–2001, also with associated increased disease severity.

Objectives: We examined the impact of pantoprazole on *C. difficile* toxin expression of cytotoxin B in a Sprague-Dawley animal model simulating human intestinal ecology and antibiotic selection pressure.

Methods: In two parallel experiments, 73 rats were infected with either the dominant, binary toxin-positive (M13) from the Montreal CDAD outbreak, a less-prevalent, binary toxin negative (M18) Montreal strain or the binary toxin-negative Calgary outbreak strain (C2007). Animals (200 g) were individually housed in wire mesh cages; barrier/contact precautions were maintained between groups to prevent cross infection. Cultures of all rats confirmed no initial colonization. After 7 days of conditioning on a mixed meat/grain diet (maintained for 20 days), the animals were given 10^3 CFU of CD spores on days 8–9, followed by Cefotaxime (15 mg q8h) with or without pantoprazole (2 mg IP/day) from days 10–20. The animals were sacrificed on day 21, and vegetative and spore counts were quantitatively assessed. Toxin titres were determined by standard cell culture methods, and pH of cecal contents was measured ex-vivo.

Results: The increase in toxin titre upon exposure to pantoprazole was statistically significant ($p < 0.01$) in the pooled M13 group (Montreal dominant clone). Conversely, toxin titres in C2007 and M18 both decreased upon exposure to the PPI, but while the decrease in C2007 was significant ($p < 0.05$), the change in M18 was not.

	TOXIN TITER	VEG. COUNT	SPORE COUNT	CECALpH
Exp #1				
M13 Cefotasxime (n=8)	4000 (±645 6)	1.1E9 (±2.6E8)	3.4E7 (±1.5E7)	
M13 Cefotaxime + Pantoprazole (n=8)	10625 (±2652.0)	4.8E8 (±1 0E8)	7.8E6 (±3 4E6)	
C2007 Cefotaxime (n=8)	6500 (±2665 9)	5.3E8 (±1 0E8)	1.1E7 (±2.1E6)	
C2007 Cefotaxime + Pantoprazole (n=8)	3750 (±700.7)	5.1E8 (±1 0E8)	1.1E7 (±2.1E6)	
Exp #2				
M13Cefotaxime (n=8)	4125 (±934.2)	3.4E8 (±8.4E7)	5.1E3 (±1.1E3)	6.08 (±0.04)
M13 Cefotaxime + Pantoprazole (n=8)	8000 (±1851.6)	4.0E8 (±1.4E8)	8.5E6 (±7.4E6)	6.93 (±0.33)
M18 Cefotaxime (n=8)	6375 (±3741.4)	5.5E8 (±2.2E8)	9.3E3 (±3.8E3)	7.04 (±0.11)
MI8 Cefotaxime + Pantoprazole (n=8)	2625 (±532 4)	4 1E8 (±7.2E7)	2.8E4 (±)1.2E4	6.98 (±0.12)
Control				
C2007 Untreated Control (n=9)	Nil	Nil	Nil	6.33 (±0.07)

Conclusion: In this animal model, the dominant strain of the 2003–4 Montreal CDAD epidemic demonstrated a 2 to 2.5 fold increase in-vivo toxigenicity in the presence of pantoprazole. It is unclear if the function of colonic Na + /K + ATPase pumps accounts for this difference. The effect appears to be strain specific. Since toxigenicity is variably linked to disease severity, these observations support of the possibility that PPIs could increase disease severity.

P1803

Ciprofloxacin impregnated carboxymethylcellulose membrane reduces adherence and intra-abdominal abscess formation and mortality rate in a lethal intra-abdominal sepsis model in mouse

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Objective: To assess the efficacy of ciprofloxacin impregnation to carboxymethylcellulose membrane in reduction of adherence, intraabdominal abscess formation and mortality rates in a lethal intraabdominal sepsis model in mouse.

Methods: A midline laparotomy was performed under general anesthesia and 0.8 ml of 3×10^8 cfu/ml *E. coli* and 0.2 ml of sterile homogenated mouse faeces as an adjuvant were inoculated after caecal abrasion in mouse. This model was found to be 100% lethal in 24 hours. Seprafilm was dissolved in 0.05 M NaOH solution by sonication for 4 hours. Ciprofloxacin and 0.05 M HCl solution were added to this solution and mixed for 2 hours. Different drug concentrations were used in the preparation of membranes. At predecided intervals, samples were withdrawn and the release of ciprofloxacin in the buffer solution was followed by UV spectrophotometer at 275 nm. The cumulative drug released into the medium from membranes was measured as a function of time.Afterwards the study was conveyed in four groups. In the first group this newly synthetised carboxymethylcellulose membrane was placed after midline laparotomy and caecal abrasion without any microbiological intervention. Three ciprofloxacin impregnated carboxymethylcellulose sheets differing in ciprofloxacin doses were used in groups 2, 3 and 4. The mice were sacrificed on day 7 and the presence of intraabdominal abscesses and adhesions were evaluated

Results: The newly synthetised carboxymethylcellulose was found to be similar to the original with respect to intraabdominal adhesions. There was no mortality in groups 2, 3 and 4. The rate of intraabdominal abscess formation and adherence were significantly reduced in study groups 2, 3 and 4. The rate of intraabdominal adhesion and abscess formation were reduced as the amount of impregnated ciprofloxacin was increased.

Conclusion: The impregnation of ciprofloxacin to newly synthetised carboxymethylcellulose reduces mortality rate, intraabdominal abscess and adhesion formation in a lethal intraabdominal sepsis model in mice.

P1804

Pharmacokinetics of IV or IM ceftazidime in relation to cytokines in septic neutropenic mice

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Objectives: A sudden high cytokine release has been reported after the first dose of beta-lactam antibiotics during treatment of Gram-negative infections. This cytokine response is a result of antibiotic-induced bacterial endotoxin release and this cascade probably worsens the infection outcome. We hypothesized that different routes of antibiotic administration have significant influence on kinetics of endotoxin- and cytokine-release patterns during the first hours of infection.

Aim: To compare cytokine-release patterns and kinetics of plasma antibiotic levels during initial treatment of Gram-negative sepsis in neutropenic mice, applying different routes of antibiotic administration.

Methods: Fifty neutropenic Swiss mice were divided into two groups with infection and one without. Two hours after inoculation of 10^7 CFU *E.coli* in the thigh muscle, mice were treated with either ceftazidime 2 mg (80 mg/kg) intravenously (IV) or intramuscularly (IM). Mice were sacrificed at T = 0,1,5,10,20,30,40,60 and 90 minutes for plasma-sample collection to determine TNF-alfa, IL-6, and antibiotic-concentrations. Bacterial counts and morphology were also assessed. In mice without infection, ceftazidime was administered IV to study healthy in-vivo kinetics of ceftazidime.
Results: Peak serum levels of ceftazidime (T = 1) in mice without sepsis were 486 mg/l. At t = 90, antibiotic concentration had decreased to 9.3 mg/l. Elimination half-life was 10 minutes. TNF-alfa levels showed a short peak, declining to undectectable values within 40 minutes in both the IV and the IM group. Plasma IL-6 levels did demonstrate a fast peak at T = 5 during IV treatment, but not after IM administration. In the latter case, a stable plateau-phase was encountered lasting the entire experiment. At T = 90, IL-6 levels were significantly higher in IM treated mice compared to IV treated mice (2595 vs 1504, $p < 0.05$)
Conclusion: In the initial treatment of Gram-negative sepsis, antibiotic route of administration significantly influences cytokine release patterns. IV treatment causes short and high peak levels, whereas IM treatment leads prolonged elevations. These differences probably indicate faster killing of bacteria after start of IV treatment with concomittant higher endotoxin release.

P1805

Effect of melatonin on *Candida* sepsis in experimental rat models

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Objectives: The aim of the present study was the evaulation of possible protective effects of melatonin against experimental *Candida* sepsis in rats.
Methods: Total 50 rats included to study at 5 different groups, as control, sepsis, melatonin-control, melatonin-sepsis without treatment and melatonin-sepsis with treatment. Melatonin (200 microgram/kg/day) was injected introperitonally (one a.day-during 3 days) three days prior to sepsis application. for sepsis *Candida albicans* ATCC 10259 strain was used intraperitonally. For treatment Amphoterisin B was used 1 mg/day intraperitonally. Plasma concentrations of cytokines and adhesion molecules were compared between rats with sepsis due to Candida albicans and control groups. Experimental data during *candidaemia* are controversial. On day 1 and day 15 of sepsis, plasma levels of IL-6, TNF-alpha, V-CAM, E- selectin were determined. All rats were investigated for physiopathologic differences in liver and spleen by using immunhistochemical dying.
Results: On day 1; IL-6, TNF-alpha, V-CAM levels were find higher in sepsis groups compared to controls($p<0.05$), while E-selectin levels were not shown significant difference. On melatonin groups, Il-6 and V-CAM plasma levels were detected significantly greater than others ($p < 0.05$). On day 15; All measurements were higher than initial levels. Plasma levels of cytokines and adhesion molecules of survivor in sepsis groups were significant higher than control groups($p < 0.05$). On melatonin groups; there was no significant difference according to day 1 plasma levels. On the treatment taken groups; 1t has been detected mean of improving time of melatonin taken group was 2.25 day shorter than normal treatment group.
Conclusions: In view of the current results; we conclude that adhesion molecule levels may use a diagnostic marker for yeast sepsis with cytokine level measurements and melatonin treatment may have therapeutic benefits in candidal sepsis in addition to classic antimycotic treatment due its capacity to reduce oxidative stress.

P1806

Effect of *Saccharomyces boulardii* on the re-establishment of hamster gut microflora perturbed by vancomycin treatment

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Studies of the intestinal microbial ecosystem by classical culture techniques suggest that only 30% of the microbiota can be cultured. PCR procedures based on 16 S ribosomal RNA gene specific for bacteria were developed to detect bacterial populations in hamster feces. 33 populations of bacteria were characterised by their genomic DNA sequences and targeted by PCR probes: Actinomyces group, *Bacteroides distasonis*, *B.fragilis*, *Bifidobacterium* group and 5 *Bifidobacterium* species, Campylobacter group, Citrobacter group, *C.freundii*, Clostridium group and 5 Clostridium species, *Enterobacteriaceae* group, *Enterobacter cloacae*, *Escherichia* group, *Faecalibacterium prausnitzii*, *Lactobacillus* group, *Morganella morganii*, *Peptostreptococcus* productus, *Porphyromonas gulae*, *Propionibacterium* group, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Ruminococcus bromii*, *R. obeum*, *Salmonella* group and *Staphylococcus* group.After oral antibio-therapy, with 200 mg/kg vancomycin (ATB) daily for 2 days, some bacterial groups were partially or totally eradicated. Qualitative variations were observed for 4 or 5 days: Bacteroides distasonis, *B. fragilis*, Bifidobacterium *angulatum*, *Clostridium coccoides*, *C. clostridiiforme*, *C. leptum*, *C. perfringens*, *Faecalibacterium prausnitzii*, *Peptostreptococcus* productus, *Staphylococcus* group. No antibiotic effect was observed on levels of dominant faecal groups: Bifidobacterium group, Citrobacter group, Enterobacteriaceae group, Escherichia group, *Proteus mirabilis*. In order to better understand the *Saccharomyces boulardii* (S.b.) mechanism of action in prevention of antibiotic-associated diarrhoea, 4.109 CFU/kg daily for 5 days of S.b. were administered to hamsters during oral ATB treatment. The results showed that populations eradicated with ATB treatment remained expressed and stabilised with concomitant S.b. treatment. More effective protection were showed for 2 or 3 days with S.b and ATB groups than with ATB only treatment on the following fecal microbiota: *Bacteroides fragilis*, *Clostridium coccoides*, *C.clostridiiforme*, *C.leptum*, *C.perfringens*, *Staphylococcus* group. These observations are in agreement with the well accepted notion of an equilibrium of the total *fecal microbiota*. S.b. induces a functional resilience (re-establishment) among the main groups of the flora: the Bacteroides, the *C. coccoides* and the *C. leptum* subgroup. These PCR results will be used to quantify the effects of S.b. on the intestinal microflora by DNA microarray analysis.

Non-fermenter Gram-negative bacilli: epidemiology and resistance

P1807

Characterisation of a hospital outbreak of multi-resistant *Acinetobacter baumannii* and prevalence of class 1 integrons

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Objectives: The aim of this study was to investigate the possibility of nosocomial transmission of one or more epidemic strain(s) using phenotypic and genotypic typing methods and to detect the presence of class 1 integrons in these strains.
Methods: Nine multi-resistant *Acinetobacter baumannii* isolates were recovered from patients over a two-month period between May and June 2003, in an intensive care unit (ICU) of a Greek tertiary hospital. The strains were identified by commercial ID panels and susceptibility to a broad panel of antimicrobial agents was determined by broth micro dilution method (MIC panels) according to NCCLS guidelines. Plasmid DNA fingerprinting, REP-PCR and PFGE analysis were used for molecular typing. Class 1 integrons were detected and molecularly characterized by sequencing.
Results: All isolates were resistant to beta-lactam/beta-lactamase inhibitor combinations, cephalosporins, aztreonam, imipenem, meropenem, aminoglycosides, ciprofloxacin, trimethoprim-sulfamethoxazole and chloramphenicol. Both REP-PCR and PFGE techniques revealed identical or similar banding patterns classified as subtypes of the same clone. Plasmids were found in all isolates and two different plasmid profiles were observed with sizes in the range of 2 to >30 kb. All isolates bore class 1 integrons. An integron of 2.2 kb was the predominant, with only one isolate bearing an integron of 3 kb.
Conclusions: The molecular typing results showed that the outbreak was caused by a single strain, indicating a common sourse of transmission. The differences in the plasmid profiles reflect the exchange of genetic elements in a higly selective environment during the outbreak. The presence of integrons in all isolates proves their importance for the dissemination of antibiotic resistant genes and nosocomial spread of *Acinetobacter baumannii*.

P1808

Multi-drug resistant *Acinetobacter baumannii*: susceptibility patterns (including tigecycline) and ribotyping

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Acinetobacter baumannii is an important cause of nosocomial infections in many hospitals, most often isolated from critically ill patients admitted in intensive care units (ICUs). After the first isolation of an *A. baumanni* strain with a high-resistance profile (only susceptible to colistin) in a big hospital of Northern Italy we collected in a four-year period 44 strains from 22 different patients. In this report we present the results of the susceptibility and the molecular typing of the *A. baumannii* strains collected. Susceptibility testing was performed on the collected isolates using the standardized disk-diffusion method following the criteria of NCCLS. We evaluated the susceptibility of our isolates to beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines and to the new agent tigecycline (GAR-936) [WAY-156936]. All the strains showed a profile of multi-drug resistance except for a strain resistant only to beta-lactams. In particular, 43 out of all 44 isolates were resistant to carbapenems and all strains were resistant to tetracyclines but susceptible to tigecycline. It is noteworthy that 6 strains (isolated from two different patients) were resistant to colistin. We are currently evaluating the mechanism of resistance to this drug, since this kind of resistance is rarely reported in *A. baumannii*.
Strains typing: We performed automated ribotyping using EcoRI as restriction enzyme on 24 strains isolated from 20 different patients. We obtained two distinct patterns: all the carbapenem-resistant strains showed the same profile, whereas the carbapenem-susceptible strain had a different pattern. Both the patterns obtained were quite different from those obtained from isolates collected in the Hospital of Pavia (data presented at the 6th International Symposium on *Acinetobacter*, Dublin 2004).
Conclusions: Our report confirms the wide circulation of a carbapenem-resistant clone of *A. baumannii*, that was different from those isolated in a different hospital of the same geographical area. We reported also an unusual high number of colistin-resistant strains. The only 100% effective drug was the new compound tygecicline, that is not yet used in clinical practice in our country.

P1809

Molecular epidemiology and beta-lactamase production of multidrug resistant *Acinetobacter baumannii* clinical isolates from Italian hospitals

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Objectives: *A. baumannii* is an opportunistic pathogen responsible for a variety of nosocomial infections in intensive-care-units (ICUs) and in immunocompromised patients exposed to elevated risk factors. Nosocomial infections caused by multidrug-resistant (MDR) *acinetobacters* are difficult to treat. Carbapenems remain active in many centres, but reports of carbapenem resistance have accumulated worldwide. In this work we studied MDR *A. baumannii* clinical isolates from different Italian Hospitals to evaluate clonal relationships and beta-lactamase determinants.
Methods: From 2002 to 2004, 44 nonreplicate MDR isolates were collected from different wards (ICUs, surgical and rehabilitative units) of 3-Italian Hospitals, and studied for clonal diversity and beta-lactamase production. All the isolates were identified and tested for antimicrobial susceptibility using the GNI card (Vitek System, BioMérieux) and panels NMIC/ID4 (Phoenix System, BD). The blaVIM, blaIMP, blaTEM, blaSHV, blaCTX-M, blaVEB, and blaOXA determinants presence and nature were investigated by colony hybridization method, DNA microarray analysis, PCR and sequencing. AmpC production was determined by spectrophotometric assay. The clonal relations were analysed by REP-PCR and confirmed by PFGE after digestion with SmaI enzyme.
Results: The 44 isolates belonged in 5 different clones, of which 3 were more prevalent and were detected in more than one hospital. One dominant clone (14 of 26 isolates from Varese hospital and 1 of 13 from 'S. Maugeri' IRCCS in Pavia), resistant to cefotaxime (CTX), cefepime (FEP), ceftazidime (CAZ) and susceptible to piperacillin/tazobactam (TZP), was found to produce the TEM-92 extended-spectrum beta-lactamase. Another dominant clone including isolates closely related (8 of 26 isolates from Varese hospital and 11 of 13 from 'S.Maugeri' IRCCS), resistant to CTX, CAZ, FEP and intermediate to TZP,

produced high levels of the AmpC chromosomal beta-lactamase. The third dominant clone (5 of 5 isolates from San Giovanni Rotondo hospital and 1 of 13 from 'S. Maugeri' IRCCS), resistant also to TZP and imipenem (MIC > 32 mg/l), produced an OXA-type beta-lactamase.
Conclusions: The results showed inter-hospital spread of multiple MDR clones of *A. baumannii*, which carried different beta-lactamase determinants. This is the first report on production of the TEM-92 ESBL in *A. baumannii*.

P1810

Acinetobacter baumanii: evolution of the antimicrobial resistance of strains isolated at a central military hospital

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Objectives: To evaluate the frequency and the antimicrobial resistance evolution of *Acinetobacter baumanii* isolated from different specimens in our laboratory from September 1st 2002 to august 31 2004.
Methods: From September 1st 2002 to august 31 2004 strains of *Acinetobacter baumanii* were isolated from different clinical samples coming from inpatients hospitalised in different departments of our hospital. The strains were identified with the biochemical galleries (API NE–Biomerieux) and their susceptibility to the antimicrobial agents evaluated by the disk diffusion method according to the NCCLS.
Results: 121 strains of *Acinetobacter baumanii* were isolated during the 3 years: 11 in 2002, 85 in 2003(during this period we observed an epidemy in the ICU) and 25 in 2004.These results shown that the susceptibility to Ticarcillin, Piperacillin, Imipenem, Amikacin, and Gentamycin decreased noticeably during these 3 years, when it increased to Ceftazidim and Pefloxacine. The resistance was respectively during 2002–2004:

Ticarcillin	60%	68%	95%
Piperacillin	80%	82%	91%
Ceftazidim	100%	64%	65%
Imipenem	0	47%	46%
Amikacin	70%	64%	87%
Gentamycin	64%	60%	90%
Pefloxacin	56%	56%	46%

Conclusion: The strains of *Acinetobacter baumanii* isolated in our hospital during the period 2002–2004 in our hospital presented a high level of antimicrobial resistance which was often associated to a resistance to at least 3 antimicrobial agents. In some cases the strains were totally resistant and the treatment became problematical. The most notable resistance was to Imipenem which increased from 0% to 47% in 3 years. In front of this situation, hygiene measures have been taken in the ICU with the collaboration of the physicians and the director in the purpose to limit the diffusion of these strains. Moreover the use of the antimicrobial agents and especially Imipenem is controlled in our hospital and in the majority cases the prescription is based on the results of the susceptibility test.

P1811

Acinetobacter species and strains in a university hospital during a four-year period

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Objectives: Despite the numerous reports of outbreaks by *acinetobacters*, relatively little is known about the general incidence of *Acinetobacter* species in hospitals. The aim of the present study was to investigate the species and strain distribution of *Acinetobacter* during a four-years' period in the Leiden University Medical Center using validated genotypic methods for species and strain identification.
Methods: Isolates were identified to species by the use of AFLP analysis and/or amplified ribosomal DNA analysis (ARDRA). AFLP was also used to identify isolates at the strain level using a grouping level of ≥90% as a threshold above which organisms were considered to belong to the same strain.
Results: In total, the investigated number of isolates of the period of study were 37 out of 25 patients (1999), 79 out of 47 patients (2000), 126 out of 53 patients (2001) and 39 out of 25 patients (2002). Isolates were identified to 15 named and unnamed species with *Acinetobacter baumannii* (62 patients) and the unnamed genomic species 3 (39 patients) being the most prevalent. Isolates of 11 patients could not be identified to any of the described species of *Acinetobacter*. Cross-infection was noted for *A. baumannii* (4 strains, 35 patients), genomic species 3 (3 patients), yet unclassified strain (2 patients), and gen.sp. 10 (2 patients). In 1999–2000 one *A. baumannii* strain was epidemic in the hospital, affecting 29 patients. Exclusion of this epidemic strain from the study, revealed the more endemic level of occurrence of *Acinetobacter* species.
Conclusion: The current study provided insight in the prevalence of different *Acinetobacter* species in the hospital of study. Apart from *A. baumannii*, many other species can be found in clinical specimens, some of which may also spread among patients and may be of clinical significance.

P1812

Appearance of a pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a Portuguese university hospital

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Since 1999, sporadic and clusters of multidrug resistant *Acinetobacter baumannii* isolates (MDRAb) have been found in patients infected or colonized by these bacteria throughout the University Hospital of Coimbra (HUC), Portugal, a 1600-bed hospital. Recently, there was an increase in the isolation of these organisms, disseminated in several wards.
Objective: The main objective of this work was to determine the clonal relatedness of MDRAb isolates and to document the first appearance of a pandrug-resistant isolate in Portugal (isolates resistant to almost all commercially available and tested antibiotics).
Methods: Between November 2003 and March 2004, thirty eight MDRAb isolates were recovered from 35 inpatients of HUC. Susceptibility was determined by disc-diffusion method and VITEK 2 System (BioMérieux). Random amplified polymorphic DNA analysis (RAPD) was used to assess the genetic relatedness among the isolates.
Results: The isolates were recovered from: hemocultures (44.4%), catheters (19.4%), urine (14%), bronchial aspirates (11.1%), wounds (8.3%) and sputum (2.7%). The majority of the samples were taken from inpatients of Medicine Intensive (SMI) (43%) and Burn Unit (BU) (20%) wards. All but one isolate showed an identical resistance profile to amoxicillin, amoxicillin/clavulanate, piperacillin/tazobactam, ampicillin/sulbactam, cefalotin, ceftazidime, cefotaxime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, doxiciclin, SXT, gentamicin and netilmicin, and variable susceptibility to tobramycin (Tob) and amikacin (Amk). Thirty isolates were susceptible to Tob and

Amk (TobSAmkS). The other phenotypes observed were: TobRAmkS (n = 1), TobRAmkI (n = 3) and TobRAmkR (n = 3). Analysis of RAPD patterns suggested that the isolates were genetically related. Comparing the antibiotype and RAPD fingerprints of outbreak isolates, occurred in 1999 and 2002, suggested the persistence and dissemination of a clone, that has been acquiring more resistance determinants. It is noteworthy that isolates resistant to all antibiotics typically tested, pandrug-resistant isolates (TobRAmkR phenotype) emerged only recently.
Conclusion: The results showed that a multi-resistant *A. baumannii* clone is endemic for several years in this hospital, despite the infection control measures taken by the institution. The emergence of a pandrug resistant isolate may augur the appearance of untreatable infection by this organism.

P1813

Bacteraemia due to *Acinetobacter baumannii* in an intensive care unit

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Objectives: To analyze clinical and microbiological features bacteremias due to *A. baumannii* (ABB) acquired in an ICU.
Methods: All clinically significant bacteremias in a 8-bed ICU, from Jan 2003 to Aug 2004 were evaluated.
Results: Eighteen cases of ABB occurred in 16 patients (3.7 episodes per 1000 patients-days). All cases were hospital-acquired and occurred after a mean hospitalisation of 37.1 days. Nine cases (50%) were observed from July to September. The mean age was 72.3 years and 11 patients were males. All cases underwent invasive procedures: 4 (22.2%) surgery, 16 (88%) mechanical ventilation, 18 (100%) intravascular catheterisation (cvc), 18 (100%) urinary catheterisation. Sixteen cases (88.8%) previously received antimicrobial therapy. The sources of ABB were cvc in 10 (55.6%) cases, respiratory tract in 6 (33.3%) and unknown in 4 (22.2%). Six cases (33.3%) were polymicrobial bacteremias. All strains were resistant to piperacillin, piperacillin/tazobactam, ceftazidime, amikacin, gentamicin, ciprofloxacin and aztreonam, 37.5% were susceptible to tobramicin and 60% to imipenem. Sepsis occurred in 11 cases (61.1%), severe sepsis in 1 (5.6%) and septic shock in 6 (33.3%). Nine patients died (56.5%) and deaths were related to ABB in 8 (50%). One patient died for severe respiratory failure after 4 months. Seven patients survived. The 8 deaths directly related to ABB occurred after a mean time of 7.1 days from the onset of sepsis, in 50% of cases the source was the respiratory tract and 62.5% of isolates were resistant to carbapenems; 7 (87.5%) patients received inadequate therapy (in 5 cases the strains were multi-drug resistant), the remaining patient died for septic shock despite adequate therapy. In the group of patients who recovered from ABB the rate of carbapenem resistance was 42.8%, and the source was respiratory tract in 2 cases (28.6%): 4 patients received adequate therapy (51.1%), 3 patients had a cvc-related ABB due to a multi-drug resistant strain, but removal of intravascular catheter was effective.
Conclusions: Our study confirm that ABB has a high rate of related mortality in ICU patients. Pneumonia as source of bacteraemia and resistance to carbapenems are associated with a higher mortality. Moreover a prompt adequate antimicrobial therapy and removal of infected devices are associated with a better prognosis.

P1814

An epidemic with multiresistant *Acinetobacter baumanii*: a chain of horror?

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Objective: To investigate a cluster of MDR *A.baumanii* bacteremic infections (3) in a newly built and operating for the last 10 months, tertiary, university, general hospital.
Methods: In September 2004 two patients with acute leukaemia died of perineum soft tissue bacteremic infections due to *A.baumanii* sensitive only to colimycin and despite appropriate treatment. A third patient in the surgical ward presented concurrently with CVC-related bacteraemia. A survey of all *A. baumanii* strains isolated in the hospital since November 2003 revealed two clusters of MDR *A.baumanii* cases (colonization or infection) : one in April–May (5 pts, 2 bacteremias considered at that time imported) and the other in August–September (10 cases, 3 bacteremias). None had been in the ICU and all were in the surgical ward and the one of the 2 medical wards, admitting hematology–oncology patients.
Results: Study of the strains by REP-PCR showed 2 distinct clones crossing over between the above mentioned wards. Interestingly these clones were harboured by 2 patients previously in the ICU and subsequently transferred to the surgical and hematology unit respectively more than 4 months earlier. The same strains were found on the hands of a nurse in the medical ward and the hands of the personnel and the inanimate environment of the ICU, where MDR *A. baumanii* is endemic. They were not found on the surfaces of the wards, but thorough cleaning and disinfection had already been performed before sampling. Strict infection control and hand hygiene measures regarding also in-hospital transportation of patients are now being applied. No new case has been reported in October 2004 from the wards but *A. baumanii* is still endemic in the ICU. Probably the hands of health-care workers-HCW (surgeons and ICU doctors consulting in the medical wards) served as the vehicle spreading the pathogen from a reservoir in the ICU.
Conclusion: *A. baumanii* can be preserved in reservoirs in the hospital and cross transferred by the hands of HCW. Infection control measures must be constant and intense to eliminate this sometimes deadly chain.

P1815

The incidence and risk factors for *Acinetobacter baumannii* bacteraemia in ICU patients with *A. baumannii* respiratory tract colonisation

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Objectives: The aim of the study was to determine the impact of *Acinetobacter baumannii* respiratory tract colonization in patients admitted to the ICU, and the incidence and the risk factors for the development of *A. baumannii* bacteraemia in this group of patients.
Methods: During a 4-month period all patients with ICU stay more than 48 hours, in the 30-bed multidisciplinary ICU of Evangelismos hospital, were studied. Clinical, laboratory, and demographic characteristics, the time of respiratory tract colonization with *A. baumanni*, the development of *A. baumannii* bacteraemia, and the susceptibility profiles were recorded.
Results: During the study period 63 (44 male, 19 female), of the 152 ICU patients, (41%), became respiratory tract colonized with

A. baumannii. Admission diagnosis was surgical in 35 of them and medical in the remaining 28. Subsequently, 20 of the above group of patients (32%) developed *A. baumannii* bacteraemia. The time between admission and colonization or bacteraemia was 6.5 days and 10.5 days respectively (median value). All *A. baumannii* strains were resistant to aminoglycosides and 20.6% of them were resistant to ampicillin plus sulbactam. Patients with *A. baumannii* respiratory tract colonization who developed *A. baumannii* bacteraemia had significantly a higher Apache II score on admission than those with *A. baumannii* respiratory tract colonization who didn't developed *A. baumannii* bacteraemia (22.2 + 1.7 vs. 17.2 + 1.0, $p = 0.014$). Multiple logistic regression analysis showed that independent risk factors for *A. baumannii* bacteraemia in the respiratory track colonized patients with *A. baumannii*, were Apache II score (OR = 1.1, $p < 0.05$), and admission albumin (OR = 0.3, $p = 0.03$).
Conclusion: The incidence of *A. baumannii* bacteraemia in the ICU patients with *A. baumannii* respiratory tract colonization is high (32%). The severity of the disease and the degree of hypoalbuminaemia on admission are independent risk factors for the development of *A. baumannii* bacteraemia in ICU patients with *A. baumannii* respiratory tract colonization.

P1816

Nosocomial bacteraemia due to *Acinetobacter baumannii* in a university hospital

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Objectives: To determine clinical features, risk factors, and outcome of bacteraemia due to *Acinetobacter* species detected in a university hospital.
Methods: For a 12-month period during year 2003, 44 bacteraemia episodes due to *Acinetobacter baumannii* detected as a part of nosocomial infection surveillance study of Çukurova University Hospital, a 1100-bed tertiary health care institution, investigated retrospectively.
Results: Of all patients whose mean age was 35 ± 25 (min 1, max 85) 63.6% (n:28) were male and 36.4% (n:16) female. Mean growth time was 24 days after admission. Thirty eight patients (86.4%) were hospitalized in intensive care units. Of bacteraemia episodes 59.1% (n:26) were polymicrobial. No focus of infection was detected in 65.9%. Of the remainders (34.1%), infection focus determined as follows: pneumonia 20.5%, urinary tract infection 4.5%, burn infection 4.5%, and catheter related bloodstream infection 4.5%. None of the patients had endocarditis. Fifteen patients (34.1%) were colonized previously by *Acinetobacter baumannii*. All of the patients have at least one risk factor described in the literature. Risk factors detected were previous antibiotic use 79.5%, central venous catheter 54.5%, mechanical ventilation 50.0%, surgery 43.2%, trauma 25.0%, neurological disorder 20.5%, burn 11.4%, solid organ transplantation 9.1%, haemodialysis 11.0%. Despite appropriate therapy%61.4 (n:27) of patients were died.
Conclusions: In recent years, bloodstream infections due to *Acinetobacter baumannii* became an important cause of mortality in patients who requires intensive care and hospitalized long period. Besides strict infection control procedures to prevent acquisition of this pathogen, payin attention for early determination of patients with high risk and appropriate therapy would reduce mortality.

P1817

Genotyping analysis of *Acinetobacter* isolated from bloodstream infections in a Turkish university hospital

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Objectives: To evaluate the genetic relatedness of *Acinetobacter* spp. isolated from blood cultures.
Methods: This prospective study was conducted from March 2002 to March 2003 in a University Hospital in Turkey. During this period, all patients with BSI caused by *Acinetobacter* spp. were included in the study. Blood cultures were processed by using the BACTEC 9240 blood culture system (Becton Dickinson, Sparks, MD, USA). *Acinetobacter* spp. isolates were identified according to their gram-negative morphology, based on standard biochemical reactions (API ID 32E system; bio-Merieux). For susceptibility testing and genetic analysis, only the first isolate of a bacteremic period was included. Antimicrobial susceptibility testing was determined by Kirby–Bauer disk diffusion testing according to NCCLS recommendations. The genetic analysis of isolates was performed by RAPD-PCR and pulsed-field gel electrophoresis (PFGE) at the University Medical Centre, Nijmegen. Eric 1 and d8635 primers were used in RAPD-PCR and SmaI was used in PFGE.
Results: During a one-year period, 41 patients acquired a nosocomial bloodstream infection caused by *A. baumanii*. More than 80% of these infections (36 of 41) occurred while the patients were treated in the intensive care unit. Nearly 80% of the isolates belonged to three genotypes, suggesting cross-transmission in ICU settings where infection control practices are poor. Most *Acinetobacter* isolates were multi-resistant. The crude mortality of patients infected with *A. baumanii* was 80.5%.
Conclusion: BSIs due to *A.baumannii* are mostly ICU-acquired. Moreover nosocomial transmission may lead to the spread of one or more epidemic strains. Early detection and strict compliance with standard precautions and contact isolation, are essential components of infection control programmes to prevent nosocomial transmission of *A. baumannii*.

P1818

Attributable mortality of *Acinetobacter baumannii* bacteraemia

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Objective: This study was designed to determine the attributable mortality resulting from *A. baumannii* bloodstream infection (BSI).
Methods: The Rambam medical Center is a 900-bed teaching hospital providing tertiary care for persons in the Northern of Israel. A matched, retrospective, case-control study was performed. A case patient was defined as any adult patient developing signs and symptoms of BSI > 48 hours after admission to the hospital, and for whom 1 or more blood culture yielded *A. baumannii* between January 1, 2001 and December 31, 2003. Patients with polymicrobial bacteraemia were excluded. A control was defined as a patient similar to a case but without evidence of *A. baumannii* BSI during hospitalization. We carefully matched each case patient to a control patient using a 3-stage stepwise procedure. First, using a computerized database cases were matched by period of hospitalization (±3 years), age (±10 years), sex, length of time at risk (length of stay for each control equal or greater than the time of onset of *A. baumannii* BSI for the matched case), and primary diagnosis at admission. Second, the ICD-9 diagnostic codes and medical records were

reviewed to match for underlying diseases(s), primary and secondary diagnosis, and surgical procedure(s). Finally, candidate records were reviewed to identify the best possible match based on the severity and duration of illness, without knowledge of outcome for either case or control.
Results: 52 patients fulfilled our inclusion criteria for *A. baumannii* BSI. We matched the 52 case patients with 52 control patients. All case and controls patients were correctly matched for major underlying disease, age, sex, period of hospitalization and median duration of hospital stay before the onset of BSI. Most blood culture isolates were multiple-drug resistant. Of the 52 cases, only 18 (34.7%) and 34 (65.4%) were susceptible to imipenem and ampicillin-sulbactam, respectively. Of the 52 cases, 29 died during hospitalization, for a crude mortality of 55.7%. The mortality rate found in the control group was of 19.2% (10 of 52 patients). Therefore, the attributable mortality was 36.5% (95% CI, 27%–46%) and the mortality risk-ratio was 2.9 (95% CI, 1.58–5.32).
Conclusion: *A. baumannii* is a multiple drug resistant pathogen commonly isolated from nosocomial BSI and is associated with mortality in excess of that due to the underlying diseases alone.

P1819

Cure of multidrug-resistant *Acinetobacter baumannii* prosthetic material-related orthopaedic infections with intravenous colistin

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Background: Multidrug-resistant (MDR) Gram-negative bacteria, including *Pseudomonas* and *Acinetobacter* spp., are frequent causes of osteomyelitis, and septic arthritis now-a-days. For such infections, colistin has not been considered as an alternative to the conventional therapeutic intervention, mainly due to its reported in the old literature inadequate penetration in these tissues.
Methods: We describe two cases of MDR *Acinetobacter baumannii* prosthetic material-related orthopaedic infections which where successfully treated with intravenous colistin.
Results: A 53-year-old male was admitted with complicated left proximal femur fracture. He underwent surgical debridement of the necrotic tissues and external fixation of the femur. However, during the ensuing days his clinical condition worsened and he became febrile. A specimen culture obtained during consecutive surgical debridement grew an *Acinetobacter baumannii* strain. This strain exhibited sensitivity only to colistin (MIC 0.5 mg/l) and intermediate sensitivity to gentamicin, tobramycin, and meropenem. The patient received a bolus intravenous injection of colistin 1 million IU followed by 6 million IU per day continuous infusion for 36 days. His clinical condition gradually improved. The second case was a 37-year-old male who was admitted because of fractures of both left tibial condyles. He underwent surgical treatment, which consisted of an open reduction and internal fixation. However, he became febrile 7 days after his discharge and 5 days later he was readmitted to our hospital. Physical examination showed erythema and tenderness at the region of the left knee. The patient underwent surgical debridement of the affected area. A specimen obtained from the surgical site grew an *Acinetobacter baumanii* strain and a *Staphylococcus lugdunensis* strain. The *Acinetobacter baumanii* strain exhibited sensitivity to colistin and gentamicin and intermediate sensitivity to meropenem and tobramycin. Subsequently, the patient was given intravenous treatment with colistin 2 million IU q8h for 22 days, and vancomycin 1gr q12h for 13 days. The patient became afebrile and was discharged after 29 days of hospitalization. No serious colistin associated toxicity developed and no recurrence of the infection occurred on follow up in both patients.
Conclusion: Colistin may consider as a valuable therapeutic option in cases of prosthetic material-related orthopaedic infections due to MDR *Acinetobacter baumannii* strains.

P1820

Colonisation with vancomycin-resistant *enterococci* and methicillin-resistant *Staphylococcus aureus* increases the risk for multidrug-resistant *Pseudomonas aeruginosa* colonisation

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Background: Vancomycin-resistant *Enterococci* (VRE) and methicillin-resistant *S aureus* (MRSA) have emerged as major infections control problem worldwide. Recently, a dramatic increase in multi-drug resistant gram-negative bacteria, mainly *P. aeruginosa* (MDR-PA), has been reported. No data are available on the prevalence and risk factors for MDR-PA colonisation.
Design: A rectal swab culture survey was conducted during a 1-week period (March 2004) to determine the point-prevalence of stool colonization with VRE and MDR-PA in hospitalized patients. Two case-control studies were designed to identify risk factors for colonisation. Patients colonised with VRE (group-1) and with MDR-PA (i.e. resistance to >2 drug classes; group-2) were compared to patients with negative rectal screening (group-3) using a logistic regression analysis.
Patients: All patients hospitalised in a 700-bed University Hospital.
Results: Of the 1,036 patients screened (581 in medical and 414 in surgical wards, 41 in ICU), 26 (point-prevalence: 25/1,000 hospital admissions) were colonised with VRE and 16 patients (15/1,000 hospital admissions) with MDR-PA. One-hundred and sixty-five controls, randomly selected among patients with negative screening cultures, were compared to VRE+ and MDR-PA+ patients. The mean age ±SD was 55 ± 15 in group-1 and 65 ± 15 in group-2 (P = ns vs group-3). The mean Charlson score was 2.1 ± 2.2 (P = ns vs group-3) and 3.7 ± 3.1 in group-2 ($P < 0.001$ vs group-3). Five (8%) patients were co-colonised with VRE and MDR-PA and 12 (29%) had VRE or MRSA isolated within 6 months (6 in group-1 and 6 in group-2, $P < 0.01$ vs group-3). Risk factors for both VRE and MDR-PA colonisations were: (1) antibiotic therapy within 30 days ($P < 0.01$ group-1; P=.01 group-2); (2)previous MRSA isolation ($P < 0.001$ both groups), and (3) previous VRE isolation within 30 days ($P < 0.001$ both groups). Wheel-chair and bed-bound status was independently associated with MDR-PA colonisation ($P < 0.001$) while hospital admission from long-term care facility, nursing home, or other hospitals to VRE colonisation ($P = 0.001$).
Conclusion: The prevalence of MDR-PA colonisation is high in hospitalised patients. Previous antibiotic therapy and colonisation or infection with VRE and/or MRSA increase the risk for MDR-PA colonisation. This data suggests that alert systems and isolation measures necessary to control VRE and MRSA may provide other benefit in controlling the spreading of MDR gram-negative bacteria into the hospital.

P1821

A seven-year review of neonatal sepsis due to multidrug resistant *Pseudomonas aeruginosa* in a regional NICU

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Objectives and methods: Analysis of epidemiology, clinical symptoms, laboratory findings and outcome of neonatal

septicemia due to *Pseudomonas aeruginosa* in tertiary regional NICU. Retrospective chart review of preterm and full-term neonates diagnosed with this sepsis between January 1997 and December 2003.

Material: Among 549 septic neonates 47 (8.6%) had *Pseudomonas aeruginosa* in blood culture. Early-onset septicemia (≤3 day of life) in 4 (8.5%) and late-onset in 43 (91.5%) neonates were diagnosed. Prematurity in 87%, intrauterine hypotrophy in 21%, severe birth asphyxia in 24%, very low birth weight (≤1500g) in 58%, extremely low birth weight (≤1000g) in 30% and male sex in 51% of cases were found. In all neonates with late-onset *Pseudomonas aeruginosa* sepsis the endotracheal intubation and total parenteral nutrition in first week of life were applied.

Results: Most frequent clinical symptoms included bilateral pneumonia (96%), septic shock (66%), urinary tract infection (43%), DIC (40%), purulent meningitis (21%) and necrotizing enterocolitis (21%). Metabolic acidosis (83%) and significant increase of serum CRP concentration (92%) as biochemical disorders were most often stated. Thrombocytopenia in 58%, marked decrease of AT III activity in 51% and neutropenia in 19% of cases were found. *Pseudomonas aeruginosa* isolated from blood were resistant to gentamycin in 100%, to amikacin, tobramycin, ciprofloxacin, imipenem/clastatin, meropenem and cefepim in 75%, to ceftazidim, piperacillin, ticarcillin/clavulanic acid in 50%. All strains were sensitive to colimycin and piperacillin/tazobactam. In 36% septic mechanically ventilated neonates *Pseudomonas aeruginosa* in tracheobronchial aspirates were also isolated. Mortality rate was 34%. All 16 neonates who died had low birth weight (including 9 with birthweight less than 1000g) and late-onset sepsis. Nine of them suffered from others significant diseases such as bronchopulmonary dysplasia, necrotizing enterocolitis, congenital defects and/or severe intracranial hemorrhages.

Conclusion: Multidrug resistant *Pseudomonas aeruginosa* is potentially important pathogen in the etiology of severe neonatal sepsis, especially late-onset with bad prognosis.

P1822

Carbapenem-resistant *Pseudomonas aeruginosa* from clinical specimens over two years

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Objectives: To analyze occurence and susceptibility of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) in a university hospital.

Methods: The analysis of microbiological records with P. *aeruginosa* resistant to imipenem or meropenem from 2002 to 2003 was done. We used abbreviated methods for identification (NCCLS) and disk-diffusion technique (NCCLS) to determine susceptibility to selected antipseudomonal drugs.

Results: There were 515 and 584 one-per-patient P. *aeruginosa* isolates in 2002 and 2003, respectively. CRPA was isolated from 190 (102-2002 and 88 - 2003) patients. They were hospitalised in surgery departments (58), ICU (51), general medicine (36), haematology (15) and other units (30). Majority of CRPA were from respiratory tract specimens 29%, wound and urine, each 28%. The most active antibiotics against CRPA were caftazidime 73%, amikacine 67%, cefepime 62%, piperacilline/tazobactam 59%, and piperacilline 46%.

Conclusion: CRPA were isolated from about 20% patients with P.*aeruginosa* infection/colonisation. Patients from surgery and ICU are at the greatest risk of acquiring CRPA during their hospitalisation. Ceftazidime retains high activity against this resistant group of *Pseudomonas* isolates which makes it the drug of choice in those kind of infections.

P1823

Three-year resistance trend among P. *aeruginosa* isolated from Brazilian intensive care units: results from the MYSTIC Program

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Objective: To evaluate the resistance trends for P. *aeruginosa* responsible for nosocomial infections in intensive care units (ICUs) participating in 2001, 2002, and 2003 editions of the MYSTIC Program in Brazil.

Methods: Four hundred and ninety-four P. *aeruginosa* clinical isolates were collected by the six ICUs participating in each of the three yearly editions of the MYSTC Program (217 were collected in 2001, 131 in 2002, and 146 in 2003). Minimum inhibitory concentrations (MICs) were determined at a central laboratory by E-test methodology according to manufacturer's instructions to: cefepime, ceftazidime, piperacillin/tazobactam, imipenem, meropenem, tobramycin, gentamicin, and ciprofloxacin. Interpretations followed the NCCLS document M100-S14. A chi-square for trend test (Altman, 1999) was applied to identify ordered differences in the rates along the 3 years studied. P values below 0.05 were considered significant.

Results: The table presents susceptibility rates of all P. *aeruginosa* clinical isolates collected from Brazilian ICUs according to the year of isolation and their respective p value for the chi-square for trend test. Piperacillin/tazobactam and meropenem presented the higher susceptibility rates at all years. However, the chi-square for trend test yielded a p value of 0.04 for both, meropenem (59.9%/2001, 56%/2002, 49.3%/2003) and piperacillin/tazobactam (63.1%/2001, 62.5%/2002, 52%/2003). All other yearly comparisons showed a p value above 0.05, although ciprofloxacin (46%/2001, 45%/2002, 38.4%/2003) presented a p value close to the cut-off (0.07).

Percentage of antimicrobial susceptibility of 494 clinical isolates of *P. aeruginosa* collected from Brazilian ICUs according to the year of isolation MYSTIC Program 2001–2003

	Year of isolation/ Susceptibility %			
Antimicrobial class/agent	2001 (N-217)	2002 (N = 131)	2003 (N = 146)	P value[a]
β lactams				
Cefepime	52.0	52.6	48.6	0.54
Ceftazidime	45.1	48.8	45.2	0.93
Piperacillin/tazobactam	63.1	62.5	52.0	0.04
Imipenem	43.7	52.6	43.1	0.94
Meropenem	59.9	54.1	49.3	0.04
Aminoglycosides				
Gentamycin	35.9	47.3	42.4	0.15
Tobramycin	38.7	43.5	41.7	0.51
Fluomquiaaloaei				
Ciprofloxacin	46.0	45.0	38.3	0.07

[a]P values calculated through chi-square for trend test.

Conclusions: Decreasing susceptibility rates were observed among all antimicrobials evaluated in each year, but only meropenem and piperacillin/tazobactam presented a significant ordered decline in their rates. Although resistance for these antimicrobials seems to be increasing, they were still the most active drugs for P. *aeruginosa* in this setting. Nevertheless, it should be noted that previous editions of the program detected P. *aeruginosa* clonal spread among these centres. Thus, biased samples cannot be ruled out, due to either possible clonal spread or overvalued resistant clinical isolates.

P1824

Carbapenem- and pandrug-resistant *Pseudomonas aeruginosa* causing nosocomial infection at a university hospital in Taiwan

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Objectives: To investigate the epidemiology of carbapenem- and pandrug-resistant *Pseudomonas aeruginosa* (resistant to all cephalosporins, piperacillin-tazobactam, aztreonam, carbapenems, ciprofloxacin, and aminoglycosides) (CRPA and PDRPA) in a university hospital in Taiwan.
Methods: Data on the routine disk diffusion susceptibilities of P. *aeruginosa* recovered from 1993 to 2002 were evaluated to determine the secular trend of resistance. Time-kill study was used to evaluate the synergy of antibiotic combinations for two PDRPA isolates. Genotypes of PDRPA isolates were determined by pulsed-field gel electrophoresis (PFGE).
Results: The rapid emergence of CRPA isolates (from 8.1% in 1999 to 13.0% in 2002) causing nosocomial infections was associated with the increased use of carbapenems and extended-spectrum cephalosporins. PDRPA was first found in 1996 and reemerged thereafter (1.4% in 1999 to 2.7% in 2002). Among the 26 PDRPA isolates recovered from 16 patients, 50%, 27%, 12% and 8% were intermediate to amikacin, aztreonam, cefepime, and ceftazidime, respectively. Time-kill curve study at one-fourth the minimum inhibitory concentrations (MICs) for two PDRPA isolates demonstrated synergism for cefepime-amikacin combination at 24 hours. Nineteen (73%) of the isolates possessed blaVIM-3 and 25 (96%) had class I integrons (intI). Pulsed-field gel electrophoresis analysis of the 26 isolates disclosed 12 pulsotypes. Several isolates from different patients had identical pulsotypes or were closely related indicating the presence of clonal spreading.
Conclusions: Selective pressure due to increasing consumption of carbapenems and extended-spectrum cephalosporins as well as clonal dissemination contributed to the spread of CRPA and PDRPA in the hospital.

P1825

Epidemiology and surveillance of MDR P. *aeruginosa* in an Italian hospital

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The spread of MDR-PA is a serious emergency causing nosocomial infections, unfortunately this problem may be observed also in small hospitals.
Objectives: To evaluate the prevalence of the MDR-PA isolates through the monitoring of laboratory data. To determine the resistance rates of PA isolates againts commonly used antibiotics. To compare the obtained data with national and international database.
Methods: The Hospital is a 510-bed tertiary care teaching hospital in Mantua with 30,000 patient admissions per year. The study has been conducted from January 2002 to June 2004. All data reported refer to non-duplicate isolates. MDR-PA was defined as resistance at least three of the following four drugs: imipenem, ceftazidime, ciprofloxacin and gentamycin. Statistical analysis were performed using 'Mercurio' (Dianoema) software. The rates of resistance to individual or combination anti-pseudomonal agents were compared using Chi-square test.
Results: 790 PA were isolated from every site; 85/790 (10.7%) were considered MDR-PA. The principal wards involved were: Intensive Care Unit (ICU), Neonatal-ICU (NICU) and Respiratory diseases, respectively in 36.4%, 21,1% and 14.1% of MDR-PA isolates.The microbiological materials predominately involved were: pus, blind endotracheal suctioning and sputum respectively in 22.3%, 21.1% and 15.3% of cases. The resistance rates to anti-pseudomonal agents increased substantially over the considered period for the ciprofloxacin, aztreonam and imipenem. Resistance to ciprofloxacin increased from 11.7% to 40.0% ($p < 0.001$); to imipenem from 35.3% to 40% (p = n.s.) and to aztreonam from 23.5% to 56.2% ($p < 0.001$). The agents with the lowest rates of MDR-PA were amikacin and piperacillin/tazobactam. Empiric drugs combinations were considered to valuate the frequency of the resistance to both agents. The cross-resistance rates between ciprofloxacin and the beta-lactam antibiotics (aztreonam, imipenem) increased significantly; also for regimen with cefepime plus gentamicin. A combination that included piperacillin/tazobactam and amikacin resulted in the lowest resistance.
Conclusion: The rates of resistance using antipseudomonal agents increased in period considered and reflected the results of the international studies. The surveillance based on the laboratory data indicates the true magnitude of local resistance problems and let the selection of an appropriate empiric therapy.

P1826

Emergence of multi-drug resistant *Pseudomonas aeruginosa* isolates in neonatal septicaemia

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The emergence of multi-drug resistant (MDR) strains of *Pseudomonas aeruginosa* has complicated decision for treatment and may lead to failure in treatment. In this study, we evaluated the prevalence of resistance to Amikacin, Ampicillin, Carbenicillin, Cefixime, Cefotaxime, Ceftazidime, Ceftizoxime, Ceftriaxone, Gentamicin, Imipenem, and Trimethoprim/ Sulfamethoxazole in sixty-nine strains of *P. aeruginosa* isolates from neonates with septicemia in Kashan, Iran from April, 2000 to June 2004. In assessment of the current breadth of multi-drug resistance in *P. aeruginosa* isolated from neonates with septicemia, 4.3% were susceptible to all studied agents, 10.1% were resistant to a single agent, MDR isolates accounted for 73.9% (51 out of 69) of isolates. The majority MDR isolates (41.2%) were resistant to three antimicrobial agents, which accounted for 30.4% of all isolates. Nineteen MDR isolates from fifty-one (37.3%) were resistant to four agents (19 out of 69; 27.5% of all isolates) and 21.6% of MDR isolates to five agents (15.9% of all isolates). Statistical analysis confirmed that there were no significant differences between multi-drug resistance phenotypes of isolates with age, gender, gestational age, outcome of septicemia, and application of respirator in neonates. Continued local surveillance studies are urged to monitor emerging antimicrobial resistance and to guide interventions to minimize its occurrence.

P1827

Determination of drug resistance in *Pseudomonas aeruginosa* isolated from burn patients in Sari, Iran 2004

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Objectives: *Pseudomonas aeruginosa* is the main opportunist pathogen One of the features of this organism is resistance to most of the current antibiotics, as a result creates problems in tratmentent . This sudy is on the determination of MIC of

currently used antibiotics to *Pseudomonas* .Therefore, it is necessary to have study on this phenomenon. We tried to have a regional on the drug resistance in the *pseudomonas* isolated in burn patients referring to burn treatment centre of zareh hospital ,in order to the patients be benefited from the more effective antibiotic treatment.

Methods: With the help of sterile swab, sample from the burn was collected, kept in tube containing sterile saline and transported to the laboratory .cultured on blood agar and EMBagar,incubated at 37°(D.C) centigrade for 24 hours. Gram staining was done from the.colonies belonging to the gram negative and oxidas positive *bacilli* were selected and cultured on simmon citrate and TSI, incubated at 37 D.C for 24 hours. The confirmed *P. aeruginosa* colonies were inoculated in sterile saline, suspension was prepared and compared with 0.5 standard of Mac Farland . Then cultured by streak method on Muller–Hinton. Antibiogram was done using disk of antibiotic prepared from Padten Teb company ,Iran. Kept at 37D°C for 24 hours. The resistance and sensitivity to different antibiotics were determined in millimeters comparing with the chart provided from the manufacture.

Results: In this study samples were collected from 250 patients admitted in burn unit of zareh hospital. 60% of the patients under study had *Pseudomonas* infection, There was significant difference between prevalence sex, Geographic condition hospital stay period. It was shown that resistance to Amikacin, Gentamycin, trimethoprim sulfo methoxazole, cephoxidim, ceftazidim. Ciprofloxazin, cephipenem, emipenem, and cephriaxon, 72.9% , 63% , 41.6% , 33.3% , 30% , 29.1% , 27% , 25% , and 15% respectively.

Conclusion: Result of the studies on resistance of the organisms to antibiotics differs in different countries. These are as follow: To Amikacin 11%(in spain), 1% in Germany , 67% Tehran (Iran) and in this study 72.9%. Also resistance to Gentamicin 65.5% in zimbabveh, Tarbiat modarres university (Iran)78%, and in this study 63%. Considering the obtained results on the resistance of this organism to antibiotics, regional study on determination of drug resistance is necessary.

P1828

Antimicrobial susceptibility among *Pseudomonas aeruginosa* isolates in Centro Hospitalar de Coimbra, central Portugal

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Objective: *Pseudomonas aeruginosa* (PA) infections are of great concern for hospitalized patients, particularly those in intensive-care units. This bacterium is the leading cause of nosocomial respiratory infections. The aim of this study was to determine antimicrobial susceptibility of *PA* clinical isolates obtained from Centro Hospitalar de Coimbra to ascertain resistance patterns.

Methods: Bacterial isolates (n = 416) were collected during April 2003 to April 2004 (one year). They were identified with VITEK (BioMérieux) and MicroScan WalkAway (Dadebehring) and susceptibility patterns were determined with these panels. Susceptibilities to Piperacillin (PIP), Piperacillin plus Tazobactam (TZP), Aztreonam (AZT), Ceftazidime (CAZ), Imipenem (IP), Meropenem (MP), Amikacin (AMK), Tobramicin (NN), Gentamicin (GN), and Ciprofloxacin (CIP) were guideline by NCCLS.

Results: In the set of 416 clinical isolates two hundred and seventy three were obtained from nosocomial infections and one hundred and forty three from community acquired infections of different clinical specimens, including urine (16.5%, 39.8%, respectively), sputum (44.3%, 27.3%), exudates (13.6%, 16.1%), blood (6.6%, 4.9%), and others sources (19.0%, 11.9%). Among all isolates MP was the most potent antibiotic (91.6% susceptible), GN and CIP were the worst agents (66.8% and 69.2% of susceptibility, respectively). Susceptibilities rates varied significantly between nosocomial and community acquired infections. PIP and IP had same susceptibility in hospital infections (80.2%), but CAZ and TZP had better activities (82.8%, 81.7%, respectively), MP was the best agent (88.3%), and AZT was a weak one (71.1%). Aminoglycosides presented light activities, 78.4% for AMK and 61.5% for GN. The quinolone, CIP, inhibited only 64.1% of these isolates. Ambulatory isolates were more susceptible to these antibiotics. MP was the best agent (97.9%) followed by IP (96.5%), CAZ and TZP (95.8%), PIP (94.4%) and AMK (94.4%). GN and CIP presented sensibilities like 76.9% and 79.9%, respectively.

Conclusions: Considering the results and the high prevalence of multidrug resistant strains (9.6%) (resistance to three or four of these agents: PIP, CAZ, IP and CIP) in this hospital environment, it is obviously necessary to detect and follow sources of infection, in order to prevent the spreading of these strains and its transmission. Susceptibility rates should be determined and effective antibiotics usage policy should be performed.

P1829

Antibiotic resistance of *Pseudomonas aeruginosa* strains isolated from patients in intensive care unit

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Objective: The aim of the paper was the epidemiological investigations of multidrug resistance among *P. aeruginosa* strains isolated from patients hospitalized in AICU of Rydygier's Hospital in Kraków between 2000–2004.

Materials: The subject of the analysis was 1008 *P. aeruginosa* strains isolated from blood, respiratory tract, urine and wounds.

Methods: The isolates were identified in the ATB system (bioMerieux) using ID 32 GN strips. The sensitivity of strains to selected antibiotics (piperacillin/tazobactam, ceftazidime, imipenem, meropenem, gentamicin, tobramycin and ciprofloxacin) was performed with disc diffusion method and with interpretation according to NCCLS. The E-tests MBL (AB BIODISK, Sweden) for detection of metallo-beta-lactamases were used. PCR analysis was performed for the estimation of genotypes of chosen *P. aeruginosa* strains isolated from patients of AICU, which represented different and changing patterns of sensitivity during hospitalization and applied therapy and for comparison strains isolated from persons from other hospital wards. Analysis for the detection of genotypes of strains was carried out with primers PAL-2 (5'-CTTCTTCAGCTCGACGCGACG-3') and ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3').

Results: *P. aeruginosa* strains isolated in the period of five years showed the resistance to piperacillin/tazobactam and ceftazydime in the range respectively about 11% and 21%. The resistance to carbapenems was very similar among strains every year and was estimated in the range 22% to 29%. The decrease of resistance to aminoglicosides : gentamycin and tobramycin in the range 21% to 2% was notified. The resistance to carbapenems and/ or to ceftazidime in some strains was metallo-beta-lactamase dependent. Multidrug resistant strains were also identified. Genotyping of 20 selected phenotypes of *P. aeruginosa* confirmed the big heterogenicity of strains.

Conclusions: The E-test MBL can be used in microbiological laboratory to monitor the emergence of metallo-beta-lactamases. Estimation phenotypes of resistance and genotypes among the

most frequently isolated in ICU *P.aeruginosa* strains is very important in epidemiological surveillance of hospital infections.

P1830

Worldwide antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* causing intra-abdominal infections: results from SMART 2003

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Background: SMART (Study for Monitoring Antimicrobial Resistance Trends) is an ongoing global antimicrobial surveillance program focused on clinical isolates from intraabdominal infections (IAI). The aim of this interim analysis was to assess antimicrobial susceptibility patterns among *P. aeruginosa* from 4 different regions of the world during 2003.
Methods: A total of 71 hospitals in North America (NA), Latin American (LA), Europe (EU), & Asia/Pacific (A/P) tested the in vitro activity of 9 antipseudomonal antibiotics commonly used to treat IAI against consecutive unique *P. aeruginosa* isolates from IAI using microdilution techniques according to NCCLS guidelines & breakpoints. Isolates recovered within 48 hr of hospitalization were considered community-acquired (CA).
Results: 559 isolates of *P. aeruginosa* were recovered from 4478 patients (12%). 188 of these isolates (34%) were CA. The% susceptible are displayed by region:

	NA (N = 139)	LA (N = 64)	EU (N = 209)	A/T (N = 147)
Imipenem	37	88	78	81
Mercpenern	39	91	83	85
Ceftazidime	33	89	79	75
Cefepime	34	89	80	79
Tiperacillin-Taxobactam	38	98	91	89
Tooramycin	39	38	82	80
Amikacin	98	94	83	85
Letrofloxacin	76	59	78	83
Ciprofloxacin	77	57	77	82

Conclusion: *P. aeruginosa* appears to be a relatively infrequent pathogen in IAI. Resistance rates among *P. aeruginosa* causing IAI exceeded 10% for many antibiotics. In NA & LA, fluoroquinolone resistance was more prevalent than resistance to antipseudomonal cephalosporins, Group II carbapenems, piperacillin-tazobactam, & aminoglycosides.

P1831

Multi-resistance in *P. aeruginosa* : epidemiological results from the GENARS Project

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Objectives: Natural resistance of *P. aeruginosa* to many antibiotics reduces the usefulness of many drugs for treatment of infection with *P. aeruginosa* . Most statistics on resistance report only resistance to specific drugs. However, more important is the knowledge of the epidemiology of multi-resistant strains, because these usually cause difficulties in antibiotic treatment. The GENARS-project (German Network for Antimicrobial Resistance Surveillance) is designed to provide epidemiological data for German university hospitals. Since 2002 resistance data are collected for all clinical relevant pathogens.
Methods: Analysis was based on first isolates of *P. aeruginosa* from six laboratories, collected from January 2002 to June 2004. Minimal inhibitory concentrations (MICs) were determined by broth micro dilution method (DIN) for ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MER) and piperacillin (PIP). Resistance patterns were evaluated by using breakpoints according to DIN, grouping resistant and intermediate as non-susceptible; multi-drug resistance was defined as non-susceptibility to at least four of the five agents.
Results: A total of 6,150 isolates was analysed. 82% of these isolates were non-susceptible to at least one agent: The most common pattern was a mono-drug resistance to GEN (34.5%) followed by co-resistance to GEN and PIP (14.3%). 9.8% of the isolates were classified as multi-resistant, 3.2% were resistant to all five class representatives. Significant differences in multi-drug resistance rates were associated with ward type with highest rates for ICU-patients (16.9%). Higher-than-average rates of multi-resistant strains were observed in departments for cystic fibrosis (14.2%) as well as in surgical departments (12.1%). Furthermore, multi-resistance rates varied significantly between the centers involved with a range from 6.1% to 14.9%. For one centre the distribution of resistance patterns over time revealed an outbreak for one phenotype (GEN/PIP/CIP/MER).
Conclusions: The relevance of multi-resistance in *P. aeruginosa* as a major clinical problem is proved by an overall rate of almost ten per cent for German university hospitals and an even higher proportion for ICU-patients. Among the agents tested gentamicin plays an eminent role in regard to its mono-resistant rate as well as a component of the most frequent resistance patterns.

P1832

Occurrence of meropenem-resistant *Pseudomonas aeruginosa* in a university hospital

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Objectives: The aim of this study is to asses the antimicrobial susceptibility of *Pseudomonas aeruginosa* clinical isolates and to determine carbapenemases producing strains in MMA, Sofia.
Methods: A total of 346 Gram negative nonfermentors isolates was investigated by Mini API System (BioMerieux, France). Sixty five multidrug resistant *Pseudomonas aeruginosa* from hospitalised patients during the last one year were analysed. The MICs of meropenem (MP) and eight antimicrobials were determined by E-test (AB, Biodisk, Sweden) as follow: cefepime, cefpirome, ceftazidime, ciprofloxacin, piperacillin/tazobactam, gentamicin, tobramycin, cefoperazone/sulbactam. Detection of metallo-beta lactamases (MBL) was performed using E-test 'double-ended' strip, loaded with imipenem (IMP) and IMP/EDTA.
Results: MICs of MP were ranged from 0.25–32 mg/l. In *Pseudomonas aeruginosa* isolates 18 (5.2%) of total 65 showed positive result of E-test MBL. The majority of them originated from ICU. The MBL-producers were more resistant than non-producers to gentamicin, piperacillin/tazobactam and ciprofloxacin: 89%, 72%, 97,2% and 67%, 38,6%, 56,3%. Both groups showed similar low resistant rate to cefoperazone/sulbactam. Isolates were most often recovered from respiratory tract (47.9%), urine (31.6%) and blood culture (5.1%).
Conclusions: This first in our country prevalence study confirm the appearance and increasing occurrence if carbapenem resistant *Pseudomonas aeruginosa* and suggests the further investigations of type and epidemiology of isolates.

P1833

Pseudomonas aeruginosa resistance: audit in a tertiary intensive care unit

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Objectives: To determine the rate of antibiotic resistant *Pseudomonas aeruginosa* from various isolates, and to find possible relationship between duration of hospital stay and development of multidrug resistant (MDR) *Pseudomonas aeruginosa*.
Methods: Prospective analyses of all microbiological isolates from 61 adult patients hospitalized in tertiary intensive care unit more than 10 days. Following samples have been taken: endotracheal tube/tracheal cannula aspirate, BAL samples, blood culture, wound isolate, urine and intravenous line *central and peripheral samples. Forty two (68.8%) patients were intubated/tracheostomized more than two weeks. Data of previous antibiotic therapies were noted as well.
Results: 61 patient ages from 19 to 79 years (41 male, 20 female) hospitalized more than 10 days in intensive care unit were followed regarding incidence of *Pseudomonas aeruginosa* isolation, and its sensitivity/resistance to antibiotics, considering length of stay in ICU (average stay: 22.4 days). *Pseudomonas aeruginosa* was isolated in 31(50.8%) patients, most of them from tracheal aspirate (28 patients). In 10 patients (6.1%) *Pseudomonas aeruginosa* was isolated from both, tracheal aspirate and wound samples. Resistance during appropriate antibiotic therapy (gentamycin, cephtriaxon) developed in 14 patients (45%) after 10 days of treatment. In 9 patients (29%) after 20 days of hospitalization in ICU resistance to amikacin therapy developed. Multiresistant *Pseudomonas aeruginosa* (MDR) has been isolated in 5 patients (16.1%), after 20 days of stay, where resistance developed during therapy (cephalosporines I,II,III generation, aminoglycosides, chynolones, cephtazidim). They were only sensitive to carbapenemes. Overall mortality in a study group was 47.5%.
Conclusions: Development of multidrug resistance (MDR *Pseudomonas aeruginosa*) during therapy in intensive care unit is a real possibility. Appropriate microbiological monitoring and changes of antibiotics regime are suggested in order to prevent serious complications caused by this germ.

P1834

The risk factors for imipenem-resistant *Pseudomonas aeruginosa* in the burn unit

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Objective: This study was conducted to determine the risk factors for acquisition of imipenem-resistant *Pseudomonas aeruginosa* in the burn unit.
Methods: The patients hospitalized in the burn unit from July 2003 to November 2004 were included in this study. The features of the patients with isolated imipenem-resistant *P.aeruginosa* (IRPA) were compared with those of patients with isolated imipenem-susceptible *P. aeruginosa* (ISPA). Demographic features, greatness of burn surface, burn deepness, antimicrobials used previously and presence of other microoorganism included risk factors analysis. T test, chi-square test and logistic regression analysis were used for statistics.
Results: ISPA was recovered from 137 patients, and IRPA was recovered from 40 patients in this period. There were no relation between age, gender, greatness of burn surface, burn depthness with aquisition of IRPA. Hospitalization time before isolation ($p = 0.12$), previous carbapenem use ($p = 0.000$), broad-spectra antibiotics use ($p = 0.000$), and previous presence of ISPA ($p = 0.002$) were associated with aquistion of IRPA.
Conclusion: Long hospitalization time, previous imipenem/meropenem use, previous broad-spectra antibiotics use and presence of ISPA previous were associated with aquistion of IRPA in the burn unit. For decresing incidence of IRPA infections the usage of broad-spectra antibiotics and especially carbapenem should be restricted, and if possible hospitalization time should be short.

P1835

Genotypic diversity and virulence parameters variation within samples of nosocomial populations of *Pseudomonas aeruginosa*

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Objectives: The objective of this study was to evaluate the differences in genotypic diversity between sources and among several virulence parameters, namely adhesion to polystyrene, hexadecane and silicone, initial biofilm formation and motility. A total of 96 strains of *P. aeruginosa* collected at a Portuguese Central Hospital and isolated from 5 different sources were examined: Urine (U), Bronchial Secretion (BS), Catheter (C), Exudate (E) and Reference Strains (R).
Methods: A combination of two genomic typing systems, the minisatellite-primed PCR (MSP-PCR) and the enterobacterial repetitive consensus sequence PCR (ERIC-PCR), was used to discriminate the 96 strains. The data were analysed using BioNumerics software. Bacterial adhesion (2 h) and initial biofilm formation (6 h) were studied by growing bacteria in LB in a modified microtiter-plate assay. Strains were screened for their capacity to adhere to hydrophobic biomaterials such as silicone and hexadecane using a biphasic separation method. The opportunistic *P.aeruginosa* were also screened for their capacity to swim (flagella) and twitch (pili). All tests were run in triplicate. For quantitative comparisons of genotypic diversity among samples, Stoddart's and Simpson's indices were used.
Results: Our results show that genotypic groups including several sources and clusters of source-specific genotypes are dispersed throughout the phenogram, regardless of isolation source. In 96 strains, 59 (61%) correspond to genotypic groups that include strains from all 5 sources; the other genotype groups were shared by strains from 2 to 4 of the 5 sources and accounted for a total of 28 isolates (29%); the remaining 9 isolates (9%) were included in genotypes with only one source (BS or U or R). Both diversity measures yielded similar results; the population from R and BS had the highest diversity. In general, all the pairwise differences in genotypic diversity between sources were not statistically significant ($P > 0.05$), except the pairwise difference between BS and C sources. None of the pairwise differences in all the parameters tested between sources were statistically significant ($P > 0.05$).
Conclusions: There were no significant differences in genotypic diversity among samples from the 5 different sources. Strains from each of the 5 sources exhibited wide variations in the virulence parameters tested.
Acknowledgments: This study was supported by a research grant from Fundação Calouste Gulbenkian.

P1836

Inter-laboratory comparison of the results of RAPD typing of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*

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Objectives: Macrorestriction (i.e. PFGE) is still the gold-standard in molecular typing, and sequencing-based methods are

applied increasingly. Nevertheless, due to the ease and speed of performance, PCR-based typing methods (e.g. random amplification of polymorphic DNA, RAPD) are widely used. However, the value RAPD is thought to be limited by a lack of reproducibility. The aim of this study was to assess the inter-laboratory concordance of RAPD results.

Methods: Each participating laboratory colleted 30 clinical isolates of *P. aeruginosa* , *A. baumannii*, (including 18 outbreak-isolates from Vienna), and *S. maltophilia*, respectively. For DNA extraction, colonies were suspended in lysis buffer containing 0.05 M NaOH and 0.25% (vol./vol.) SDS and incubated at 95°C for 15 min. The isolates were subjected to RAPD using the previously described primers P15 (5′-AAT GGC GCA G-3′) and 272 (5′-AGC GGG CCA A-3′) according to a standardized protocol (using Ready-to-Go™ PCR beads; Amersham) in the collecting laboratory. Subsequently, the sets of isolates were exchanged and typed in the other laboratory (and vice versa). Finally, the results of RAPD typing were analysed by the Bio Numerics™ software (Bio-Maths) by the use of dendrograms derived from the Dice coefficient-unweighted pair group method with arithmetic averages (UPGMA). Automated ribotyping was performed as reference method.

Results: In none of the six series of isolates the RAPD results were completely concordant. The median difference in the strains identified as unique genotypes was 3.5 isolates. Overall there was no difference regarding the discriminatory power of the assays performed in the different laboratories (median Simpson's index of diversity: 0.647 vs. 0.64) or using the different primers (median SID: 0.604 vs. 0.641).

Conclusion: These results are not in favour of the use of RAPD for large-scale epidemiological investigations. Several parameters in addition to those which were standardized in the present study have to be optimized in order to achieve stable RAPD results in different laboratories. With regard to the strict adherence to the predefined protocol in this study, we speculate that the quality of DNA extraction (i.e. the concentration of the DNA solution subjected to PCR) and the processing of the electronic version of the gels (with regard to brightness and contrast), which were essentially dependent on the individual researchers, are most important in this context.

P1837

Outbreak of nosocomial bacteraemia due to *Burkholderia cepacia* associated with contamined soap

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Objectives: To describe an outbreak of nosocomial bacteremias due to *Burkholderia cepacia* among low risk patients, in a 400 bed teaching hospital, associated with contaminated soap.

Methods: Recovery of historical incidence of blood culture positivity due to *B. cepacia*, retrospective analysis of medical and nursery charts, and a review of literature were done. In agreement to these findings, samples of antiseptic solutions were cultured, and a pulsed-field gel electrophoresis (PFGE) was done.

Results: The analysis of historical endemicity curve of bacteraemia due to *B. cepacia* revealed an outbreak scenario, with 5 cases per month between June and July-2004, comparing with a incidence of 0.8 cases/month in the last six months of 2003. Patients didn't have any known risk factor for *B. cepacia* infection, like cystic fibrosis, use of broad-spectrum antimicrobials or central venous catheter. Furthermore, the outbreak occurred in low risk clinical, surgical and paediatric wards, all of them using soap for hand hygiene; but not in high risk wards like bone marrow transplantation unit, that routinely uses chlorhexidine for this purpose. *B. cepacia* grew in cultures of soap dispenser for health care workers use from several units, but not in cultures of others antiseptic solutions, like alcohol and iodine. Genotypically identical strains of *B. cepacia* were identified from blood samples and soap dispenser by PFGE. Only one blood isolate from a patient with pulmonary neoplasm, and another sample of maxilar sinus puncture in an allogeneic bone marrow transplantation recipient with community acquired sinusitis were genotypically distincts. Prompt cleansing and disinfecting of all soap dispensers and continuous education of health care workers provided no new cases of *B. cepacia* bacteraemia in the further 3 months.

Conclusion: It's known that *B. cepacia* can degrade parabens, the stabilizing agents of a variety of soaps. Soap contamination was the source of this outbreak, probably by association with poor adherence of health care workers to standard precautions and by absence of routine cleansing and disinfecting of soap dispensers, emphasizing the importance of continuous education of these professionals.

P1838

The virulent properties of nosocomial strains of *Burkholderia cepacia* complex, isolated from patients of Moscow hospitals

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Objective: The development of the complex microbiological approach for the indication of the source of hospital infection, caused by *Burkholderia cepacia* complex strains.

Materials and methods: We have studied hospital strains from Moscow clinics. Different phenotypic and genotypic methods were used. We used *Burkholderia cepacia* selective agar, commercial bacterial identification systems ('Biomerje', 'Crystal'). Antibiotic resistance was studied by method of series dilutions. Genotypic methods included specific PCR for various genomovars (I-IX), RAPD-PCR, PCR for the identification of epidemic markers: cbl-gene, *Burkholderia cepacia* epidemic strain marker (BCESM), IS-hybrid marker, and the PCR for the indication cepI and cepR genes, the components of 'Quorum sensing' system, which regulated the production of pathogen factors.

Results: Phenotypic methods allowed us to differentiate Russian strains and to determine these strains to the group of three genomovars (I, III, IV). The comparison of the results of determination of antibiotic resistance did not show the clonal character of examined strains, but permitted to discover the growth of antibiotic resistance during last years. The application of molecular methods permitted to discriminate exactly Russian strains of genomovar III to the group IIIA. We revealed by typing strains with RAPD-PCR, that strains, which circulated in Moscow hospitals, divided into nature strains in concrete hospital and strains common for all Moscow hospitals. The epidemic significance of strains was confirmed by positive PCR for epidemic markers. The isolates of strains had different combinations of epidemic markers, which were specific for each hospital. Identification of cepI and cepR genes in strains of our collection permitted us to acknowledge the epidemic significance of strains, which had a potential ability due to regulatory system 'Quorum sensing' to source the infection and to persist in organs of patients. The presence of cepR gene in all strains and the absence of cepI gene among 33% of strains allowed us to suggest, that for some strains it was necessary to exist in the

presence of another bacteria , which had a full 'Quorum sensing' system. In conclusion, the complex microbiological approach with the using of the set of phenotypic and molecular methods will give the opportunity to identify more exact the source of hospital infection, caused by bacteria of *Burkholderia cepacia* complex.

P1839

Epidemiological features of nosocomial *Stenotrophomonas maltophilia pseudobacteraemia*

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Objectives: *Stenotrophomonas maltophilia* is an important nosocomial pathogen responsible for serious infections. It is associated with high morbidity and mortality especially in immunocompromised patients. It can also be a contaminant or member of the endogenous flora of hospitalized patients. In our hospital, *S.maltophilia* was recovered from the blood cultures of 7 patients hospitalized in the same Internal Medicine Ward, during a period of 40 days. We present here the clinical, microbiological and epidemiological characteristics of this outbreak.
Methods: Identification of the microorganisms was performed by the VITEK II system (Biomerieux-France). Susceptibility to antibiotics was assessed by both the disk diffusion method and MIC breakpoints determination by the VITEK II system. Molecular typing was done by the ERIC-II PCR method. The epidemiological investigation included the review of the medical records of the respective patients, as well as of the medical and nursing practices in the ward. Surveillance cultures from surfaces and also from hospital personnel (hand and throat swabs) working in the specific ward were also performed.
Results: All strains were multiresistant and shared the same phenotype being sensitive to quinolones, ticarcillin-clavulanate and piperacillin-tazobactam and resistant to all other groups of antibiotics tested. Molecular analysis showed that all *S.maltophilia* strains had identical patterns. None of the 7 patients had fever or other signs of infection during the respective period. Moreover clearance of bacteraemia occured spontaneously and without any specific treatment in all cases. All surveillance cultures were negative. The epidemiological investigation revealed that a particular povidone-iodine solution was used on all infected patients used during the process of blood culturing. Although the solution was not available for culturing, it was concluded that all data are indicative not of a real outbreak but of a pseudo outbreak, due to the contamination of blood cultures from the solution.
Conclusions: Pseudo outbreaks due to contaminated antiseptic solutions should be suspected in the event of clustering of positive cultures without the respective clinical signs of infection.

P1840

Increasing incidence of *S. maltophilia* in bacteraemic patients

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Objectives: *S. maltophilia* has been recognized as a serious bacteraemia-associated microorganism. The predisposing factors include prolonged hospitalization, use of broad-spectrum antimicrobial chemotherapy, central iv catheters, neutropenia and malignancies. The mortality rate is high, rising up to 21%–69%. The aim of our study was to investigate the increasing incidence of *S. maltophilia* in blood cultures, of 'Sotiria' Hospital patients.
Methods: In the period 1998–2004, 88 *S. maltophilia* strains were isolated from blood cultures of patients hospitalized in 'Sotiria' Chest Diseases Hospital of Athens. Blood cultures were performed using the automated system Bactec 9240 (Becton–Dickinson). The automated system Vitek2 (Biomerieux) and Api20NE were used for identification, while susceptibility testing was performed by the disk diffusion method, following standard guidelines. Molecular typing was performed by PFGE.
Results: In total of all Gram(-) bacteria isolated from blood cultures, *S. maltophilia* isolation frequency increased from 2.5% in 1998, up to 11.0% in 2004. Most effective antibiotics proved to be co-trimoxazole (95.8%), ciprofloxacin (85.2%) and ticarcillin/clavulanic acid (61.4%). PFGE showed some variability in molecular profiles among strains, although in the total number of isolates, a particular clone was predominating.
Conclusions: The increasing incidence of *S. maltophilia* in systemic hospital acquired infections, should be alerting for both microbiologists and clinicians.

P1841

Stenotrophomonas maltophilia and antibiotic use in German intensive care units: data from project SARI (Surveillance of antimicrobial use and antimicrobial resistance in German ICUs)

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Objectives: To analyze antimicrobial use as a risk factor for the selection of an emerging multidrug-resistant nosocomial pathogen, by correlation of antimicrobial use and the percentage of *S. maltophilia* isolated in intensive care units.
Methods: SARI is a prospective unit and laboratory based surveillance system which collects data on the 13 most important pathogens responsible for nosocomial infections. Isolates are non duplicate and are not differentiated according to whether they have caused infection or colonisation. The percentage of *S. maltophilia* among these 13 pathogens was calculated if ICUs reported more than 50 isolates per year; the data was subsequently correlated with antibiotic use density (AD) calculated in DDDs/1000 patient days.
Results: From 2/2000–06/2004, 38 out of 40 ICUs providing more than 50 isolates per annum were included in the analysis. The data cover a total of 52.864 isolates (the number of isolates reported yearly ranged from 51–1098, with a mean of 341) and 697,976 DDDs (yearly AD ranges from 496.8–2764.0 with a mean of 1274.1). Of the total number of pathogens reported yearly, the proportion of *S. maltophilia* ranged from 0.0% to 13.1% with a mean of 2.5%. Calculation of yearly antibiotic use and the yearly proportion of *S. maltophilia* among the total number of isolates reported showed a significant positive correlation with the use of carbapenems (correlation coefficient (cc) 0.23; $p = 0.004$), quinolones ($cc = 0.24$; $p = 0.003$), ceftazidime ($cc = 0.44$; $p = 0.000$), glycopeptides ($cc = 0.26$; $p = 0.001$) and aminoglycosides ($cc = 0.20$; $p = 0.012$).
Conclusion: *S. maltophilia* displays intrinsic resistance to many antibiotics. Thus, prudence should be exercised in the use of broad spectrum and reserve antibiotics because a significant association and a $cc > 0.2$ are observed between the use of carbapenems, quinolones, ceftazidime and glycopeptides and isolation of *S. maltophilia* in ICUs.

P1842

Comamonas testosteroni: an emerging nosocomial pathogen?

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Objectives: *Comamonas testosteroni*, formerly known as *Pseudomonas testosteroni*, is a non-fermenting, gram(-), oxidase(+) bacterium with a wide geographic distribution in water and soil, and a little apparent capacity of causing human infections. We present two cases of *Com. testosteroni* bacteraemia, occurred to our hospital within a month's period.
Methods: Patient (A) was a 76-years old lady, admitted to 'Sotiria' Chest Diseases Hospital of Athens, in a poor condition, with recurrent acute abdominal pain, low-grade fever, up to 38°C and muscular weakness. She had a history of COPD and biliary colic, but free from any other serious underlying disease. During her hospitalization, her condition was progressively deteriorating and a diagnostic laparotomy was performed, with no significant results. A week later, although no accurate diagnosis was established, she developed a septic syndrome. A triple scheme of antibiotic chemotherapy was initiated, but unfortunately, the patient passed away. The blood cultures developed *Com. testosteroni*. Patient (B) was a 75-years old female patient, admitted for an acute skeletal back pain. She had a history of cardiac failure and cardiac valve insufficiency. She also reported COPD and recent episodes of biliary colic pain. Five days after admission, she developed low-grade fever and blood samples were taken for culture. The laboratory findings were indicative for infection, with raised CRP, WBCs and SR. She was started on tazobactam 5.2 (1×3), with complete remission of symptoms. The blood culture developed *Com. testosteroni*. The patient was discharged in a good condition.*Com. testosteroni* was identified by Api20NE system and tested for susceptibility by Kirby–Bauer disk diffusion method. PFGE revealed a high similarity level in molecular profile, of both isolates.
Conclusions: Both infections were characterized as nosocomial. The source of infection was not identified, although in literature, intra-abdominal abnormalities are reported to be associated with *Com. testosteroni* infections. These cases should alert microbiology laboratories for uncommon bacteraemia-causing nosocomial pathogens.

Immunology, host defences, immunotherapy

P1843

Comparison of the virus neutralisation test (VNT), ELISA and dot immunoassay (DIA) for detection of mumps antibodies

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Objective: Although mumps is a transmissible benign infection, it is believed to be responsible for viral meningitis as the second causative agent after entroviruses. Rapid laboratory diagnoses are required to detect viral infection.
Method: Hooshino strain of the virus was cultured in Vero cell and its titre was determined. The VNT was carried out using 100TCID50 of the virus. An indirect ELISA test was developed using the plates coated with the purified virus. The DIA test was developed with a proper quality of viral antigen attached to nitrocellulose paper followed by adding serum samples and HRP conjugated antihuman. The results were justified on the basis of brown dots appearing in the field of white paper.
Results: The sensitivity and specificity of the DIA test in 400 serum samples compared to VNT was 92.69 and 80.80 percent, respectively. The results were almost similar to those of obtained with ELISA test with 90.36% sensitivity and 81.80% specificity.
Conclusion: With respect to some notable features such as feasibility, requiring minute amount of serum and the intrinsic validity of the test ELISA can be replaced with DIA.

P1844

Evaluation of serological methods for the diagnosis of *echinococciasis*

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The *echinococciasis* (e.) is a world wide parasite zoonosos. The diagnosis is based on clinical and radiological findings and is confirmed with serological techniques which detect the specific antibodies that are produced from the presence of active echinococcus cyst. Aim of the study is the comparison between indirect hemagglutination (IHA) and ELISA-IgG in the diagnosis of e.
Material and Method: We study 40-serum samples of patients with e. The diagnosis of e. was detected with clinical and radiological findings. The control group was 10 patients with other parasite infections and 20 healthy people. The examination was done with the IHA method and with ELISA. Positive were considered the titres of IHA >1/320 and for ELISA-IgG (15 U/ml
Results: The specific antibodies of e. were observed in 37 of 40 patients with ELISA method and in 38 of 40 patients with IHA. The sensitivity of ELISA (92.5%) and of IHA (95%) and the specificity of ELISA (90%) and of IHA (96.7%).
Conclusions: The identification of antibodies with IHA seems to be more sensitive and more specific than ELISA-IgG. The combination of two serological methods seem to show a better result in e. noting that none of these methods can test 100% positive in all patients of e.
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P1845

Leptospirosis: incidence in an Athens hospital

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Leptospirosis is a zoonosos which is caused by *L. Interrogans* (L.i.) and is transmitted by direct or indirect contact from water

contaminated by rodents urine. The incidence of the disease is higher in areas where there is standing water or increase rainfall. We examined 130 samples from different Departments of our Hospital from November 2001 to October 2002 for Leptospirosis. 8 (6.1%) patients were positive. From November 2002 to October 2003 205 patients were examined for Leptospirosis and 23 (11.2%) were positive. Two methods were applied: (a) The identification of L.i. in urine sample with microscopical examination (b) The identification of IgM antibodies in serum with ELISA. In our Laboratory we observed an increase in the demand for L.i. (59.2%) and an increase of positive samples to the amount of 5.0%. For the years 2001–2002 the 91.3% of positive patients came from rural areas whereas the 2002–2003 70.4% of positive patients came from rural areas and 29.6% from whole area of Attiki. The increase of this incidence must be due to the higher percentage of rainfall for the years 2002–2003 as well as the increase in roadworks. The sewerage rodents are now coming into contact with the citizens of Attiki resulting an increase in the incidence of L.i. in this population.
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P1846

Three cases of *anicteric leptospirosis* from Turkey: mild to severe complications

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Objective: Leptospirosis is a worldwide zoonosis and among patients ill with leptospirosis, 90% have the milder anicteric form of the disease. Presumptive diagnosis may be based on the microscopic agglutination test used for determination of antibody titre and tentative identification of serotype. Three cases with positive Leptospira microagglutination titre (MAT) to *L australis* var bratislava and positive urinary IFAT are presented here.
Case 1: A 71-year-old male patient living in rural area admitted with fever, nausea, myalgia, coughing and diarrhoea. Laboratory studies revealed decreased platelet count (60000/mm^3), increased blood urea, creatinine, AST and ALT. Ceftriaxone was started with the diagnosis of pneumonia. MAT was positive at 1:400. After 2 weeks, the patient was discharged with no evidence of his illness.
Case 2: A 55-year-old female patient living in rural area admitted with fever, headache, nausea, vomiting, nuchal rigidity and lumbar pain. Laboratory studies revealed decreased platelet count (44000/mm^3), and increased blood urea, creatinine, AST, ALT, and CPK. CSF analysis revealed pleocytosis with 140/mm^3 and normal levels of glucose and protein. MAT was positive at 1:400. The patient transferred to the intensive care unit and with deteriorated respiratory and neurological findings she died on the third day of admission despite ceftriaxone therapy.
Case 3: A 32-year-old female patient with a history of travel to a rural area presented with fever, headache, nausea, vomiting, nuchal rigidity, artralgia and myalgia. Laboratory studies revealed the platelet count of 90000/mm^3 and increased blood urea, creatinine, ALT and CPK levels. CSF analysis revealed pleocytosis with 180/mm^3 and normal levels of glucose and protein. Combination therapy with ceftriaxone and acyclovir was started. MAT was positive at 1:200. The patient was discharged from the hospital without any complication on the tenth day of admission.
Conclusion: Our first case admitted with clinical presentation of pneumonia and two cases presented with aseptic meningitis. There are reported cases of icteric leptospirosis with thrombocytopenia and acute renal failure from Turkey. The three cases presented here had thrombocytopenia and acute renal failure as complication of anicteric leptospirosis. One of the cases had fatal outcome but could not be strictly correlated with leptospirosis.

P1847

Actinobaculum schaalii infections: clinical relevance, identification and antibiotic susceptibility

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Objectives: Genus *Actinobaculum* is an example of clinical important organisms that recently have been separated from a large group of poorly-defined Gram-positive rods. We here describe the clinical pictures and bacteriologic characteristics in 9 cases of *Actinobaculum schaalii* infections.
Methods: Data were collected from the clinical records. Eight strains of *A. schaalii* were available for phenotypic and molecular characterization. From one patient presence of DNA from *A. schalii* was demonstrated in pus from kidney cysts, but without bacterial growth. The strains were characterized by conventional methods, API Coryne and API Rapid ID 32A. For molecular analysis, DNA was extracted from strains and analysed by real-time PCR of part of the 16SrRNA gene (526-bp fragment). Amplicons produced were DNA sequenced and a BLAST search in the NCBI GenBank was done. Obtained results were compared.
Results: Nine strains of *A. schaalii* were identified in three Danish departments of clinical microbiology. Six strains of *A. schaalii* were from one department of clinical microbiology covering 230,000 persons and collected over a period of six months. Except for one case the urinary tract was suspected as the focus of the infection. These eight patients were elderly and predisposed to urinary tract infections (UTI) because of either urinary tract or neurological diseases. *A. schaalii* was isolated from the blood in three cases, from the urine in six cases, from kidney cysts in one case and from an intradural abscess in one case. All patients received antibiotics and recovered from their infection. The strains were identified with 16S rRNA gene sequence analysis, but identification with the API Coryne and the Rapid ID32A test systems is also possible although the numerical codes are not yet registered in the manufacturer's database. The isolates were susceptible to a wide range of antibiotics, including b-lactam agents and showed little inter-isolate variability.
Conclusion: *A. schaalii* causes UTI and search for it is especially relevant in cases of unexplained pyuria, especially when there is divergence between the microscopic findings and the growth results under aerobic conditions. Samples, then, should be cultured on appropriate media and incubated in an anaerobic atmosphere. *A. schaalii* is easily overlooked as isolation of the organism requires 24 to 48 h of incubation in an anaerobic or CO_2 enriched atmosphere and even then is easily disregarded as a contaminant.

P1848

Chronic fatigue syndrome

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Objective: To describe frequency of clinical and epidemiological features in outpatients chronic fatigue syndrome (CFS).
Methods: A retrospective observational study was performed. A cohort of 25 patients fulfilling the Centers for Disease Control and Oxford, UK criteria (1994) for CFS was included.
Results: Eighteen patients were female (72%) and 28% male ($p < 0.001$, RR 2.6) with a mean age for women 37.5 ± 11.7 years and for male patients 38.3 ± 7.6 years. The mean time before the first consult to the Institute was 6.3 months. Prior history of *C. trachomatis* infection was related in 27.7% women and 28.6% men and allergic condition in 44.4% and 57.1% respectively. In the following table are synthesized the frequency of subjective symptoms. In all patients serology was no reactive for HBV, HCV, HIV e IgM CMV. Positive serology was found for IgG toxoplasmosis (84%), IgG EBV (80%), EA EBV (32%) and brucellosis (4%). Total immunoglobulin was increased in 20% and lowered in 24%. Multitest (7/8) showed hypo or anergy in 77.8%. Active lymphocytosis was a finding in 32% and increased liver enzymes in 24%.

Symptom	Total (%)	+++ ++ + = degreee of intensity
Fatigue	100	+++ = 76% ++ = 24% -
Reduction of previous levels of activity	100	+++ = 72% ++ = 24% + = 4%
Memory impairment	100	+++ = 24 ++ = 40% + = 36%
Muscle pain	96	+++ = 4% ++ = 20% + = 72%
Depression	96	+++ = 8% ++ = 24% + = 64%
Joint pain	92	++ = 28% + = 64%
Headache	88	++ = 20% + = 68%
Unrefreshing sleep	88	++ = 16% + = 72%
Tender lymph nodes	84	+++ = 12% ++ = 20% + = 52%
Throat discomfort	70	++ = 10% + = 60%
Nocturnal sweat	64	+++ = 12% ++ = 20% + = 32 %

Conclusions: This syndrome affects quality of life and status performance in the productive age group. There was net predominance of women. It must be noted the high incidence of prior history of infection for *C. trachomatis* when compared with general adult population (3–5%) in our country.

P1849

The importance of phagocytic index for determining the early period efficacy of treatment modalities, and its relationship with metabolic control parameters in diabetic patients with foot infections

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Objectives: Diabetic patients are more prone to infections and show high morbidity and mortality rates. Prior to the advent of modern antimicrobial therapy, infection accounted for much of the morbidity in diabetics. The aim of this study was to investigate the phagocytic activity changes of neutrophils in type 2 diabetic patients with foot infections during short treatment course and to evaluate the importance of phagocytic index for determining the efficacy of the treatment modalities and its relationship with metabolic control parameters.
Methods: The phagocytic activity of neutrophils were determined in blood samples of 38 type 2 diabetic patients with foot infections (14 women and 24 men), mean age and mean duration of diabetes were 66.3 ± 9.4 and 19.1 ± 11.2 (yrs) respectively. All patients receive standard treatment (intensive insulin therapy, antibiotherapy, hyperbaric oxygen therapy and surgical debridement). Phagocytic activity of neutrophils was determined by a standard method . The phagocytic index, i.e. the number of microbial bodies absorbed by an average leukocyte, and percentage of leukocytes that have phagocytized, i.e. the number of leukocytes out of 100 that have revealed the phagocytic activity and phagocytic index, that is the correlation between the product of phagocytic index and percentage of phagocytosis. Phagocytic activity of neutrophils, C-reactive protein and metabolic control parameter (HbA1c) were determined before the therapy and 2 weeks later.
Results: We observed significant differences in phagocytic index and CRP before and after short-course therapy ($p < 0.001$, *Wilcoxon signed ranks test). The phagocytic index (%) and CRP(mg/L) before and after 2 weeks therapy were 47.7 ± 11.4 and 62.5 ± 15.6 $p < 0.001$, 41.4 ± 36.7 and 17.4 ± 18.2 $p < 0.001$ respectively. The correlation analysis showed a significant relation between phagocytic index and CRP, HbA1c ($r = 0.52$, $p < 0.05$ and $r = -0.41$, $p < 0.05$ respectively).
Conclusions: The derangement in the carbohydrate metabolism in diabetic may be the reason for the impairment in the bactericidal ability of PMN leucocytes of poorly controlled diabetic patients making them more susceptible to recurrent infections. However the importance of phagocytic activity changes in diabetics has been little studied up to the present time. Our data show that the phagocytic activity changes during short-course therapy have a significant value for determining the efficacy of treatment in diabetic foot infections.

P1851

Diagnostic power of peripheral blood phagocyte Fcg receptors expression in bacteraemia

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Background: Early markers of bacteraemia are useful for prognosis and, in decision making for i.v. antibiotic therapy.
Objectives: To assess the diagnostic power of the surface expression of Fc receptors for IgG (FcgRs) for the prediction of bacteriemia in febrile patients.
Methods: We performed a 4 year prospective case-control study on 127 consecutive patients (pts) with an episode of bacteriemia as compared to 136 randomly selected concurrent febrile pts with negative blood cultures (control). Demographic and clinical data were collected by chart review and/or questioning their attending physicians. Plasma levels of C-reactive protein (CRP), TNFg, IL-1a, IL-6, IL-8 and IL-10 were determined. The surface expression of Fc receptors for IgG (FcgRs): FcgRI, FcgRII and FcgRIII on peripheral blood monocytes (M) and granulocytes (G) was assessed by flow cytometry. These studies were done concomitantly with blood cultures.
Results: Both groups were not different for age, sex, previous administration of immunosuppressants or antibiotics, clinical

severity index or comorbid conditions. In univariate analysis, cases had significantly higher levels of CRP ($p < 0.001$), TNFg ($p < 0.001$), IL-1a ($p < 0.001$) and IL-6 ($p < 0.01$) than controls. The expression of FcgRIIA and FcgRIII by M and, that of FcgRI on G was significantly enhanced ($p < 0.001$) in bacteriemic patients as compared to culture-negative febrile pts; while the expression of FcgRIIB by either M or G was significantly decreased ($p < 0.03$). Setting a cut-off value = 25% of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence of bacteriemia of 5–10% among hospitalized patients undergoing blood cultures, results in a sensitivity, specificity, positive and, negative predictive values of: 77%, 97%, 74%, and 98%, respectively for M-FcgRIIA, 73%, 96%, 74% and 97%, respectively for M-FcgRIII, 58%, 93%, 49% and 95%, respectively for G-FcgRI and, 71%, 91%, 57% and 83%, respectively for G-FcgRIIB.

Conclusions: Our results suggest that the surface expression of Fc receptors for IgG on peripheral blood monocytes and granulocytes may help clinicians to rule out bacteraemia in febrile patients.

P1852

The influence of mixed staphylococcal populations on the activity of phagocytes

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Objectives: *Staphylococcus aureus* invades the hosts from the skin or mucosa, which are always colonized with commensal microorganisms including *S. epidermidis*. Thus at an early stage of *S. aureus* invasion polymicrobial rather than monomicrobial infection occurs. If so, the phagocytes, which are a key component of innate immunity, interact with the mixed bacterial populations. We hypothesised that the interaction of the phagocytes with a mixture of staphylococci may differ from their interaction with homogeneous populations of these bacteria.

Methods: A clinical *S. aureus* B1 strain, a parental *S. aureus* 8325-4, alpha-toxin negative mutant *S. aureus* DU 1090 and *S. epidermidis* RP12 were used for the study. The mouse granulocytes and macrophages were infected with the bacteria of each strain separately or with the mixture of two bacterial strains. FITC-labelled staphylococci were prepared to determine their adherence to the phagocytes. To estimate the ingestion and intracellular killing of these bacteria a colony forming units-assay was used. The production of IL-12 and TNF-alpha by phagocytes infected with different cultures of staphylococci was measured with DuoSet ELISA.

Results: For all strains of *S. aureus*, irrespective of the tested pair of bacteria and type of phagocytes, their adhesive properties were stronger in mixed populations than in unispecies. On the other hand, the adherence of *S. epidermidis* to the phagocytes in the presence of each *S. aureus* strain was reduced. In the phagocytic cells infected with any mixed population of staphylococci the bacterial uptake and intracellular killing were diminished as compared with the cells infected with homogeneous population of these bacteria. The production of IL-12 was below detection limit, even after 2 hours incubation of bacteria with phagocytes. The level of TNF-alpha depended on the time of incubation of the cells with bacteria, but the stimulatory effect of homo- and heterogeneous cultures of staphylococci was similar.

Conclusions: Our data suggest that phagocytes can discriminate between various strains of staphylococci. The differences in the process of phagocytosis of congeneric and mixed staphylococcal populations could also result from the intercellular bacterial communication. Collectively, our data contribute to a better understanding of the role of commensal bacteria in the interaction of the phagocytes with bacterial pathogens. Supported by Grant No. 3PO4C08124 (KBN).

P1853

Increased monocyte apoptosis correlates with improved survival in patients with ventilator-associated pneumonia and sepsis

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Objective: Monocytes constitute the main cells eliciting enormous responses of proinflammatory cytokines with an important role in the pathogenesis of sepsis. Little evidence exists on the apoptotic profile of monocytes in septic patients.

Methods: Fifty-four patients with sepsis (according to ACCP/SCCM 1992 criteria) and ventilator associated pneumonia (VAP) were enrolled in the study. Peripheral blood monocytes were isolated each second day after advent of symptoms of sepsis after density gradient centrifugation of heparinized venous blood over Ficoll and incubation for one hour in RPMI supplemented with 10% FBS and 2 mM glutamine. Non-adherent cells were discarded. Isolated monocytes were incubated with Annexin V conjugated with FITC and Propidium iodide (PI). Monocytes labeled with Annexin V but not PI, as determined by flow cytometry, were considered apoptotic, while monocytes labeled by both Annexin V and PI were considered necrotic. Apoptosis was expressed as percentage of apoptotic monocytes and comparisons were undertaken between survivors and non-survivors after follow-up for 28 days.

Results: The mean ± SE percentage of apoptotic monocytes of days 1, 3, 5 ad 7 of survivors was 60.9 ± 6.53, 44.02 ± 7.52, 43.89 ± 7.53 and 43.79 ± 9.74% respectively. Respective value in non-survivors were 34.21 ± 8.24 (p 0.029 compared to survivors), 34.26 ± 10.90 (pNS), 22.79 ± 5.17 (pNS) and 27.82 ± 7.78% (pNS).

Conclusions: Sepsis is a process attributed to an exaggerated systemic inflammatory host response, orchestrated mainly by cells of the monocytic system by the production of a wide array of pro-inflammatory cytokines. A significantly increased rate of monocytic apoptosis was correlated to survival in patients with sepsis caused by VAP. These findings indicate a beneficial effect of apoptosis of monocytes possibly due to a resulting attenuated inflammatory response.

P1854

Role of gene polymorphisms of interleukin-10 and CD14 in meningococcal disease

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Objectives: Susceptibility to, and severity of meningococcal disease has been shown to be partly genetically determined. The

quality and intensity of the proinflammatory response is a critical determinant of this infection. Interleukin-10 is an anti-inflammatory cytokine that is strongly associated with outcome of meningococcal disease. It has numerous functional single nucleotide polymorphisms (SNP) including a promoter SNP at 819– nucleotides relative to the transcriptional start site (IL10[–819]). CD14 is involved in the cellular recognition of lipopolysaccharide; it has a functional promoter SNP at –159 (CD14[–159]. We genotyped these two markers in a cohort of 1096 patients with microbiologically proven meningococcal disease presenting in England & Wales in the period July 2000 – November 2003, and 744 controls derived from anonymised northern English blood donors.

Methods: In both groups DNA was genotyped at IL10[–819] and CD14[–159] using the TaqMan allelic discrimination test.

Results: The genotyping results for both groups are shown in the following table. Genotype is denoted 11 (homozygote for the common allele), 22 (homozygote for the rare allele) and 12 (heterozygote). There were no significant differences between the genotype frequencies in disease and control groups, or related to disease severity in this study for either IL10[–819]or CD14[–159]. However there was a trend for association of death with carriage of the rare allele at IL10[–819], (OR 1.53: [95% CI; 0.97–2.43].

Table 1. Genotyping results for IL10[-819] and CD14[-159]. Genotype is denoted 11 (homozygote for the common allele), 22 (homozygote for the rare allele) and 12 (heterozygote).

IL10[-819]	Genotype	11	12	22	Totals
Meningococcal disease	Survivors	458	309	46	813
	Deaths	37	39	5	81
Anonymised blood donors	Controls	195	105	20	320
	Totals	690	453	71	1214
CD14[-159]	Genotype	11	12	22	Totals
Meningococcal disease	Survivors	137	251	119	502
	Deaths	14	27	14	50
Anonymised blood donors	Controls	160	326	152	638
	Totals	306	599	285	1190

Conclusions: The gene polymorphisms CD14[–159] and IL10 [–819] do not have a significant relationship with acquisition or severity of meningococcal disease in this group of patients, but it is possible that haplotypes of IL10 may have some involvement.

P1855

Differences in induction of ex-vivo pro-inflammatory cytokine release between susceptible and resistant isolates of *Escherichia coli*

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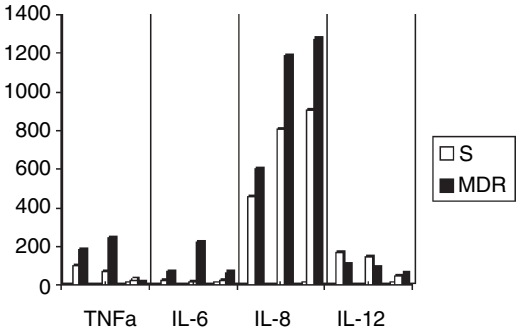
Objective: A drug resistant phenotype in isolates of *Pseudomonas aeruginosa* has been associated with decreased potential for stimulation of monocytes and attenuated virulence in experimental models. It was hypothesized that similar effects might be observed in *Escherichia coli* strains.

Method: Ten susceptible and 10 multi-drug resistant (MDR) isolates from clinical urine specimens by patients with pyelonephritis were studied. Human monocytes by a healthy volunteer were isolated. Briefly heparinized whole blood was centrifuged over Ficoll–Hypaque and the mononuclear cell fraction was incubated in RPMI supplemented with 10% FCS for 30 min. The non-adherent cells were discarded and adherent monocytes were harvested after trypsinization and resuspended in culture medium. Monocytes were triggered by an inocullum of 6 log of each *E. coli* strain for 2, 4 and 6 hours. Tumour necrosis factor alpha (TNF-a), interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 12 (IL-12) were measured by immunoassay in each supernatant.

Results: See Figure 1.



Conclusions: MDR isolates of *Escherichia coli* induce markedly increased TNF-a, IL-6 and IL-8 but not IL-12 release by human monocytes after 2 and 4 hour ex vivo stimulation compared to susceptible ones. This observation contrasts with the findings of similar studies in *P. aeruginosa*. Potential clinical implications need further study.

P1856

Cytokine profile in Mediterranean spotted fever

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Objectives: Mediterranean spotted fever (MSF) is a tick-bite rickettsiosis caused by *Rickettsia conorii*. The pathogen invades and damages the endothelial cells of the small blood vessels and gives rise to multifocal lymphohistiocyte vasculitis. The mechanism of the organism defence against rickettsiae is not fully elucidated. Similar to other diseases with obligatory intracellular pathogens, the cellular immunity is suggested to play a crucial role. Defining the cytokine profile in the early acute stage of MSF helps to elucidate certain mechanisms of the human defence against the disease.

Methods: The cytokines were analysed by ELISA with kits of BioSourse Europe S.A., Belgium. MSF was verified by IFA in the Referral Laboratory for Rickettsioses.

Results: Eighty patients with MSF were enrolled in the study. The cytokines IL-1beta, TNF-alpha, IL-6, IL-8, IL-2, IL-12, IL-10, INF-gamma are studied in the burst of the disease on the third to fourth day from the appearance of the first symptoms, just after the rash come into view. Patients show a

manifold increase in the activity of IL-6 (250.47 ± 74.26 pg/ml, $p < 0.001$), IL-8 (180.59 ± 28.68 pg/ml, $p < 0.001$) and INF-gamma (8.40 ±1.13 pg/ml, $p < 0.001$) compared with the control subjects. Statistically significant increase in the values of IL-12 (249.22 ± 21.88 pg/ml, $p < 0.001$) is also found. IL-1beta increases its activity more than 6 times (60.91 ± 13.15 pg/ml, $p < 0.001$) and TNF-alpha – about 3.5 times (22.83 ± 3.40 pg/ml, $p < 0.001$) compared with the control group. Although moderate the increase in the IL-10 level also reaches statistical significance (5.94 ± 0.83 pg/ml, $p < 0.001$), while the increase in IL-2 is short of statistical significance compared with the level in the control group (0.18 ± 0.07 pg/ml, $p > 0.05$).

Conclusion: It is postulate that the necessity of cell-mediated immune response in rickettsial infection depends particularly on the cytokine background during the induction of immune T-lymphocytes. Our study findings reveal that the culmination of MSF is characterized by a cytokine profile with prevalence of TH1 response. The elevated serum levels of the pro-inflammatory and regulatory cytokines in the acute phase of MSF probably contribute to the rickettsiae healing processes by mobilization of complex rickettsiocide biochemical and immune mechanisms.

P1857

IL-10 gene polymorphisms and susceptibility to brucellosis

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Background: *Brucella* spp. is a gram-negative facultative intracellular bacterium and causative agent of brucellosis. It is clarified that type-1 immunity is important to control *Brucella* infection. In this regard, macrophages have critical role. IL-10 is a Th2-type cytokine that inhibit macrophage activation. It is known that production of IL-10 is affected by its gene promoter polymorphisms. In this study we investigated the relationship between IL-10 gene promoter polymorphisms and susceptibility to brucellosis.

Materials and Methods: One hundred and ninety patients with brucellosis, 186 healthy individuals who were members of patients' family and 82 healthy animal husbandmen who had infected animals with *Brucella* were included in this study. All individuals were genotyped for three bi-allelic IL-10 gene promoter polymorphisms at positions (1082(G/A), (819(T/C), and (592(A/C) using PCR-RFLP.

Results: Genotype and allele frequencies of −592(A/C) and −819(T/C) were significantly different between patients and animal husbandmen groups ($P < 0.05$).

Discussion: There are some reports showed that A allele at position −592 of IL-10 gene is associated with lower IL-10 production in-vitro or in-vivo. According to the results, higher frequency of A allele at position −592 in animal husbandmen may cause these individuals more resistant to disease.

P1858

Polymorphisms of IL-10 gene promoter in patients with kala-azar

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Objectives: Visceral leishmaniasis is caused mostly by *Leishmania infantum* in south of Iran. Manifestations range from asymptomatic infection to fatal disseminated visceral disease. Protective immune response against *Leishmania* is cell-mediated immunity and it is known that IL-10 can down-regulate this kind of response. Researchers showed that polymorphisms in IL-10 gene promoter can regulate IL-10 production. The aim of this study was to determine the relationship between IL-10 gene polymorphisms and outcome of the disease.

Methods: One hundred and twenty paediatric patients involved with kala-azar, 57 healthy individuals who were patients' siblings and 102 healthy individual who lived in endemic area without any history of kala-azar or cutaneous leishmaniasis and with positive *Leishmanin* skin test were included in this study. Polymorphisms of IL-10 gene promoter (−1082G/A, (819T/C, (592A/C) were determined using PCR-RFLP.

Results: There were no significant differences in genotype and allele frequencies of investigated IL-10 gene polymorphisms between the groups.

Conclusion: It is documented that protective immunity against leishmaniasis is cell-mediated immunity. Therefore the presence of Th2-type cytokines during the disease can worsen the condition of the patients. Since the results showed no significant differences in genotype and allele distributions between the groups, study of the cytokine profiles and other cytokine gene polymorphisms are recommended.

P1859

Deoxycholate and lipid amphotericin B formulations (AMBF) regulate cytokine and chemokine expression in human monocytes

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Objectives: Innate antifungal host defense includes expression of various genes of cytokines and chemokines by phagocytes. Since amphotericin B is a microbial product, AMBF [deoxycholate amphotericin B (DAMB), lipid complex (ABLC), liposomal (LAMB), and colloidal dispersion (ABCD)] may modulate expression of such genes in MNCs.

Methods: 10×6 monocytes (MNCs) obtained from healthy donors by ficoll centrifugation/plastic adherence were incubated with 5 mg/L DAMB and 25 mg/L lipid AMBF at 37°C for 0.5, 1, 2, 6 and 22 h. Total RNA was isolated and mRNA profiles for TNF-α, IL-1β, IL-1ra, MCP-1 and MIP-1β were determined by semi-quantitative RT-PCR. Quantitation of mRNA was performed by UViDOC program. Experiments were repeated 3 times.

Results: While there was no loss of viability induced by the drug concentrations studied, there was a progressive decline of all 5 cytokine/chemokine mRNAs expressed by untreated MNCs between 0.5 and 22 h. DAMB induced a 39% increase of IL-1β as compared to untreated MNCs after short incubation (2 h). In contrast, it increased IL-1ra by 640% after 22 h, simultaneously with the return of IL-1β mRNA levels to baseline. DAMB induced maximal increases of MCP-1, MIP-1β and TNF-α at 6 h. By comparison, LAMB, ABLC and ABCD induced decreases in the IL-1β at 0.5 h by approximately 48% compared to untreated MNCs, which then returned to baseline

values. Lipid AMBF did not enhance the expression of IL-1ra within 0.5–6 h but maintained it stable as compared to decreasing mRNA levels in untreated MNCs. While MCP-1 decreased progressively in untreated cells, all AMBF maintained its mRNA levels equal to that measured in untreated cells at 0.5 h. In addition, while DAMB, LAMB and ABCD induced increased production of MIP-1β at 6–22 h, ABLC did not cause such an increase. In contrast to DAMB and ABCD that induced peak levels of TNF-α at 2 and 6 h, LAMB and ABLC did not affect TNF-α expression.
Conclusions: DAMB and ABCD induce gene expression of proinflammatory cytokines/chemokines in higher degree than ABLC and LAMB. This difference potentially may explain the differences in infusion-related reactions as well as suggesting potential modulation of antifungal host immune response due to AMBF.

P1860

A common human toll-like receptor 4 mutation is associated with increased mortality in children with invasive meningococcal disease

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Objectives: Human Toll-like receptor 4 (TLR4) is the major endotoxin-signalling receptor of the innate immune system and is required for efficient recognition of gram-negative bacterial infections. We investigated a possible association between the common TLR4 mutation Asp299Gly and mortality due to invasive meningococcal disease in children.
Methods: 197 children (ages 2–215 months, mean 59 months) with clinically and/or microbiologically proven invasive meningococcal disease from five different European countries were analysed for the Asp299Gly mutation by Lightcycler allele specific fluorescent hybridisation probe assays and direct sequencing. Genotyping results were correlated with fatal or none fatal outcome. Statistical analysis was performed applying Pearsons Chi-Square test (two-sided).
Results: The overall allele frequency of the Asp299Gly polymorphism was 9.4% (165 Asp/Asp, 27 Asp/Gly, 5 Gly/Gly) among the analysed patients with meningococcal disease. A total of 19 patients (9.6%) succumbed to the disease. Interestingly the heterozygous TLR4 Asp299Gly mutation was significantly associated with fatal disease outcome: In the none-survivor group 6 out of 19 patients (31%) had a heterozygous Asp299Gly polymorphism whereas this was only the case in 21 out of 178 patients (11.8%) in the survivor group ($p = 0.029$). There was no significant ($p = 0.459$) difference between the mutation in homozygous state in the survivor (5/178, 2.8%) and none-survivor group (0/19, 0%).
Conclusions: Our data suggest that the analysed TLR4 mutation Asp299Gly is associated with increased mortality in heterozygous state and might have an important impact on the clinical course and outcome of meningococcal disease in childhood.

P1861

Association between Manosse-binding lectin deficiency and shock in patients with acute pyelonephritis

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Background: Mannose-Binding Lectin (MBL) trigger complement activation after binding to microbial surfaces. Previous studies have demonstrated that MBL deficiency is associated with increased susceptibility to severe bacterial infections. The aim of the study is to analyze the possible association between polymorphism's of the MBL gene and the incidence of bacteraemia and shock in female patients after an index episode of acute pyelonephritis (AP) due to *E. coli*.
Methods: Blood samples from 63 patients with AP were prospectively collected. AP was defined as the presence of fever, lumbar tenderness and leukocyturia in the absence of other focus of fever. All patients had a positive *E. coli* uroculture. Six single nucleotide polymorphism's (–550 G/C, (221 C/G, 4 C/T, 52 CGT/TGT, 54 GGC/GAC and 57 GGA/GAA) in the MBL2 gene were genotyped using PCR and sequence specific primers.
Results: Mean age of the patients included in the study was 44 years (range 18–94). 27 (43%) patients developed *E. coli* bacteraemia. Comorbidity (diabetes mellitus, cirrhosis, corticosteroid treatment, renal insufficiency) was present in 14% of the non bateremic AP and in 55% of the bacteremic AP. 7 developed septic shock, 1 was a non bacteremic AP (2.7%) and 6 were bacteremic AP (22%). No significative differences in the incidence of low-producing MBL genotypes was observed in the bacteremic *E. coli* AP (7 out of 27 patients) (26%) compared with the non bacteremic AP (4 out of 36 patients) (11%) ($p = 0.18$, Fisher). 4 out of the 7 patients (57%) with septic shock had low-producing MBL genotypes while 7 out of the 56 patients (12.5%) without shock had low producing MBL genotypes and this difference was statistically significant ($p = 0.014$, Fisher). Of the 4 patients with septic shock and low producing MBL genotypes, 3 had comorbidity.
Conclusions: Our results support that MBL deficiency may predispose to septic shock in patients with bacteremic *E. coli* AP. There is a tendency towards a higher proportion of low producing MBL genotypes in bacteremic *E. coli* AP although the differences were not statistically significant.

P1862

Evaluation of treg cells in vivo and in vitro in leprosy patients

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Objective: T-cell defect is a common feature in lepromatous leprosy (LL) patients as compared to tuberculoid type (TT) patients. In this study, it was aimed to research for the role of T regulatory cells (Treg) in leprosy:40 (29 male, 11 female) inactive lepramatous leprosy patients whose mean of age 60 ± 2.28 and mean of years in diagnosis 30.66 ± 2.93 were obtained.
Method: Peripheral blood lymphocyte and peripheral blood mononuclear cells (PBMC) culture were analysed for lymphocyte subgroup and Treg cells. PBMC were stimulated in 24-well flat bottom plates with sonicated *M. leprae* extract. Cells were harvested for flow cytometric analysis on 24 hour.

Result: Mean ratio of CD4+CD25+ Treg cells in blood samples: 5.82 ± 3.11 and second ratio from culture: 10.95 ± 4.88 in percentage ($p < 0.05$). In healthy controls these ratio was found 2.85 ± 1.08 ($p < 0.05$). CD8+ cytotoxic T cells were determined higher and CD4+/CD8+ ratio lower than healthy controls ($p < 0.05$). There was no difference in CD4+ cells between patient and control group.

Conclusion: An increased surface expression of CD25 was observed when PBMC of LL (lepromatous) patients were stimulated in vitro. In PBMC culture not all CD4+CD25+ cells are Treg many of them are stimulated CD4+ cells but in patient peripheral Treg ratio was found two times higher than controls. Thus, Treg cells which produce IL-10 appears to have a little regulatory role in peripheral blood of leprosy patients.

Antibiotic resistance: nosocomial pathogens

P1863

Trends in prevalence of multidrug-resistant bacteria in patients undergoing intensive therapy

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Objectives: The analysis of change in prevalence and resistance to antibiotics of bacteria isolated from patients hospitalized in two intensive care units (ICU) of a tertiary care hospital.
Methods: The clinical specimens comprised blood, pus, sputum, bronchial aspirates, wound swabs and peritoneal swabs taken intraoperatively. An analysis comprised 1673 bacterial strains isolated over a period of two years: 861 (51.5%) strains from ICU-A and 812 (48.5%) strains from ICU-B of a university-affiliated hospital (1200 beds). Identification of the isolates were done with API and VITEK automated tests (BioMerieux), while susceptibility testing – by a disk-diffusion method according to the NCCLS recommendations.
Results: Prevalence of Gram-positive cocci in ICU-A decreased from 35.3% in 2002 to 19.1% in 2003, while in ICU-B from 27.7% to 16.4%, respectively. There was an increase in the frequency of isolation of non-fermenting Gram-negative rods (ICU-A: from 32.9% to 49.3% and in ICU-B from 41.2% to 48.8%) in the analysed period. Prevalence of Gram-negative rods of the Enterobacteriaceae family and enterococci remained stable in both ICUs. Overall a higher percentage of multi-drug resistant strains was isolated from ICU-A patients in comparison to ICU-B. An emergence of *Acinetobacter* spp. strains resistant to imipenem was recorded in ICU-A (from 3.6% in 2002 to 12.1% in 2003) and ICU-B (from 2.9% to 6.7%, respectively). There was a marked increase in HLAR strain isolation in ICU-A (from 22.7% in 2002 to 86.4% in 2003) and ICU-B (from 29.4% to 90.0%, respectively). However, frequency of methicillin-resistant *S. aureus* declined in ICU-A from 69.6% to 44.1%, while in ICU-B from 70.0% to 64.1%.
Conclusions: (1) A rise in prevalence of Gram-negative rods was observed, particularly of the non-fermenters group. (2) Overall a higher percentage of multi-drug resistant strains was isolated from ICU-A, corresponding to longer patients' mean stay in comparison to ICU-B. (3) Imipenem-resistant strains of *Acinetobacter* spp. have emerged in both ICUs. (4) Monitoring of bacterial prevalence and susceptibility patterns helps in determining optimal empiric therapy of infections in critically ill patients.

P1864

Linezolid susceptibility testing results in Europe: report from the Worldwide ZAAPS Program (2003)

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Objective: To compare and contrast susceptibility (S) testing results for linezolid (LZD) in European isolates of Gram-positive cocci (GPC) with those from the 'rest of the world' (ROW; North and South America, Asia). Results from the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program surveillance study of LZD activity initiated in 2002 worldwide (7921 strains) were compared to program results continued in 2003.
Methods: The ZAAPS Program monitors for oxazolidinone (LZD) resistance (R) in (60 medical centers worldwide, each country or site sending 200 isolates to a central laboratory for reference NCCLS testing. In 2003, a total of 8089 strains were tested, 1349 strains from Europe (7 countries; 18 sites). Interpretive criteria of the NCCLS M100-S15 (2005) were applied (LZD-R or non-S at ≥8 mg/L) for staphylococci and enterococci; and ≥4 mg/L for streptococci. LZD-R strains were studied to determine the mechanism via target site sequencing, and patient demographics were also sought.
Results: Six groups of GPC were monitored: *S. aureus* (SA), coagulase-negative staphylococci (CoNS), *Enterococcus* spp. (ENT), *S. pneumoniae* (SPN), viridans group streptococci (VgS) and beta-haemolytic streptococci (BST). The 1349 European strains were processed and results compared to ROW data, see table: Characteristics of the European collection included: (1) 26.7% MRSA; (2) 76.4% MR-CoNS; (3) 5.3% VRE, 58% vanA; (4) SPN penicillin-R and macrolide-non-S at 17.2% and 25.0%, respectively; and (5) macrolide-R BST at 29.0%. Seven LZD-R ENT had G2576U ribosomal target mutations (6 from USA, 1 from Greece; separately submitted, not in ZAAPS monitored nations). No significant changes in LZD potency by organism group or in rates of LZD-R strains were detected between 2002 and 2003 samples.

	LZD MIC (mg/L)			% S	
Organisms (no. tested)	Range	50%	90%	Europe	ROW
SA (375)	0.12-2	2	2	100.0	100.0
CoNS (263)	0.25-2	1	1	100.0	100.0
ENT (264)	0.5-2	2	2	100.0	99.2
SPN (307)	0.12-2	1	1	100.0	100.0
VgS (62)	0.25-2	1	1	100.0	100.0
BST (78)	≤0.06-1	1	1	100.0	100.0

Conclusions: LZD, as monitored by the ZAAPS Program, remained highly active against European isolates of GPC, having a potency identical to that observed in other geographic regions and in various pre-marketing trials (ZAPS). LZD-R strains (ENT) appeared in patient infections in the USA, each with established ribosomal target alternatives. Continued monitoring seems prudent, but LZD-R rates remain very low at 0.07%.

P1865

The importance of distinguishing between bacterial colonisation versus infection in assessing risk factors

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Objective: The majority of published studies use infection or clinical cultures as the outcome of interest when assessing risk

factors for antimicrobial-resistant pathogens. We hypothesize that risk factors differ between colonization and infection.
Methods: Patients with *Pseudomonas aeruginosa* (PA) bloodstream infections (BSI) were identified during a prospective surveillance study (2002–2004) in a large University hospital, and pts with and without PA rectal colonization, respectively, were identified from a cross-sectional rectal screening of 1036 hospitalized pts. Two case-control studies were performed which included 87 randomly selected pts with PA-BSI (group-1), 29 with PA positive rectal screening (group-2) and 87 randomly selected pts with negative rectal screening (group-3). Exclusion criteria for group-2 and -3 were the detection of a clinical culture positive for PA during hospitalization.
Results: Mean age ±SD of population was 58 ± 16 in group-1 and 66 ± 8 y.o. in group-2 (P = ns vs group-3) while the mean length of hospitalization before entering the study was 27 ± 44 in group-1 (P < 0.01) and 35 ± 37 in group-2 (P < 0.01). Resistance to the following drugs was detected more frequently among infecting than colonising strains: imipenem (28% vs 24%, P = ns), ciprofloxacin (55% vs 24%, P < 0.01), ceftazidime (32% vs 31%, P = ns), gentamicin (26% vs 17%, P = ns) , and piperacillin (18% vs 10%, P = ns). Multidrug resistance (2 classes of drugs) was reported in 50% of PA in group-1 vs 27% in group-2 (P = 0.04). At multivariate regression analysis, prolonged length of hospitalization (>17 days; group-1, OR = 2.8; group-2, OR = 3.7) and previous use of cephalosporins (group-1, OR = 6.9; group-2, OR = 9.3) and aminoglycosides (group-1, OR = 35.1; group-2 OR = 12.4) were independent risk factors for both PA colonisation and BSI (P < 0.05). Patients with PA-BSI had a higher Charlson score (>3; OR = 20.5) and were more likely to have had a ICU admission (OR = 5.8) and quinolones therapy within 30 days (OR = 4.3) (P < 0.01). The presence of wheel chair or bed-bound status was independently associated with PA colonisation (OR = 10.6, P < 0.01) but not with BSI.
Conclusion: This study provides novel information that may be used to better design future case-control or cohort studies analysing the epidemiology of antibiotic-resistant infections. Taking into consideration the differences between colonization or infection as the outcome of interest may reduce bias and help better design hospital interventions.

P1866
Significant increases in the rate of fluoroquinolone resistance among Gram-negative bacilli: six-year (1999–2004) report from the USA MYSTIC Programme

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Objective: To utilize the Meropenem (MEM) Yearly Susceptibility Test Information Collection (MYSTIC) Programme, a global longitudinal surveillance network of (100 medical centers, to monitor the activity of broad-spectrum agents in hospitals actively using carbapenems. Between 1999 and 2004, 10–16 USA medical centers referred 200 consecutive, non-duplicate isolates from clinical infections to a central laboratory.
Methods: 15,990 strains were submitted over 6 years. Enterobacteriaceae (ENT; 7229), *P. aeruginosa* (PSA; 2254) and *Acinetobacter* spp. (ASP; 489) strains were tested for susceptibility (S) using current NCCLS reference methods and interpretative criteria (M100-S15; 2005). Antimicrobials tested included: MEM, imipenem (IMP), ceftriaxone, ceftazidime, cefepime, aztreonam, gentamicin (GENT), tobramycin (TOB), piperacillin/tazobactam, ciprofloxacin (CIPRO), and levofloxacin (LEVO). Strains demonstrating multi-drug R (MDR) from the same site were further characterized by automated ribotyping and PFGE to determine clonality.
Results: The rates of CIPRO R within ENT increased from 3.7% in 1999 to 12.9% in 2004 (range, 0.0–4.3%; average 1.8% increase/year). The greatest increases in CIPRO-R were noted among indole-positive Protease (IPP; +24.0%), *E. coli* (EC; (16.6%), *Enterobacter* spp. (EBS; +8.8%) and *P. mirabilis* (+4.9%). Between 2003 and 2004, an 89.9% increase in CIPRO-R was observed for EC. During the same period, the% R for the comparators against ENT remained stable (range, (0.3 to (+0.7%) except for GENT (+4.1%) and TOB (+2.3%). Among PSA, the CIPRO-R rate increased from 11.9% in 1999 to 25.3% in 2003, but decreased in 2004 to 21.2%. Percent R for IMP and MEM also decreased 13.6% and 10.3%, respectively. The CIPRO-R rate among ASP strains increased from 25.0% to 44.4% (average 3.9% increase/year). LEVO-R rate (2003–2004) was consistently lower than the CIPRO-R rate for all ENT and ASP, but slightly higher against PSA. In 2004, clonal spread of MDR (including FQ-R) strains was detected in 12 of the 15 hospitals (EC and ASP clusters in 6 sites each).
Conclusions: During the monitored period (1999–2004), the rate of FQ-R in ENT tested within the MYSTIC Programme has increased most dramatically for IPP and EC strains. FQ-R has emerged to an even greater degree amongst ASP and PSA. Continued surveillance within the MYSTIC Programme participant sites is warranted to monitor the escalating loss of FQ activity against Gram-negative pathogens.

P1867
Antibiotic resistance in the south-eastern Mediterranean: preliminary results from the ARMed Project

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Objectives: Few standardised studies have looked at the prevalence of antibiotic resistance in the south-east Mediterranean. The 'Antibiotic Resistance Surveillance & Control in the Mediterranean Region' project (Acronym: ARMed) was initialised in January 2003 and is financed by the European Commission under INCOMED-FP5. This project extends European surveillance studies – EARSS, ESAC and HARMONY – to southern and eastern Mediterranean partner countries to improve epidemiological analysis of antimicrobial resistance and antibiotic consumption and to identify infection control practices in participating centres [www.slh.gov.mt/armed].
Methods: A total of 29 laboratories from Cyprus, Egypt, Jordan, Malta, Morocco, Tunisia and Turkey are taking part. Targeted surveillance of antibiotic resistance is being undertaken by means of collection of comparable antimicrobial susceptibility results through the use of a standardised protocol and selection of specific medically important bacteria isolated from blood cultures and cerebrospinal fluid. A methodology conformant with that adopted by the European Antimicrobial Resistance Surveillance System (EARSS) [www.earss.rivm.nl] has been agreed. Results are validated by a concurrent quality assurance and proficiency testing programme.
Results: Methicillin resistance in *Staphylococcus aureus* appears to be the major challenge of the region with rates ranging from 17.8% to 66.1%. In four of the participating countries, methicillin resistance was detected in more than 40% of isolates. Resistance in other nosocomial isolates was significantly more heterogenous. Third generation cephalosporin resistance in *Escherichia*

coli varied widely from 3.7% in Malta up to 76.6% in participating Egyptian hospitals. There was less variation in quinolone resistance within the same species with a range of 12.5–39.6%. Vancomycin resistance in enterococci is relative rare with only Turkey reporting 1.1% of strains showing glycopeptide non-susceptibility. Penicillin resistance in *Streptococcus pneumoniae* is on the whole low, with Jordan and Egypt being the only two countries where levels exceed 15%, although macrolide resistance is more common.

Conclusion: This initial antimicrobial susceptibility data indicates that the epidemiology of resistance in the south-eastern Mediterranean shows a significant heterogeneity but is on the whole equal or at times higher than identified in northern countries of the same region by the EARSS network.

P1868

Risk factors associated with the development of multiresistant micro-organism bacteraemia in an intensive care unit

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Objectives: The development of a Multiresistant bacteria infection has become in one of the most important problems in critically ill patients due to its high morbidity and its influence on outcome. The aim of this study was to know the prevalence of bacteraemia due to multiresistant microorganisms (MRB) in an ICU, to describe its main clinical features and etiologies, and finally to identify independent risk factors for developing MRB, in order to prevent it.

Material and methods: Prospective study of all the clinical significant bacteremias in a medical-surgical intensive care unit in a teaching hospital during five years (from 1999 to 2004). The resistance pattern of the isolated pathogens was considered as Multiresistant when the strain was not susceptible to two or more antibiotic families tested. To analyze the predisposing factors for developing MRB appropriate clinical and epidemiological variables were recorded from clinical charts. We used stepwise logistic regression analysis to determine independent predictors of MRB. SPSS (9.0) software was used for data analysis.

Results: Fifty-three (37.8%) of 140 ICU bacteremias were MRB. The mean age of patients with MRB was 63.7 ± 11.7 years and the relation between men/women was 2.1. The principal origins of MRB were: respiratory (39.6%), unknown (28.3%) and catheter (22.6%) Their main etiologies were: *Acinetobacter baumannii* (51%), CNS (26.4%) and *Pseudomonas aeruginosa* (7.5%). Septic shock was present in 54% of MRB. The mean length of stay in the hospital was 44.5 ± 35 days. The global and related mortality rate for MRB was 66% and 33.9%.Risk factors for MRB in the multivariate analysis were nosocomial origin of bacteraemia (OR 4.02; IC 95%1.05–17.1:p = 0.04), ventilator associated pneumonia (VAP) as focus of bacteraemia (OR 3.10; IC 95% 1.15–8.30: p = 0.02), catheter related bacteraemia (OR 7.60; IC95% 1.72–32.7: p = 0.006), and the presence of abdominal drainage (OR 4.40; IC 95% 1.19–16.2: p = 0.02). The previous use of betalactams or quinolones and the length of stay were not associated with the development of MRB.

Conclusions: The prevalence of MRB was excessively high in our study. MRB must be suspected when VAP or catheter were the source of bacteraemia in order to avoid inadequate empirical antibiotic treatment. VAP and catheter infection preventive measures are needed to diminish the incidence of MRB.

P1869

Prevalence and antimicrobial resistance of pathogens isolated from patients with bacteraemia in an intensive care unit

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Objectives: To study the prevalence and antimicrobial resistance of pathogens isolated from patients with bacteraemia in the ICU.

Methods: From January to September 2004, a total of 73 patients presented one or more episodes of bacteraemia. The identification and MIC determination of the isolated strains were performed by the VITEK II system (bioMerieux, France), conventional methods and the E test method (Solna, Sweden).

Results: Bacteraemia was detected in 73/289 (25%) patients. Polymicrobial bacteraemia was observed in 18/73 (25%). A total of 302 strains were isolated. Of them, 146/302 (48.34%) were gram-positive, 149/302 (49.34%) gram-negative bacteria and 7/302 (2.32%) yeasts. Among Gram-positive, the most prevalent were coagulase-negative Staphylococci (CNS), 116/146 (80%), followed by *S. aureus* 12/146 (8%) and Enterococci 17/146 (12%). The most prevalent of CNS was *S. epidermidis* 84/116 (72%), oxacillin resistant in 96%. Two strains (2.4%) were identified as glycopeptide intermediate *Staphylococcus epidermidis* (GISE). *Enterococcus faecalis* was the most common and Van A phenotype resistance was observed in 5/17 (29%). The most frequent among Gram-negative bacteria were *Acinetobacter baumanii* 48/149 (32%), *Klebsiella pneumoniae* 44/149 (29%), *Pseudomonas aeruginosa* 36/149 (24%) and *Proteus mirabilis* 6/149 (4%). 9/48 (18.7%) *Acinetobacter baumanii* strains were multiresistant. Their resistance rate (%) was: Imipenem 70, ceftazidime 97, ciprofloxacin 97, piperacillin/tazobactam 92.7 and ticarcillin/clavulanate 97.5. *Klebsiella pneumoniae* strains produced ESBLs and Amp C-b-lactamases in 41/44 (93.2%). Resistance to carbapenems was observed in 26/44 (59%). Five out of 36 (13.8%) *Pseudomonas aeruginosa* strains were multiresistant. Their resistance rate was (%): Aztreonam 77, ciprofloxacin 67, ceftazidime 55, imipenem 52.5. One out of 6 strains *Proteus mirabilis* was resistant to imipenem.

Conclusion: A very high incidence of multiresistant strains causing bacteraemia were isolated from patients admitted to ICU in our hospital. Continuing regular monitoring for the emergence of further development of bacterial resistance to antibiotics is mandatory.

P1870

Comparison of antibiotic resistance patterns in community and hospital urinary isolates of Gram-negative bacilli in Toronto, Canada

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Background: The purpose of this study was to compare resistance patterns in relevant antibiotics to common community and hospital urinary tract isolates of gram negative bacilli in Toronto, Canada to determine if similar patterns existed.

Methods: Between Dec 2003 and Jan 2004 in the Greater Toronto Area, 1975 consecutive outpatient urinary tract isolates were collected by MDS Laboratories (which services the community), and 2038 consecutive inpatient isolates were collected from the Toronto Medical Laboratory/Mt. Sinai Hospital Microbiology Department (which services 11 hospitals). Antimicrobial

susceptibility of isolates was tested by NCCLS broth microdilution methods and resistance rates were determined using NCCLS criteria.

Results: The frequency of organisms identified of the 4013 total isolates included: *E. coli* (71.8%), *K. pneumoniae* (11.1%), *P. mirabilis* (5.4%), *P. aeruginosa* (2.7%), SPICE organisms (7.0%). SPICE organisms included: *Serratia* spp., *Providentia* spp., *P. vulgaris*, *M. morgagni*, *Citrobacter* spp., and *Enterobacter* spp.. Antibiotic activity against these isolates to beta-lactams, fluoroquinolones (FQ), nitrofurantoin, gentamicin and trimethroprim–sulfamethoxazole (TMP/SMX) are listed in the Table (see graphic) below.

Organism (Com/Hosp)	*E. coli* n=1540/1341	*P. mirabilis* n=79/136	*K. pneumoniae* n=167/ 262	*P. aeruginosa* n=27/ 81	*SPICE* n=105/176
Amoxacillin	30.3 / 35.7 (p=0.002)	12.6 / 18.4 (p=NS)	95.7 / 97.7 (p=NS)	No NCCLS breakpoints	90.48 / 93.2 (p=NS)
Amox/clav.	4.3 / 8.1 (p< 0001)	1.2 / 1.5 (p=NS)	1.6 / 2.7 (p=NS)	96.3 / 100 (p=NS)	67.7 / 78.9 (p=0.03)
Cefazolin	4.1 / 7.5 (p<0.0001)	6.3 / 4.4 (p=NS)	1.6 / 11.5 (p<0.0001)	No NCCLS breakpoints	75.2 / 84.6 (p=0.05)
Cefprozil	4.6 / 9.2 (p<0.0001)	2.5 / 2.9 (p=NS)	2.1 / 5.7 (p=NS)	No NCCLS breakpoints	68.5 / 78.4 (p=NS)
Ceftriaxone	0.8 / 1.9 (p=0.007)	0 / 0.74 (p=NS)	0 / 3.1 (p=NS)	44.4 / 50.6 (p=NS)	5.7 / 11.4 (p=NS)
Norfloxacin	7.9 / 15.7 (p<0.0001)	6.3 / 7.4 (p=NS)	1.1 / 5.0 (p=0.02)	22.2 / 30.9 (p=NS)	0.95 / 10.8 (p=0.002)
Ciproflox.	8.2 / 15.9 (p<0.0001)	6.3 / 12.5 (p=NS)	1.1 / 4.0 (p=0.02)	22.2 / 32.1 (p=NS)	2.9 / 11.3 (p=0.003)
Gentamicin	2.8 / 6.9 (p<0.0001)	1.3 / 2.9 (p=NS)	0 / 2.7 (p=NS)	7.4 / 12.4 (p=NS)	0.95 / 9.7 (p=0.004)
Nitrofur.	1.3 / 1.8 (p=NS)	92.4 / 96.3 (p=NS)	39.0 / 32.4 (p=NS)	No NCCLS breakpoints	38.1 / 39.9 (p=NS)
TMP/SMX	16.8 / 21.3 (p=0.002)	15.1 / 16.1 (p=NS)	8.0 / 9.6 (p=NS)	96.3 / 88.9 (p=NS)	7.62 / 15.9 (p=0.04)

Conclusion: The rate of FQ resistance is significantly higher in hospital urinary isolates of *E. coli*, *K. pneumoniae* and SPICE organisms, compared with the community. Rates of resistance in *E. coli* isolates to all antibiotics (beta-lactams, FQ, gentamicin and TMP-SMX) other than nitrofurantoin are significantly higher in hospital isolates compared with the community. This indicates a compromised spectrum of first-line agents in both inpatients and outpatients.

P1871

Microbiological surveillance of blood cultures in an intensive care unit in a Greek hospital

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Objectives: To investigate the pattern and antibiotic resistance of blood culture isolates from an intensive care unit (ICU) in a tertiary care hospital in Greece.

Methods: Blood cultures from ICU patients were performed using the BacT Alert 30 automated system. Vitek 2 automated system (bioMerieux, France) was used for identification and susceptibility testing.

Results: A total of 497 bacterial strains were isolated from blood cultures, originating from ICU patients, between January 2001 and October 2004. *Acinetobacter baumannii* was the most prevalent pathogen (42.2%), followed by *Klebsiella* spp (15.3%), *Pseudomonas aeruginosa* (13.2%), *Enterococcus* spp (12.2%), coagulase negative staphylococci (6.4%), *Proteus mirabilis* (2.4%), *Enterobacter* spp (2.2%), *Stenotrophomonas maltophilia* (2%), *Staphylococcus aureus* (1.8%), *Citrobacter* spp (1.4%), *Serratia marcescens* 0.2%. In 32 (6.4%) positive blood cultures, more than one microorganism was isolated. Resistance rates to commonly used drugs like ceftazidime, amikacin, ciprofloxacin and imipenem were 96.2%, 67.6%, 97.2% and32% respectively for *A. baumanni* and 66.6%, 77.3%, 71.2% and 36.4% respectively for *P. aeruginosa*. *Klebsiella* spp isolates presented 90.9% resistance to ceftazidime, 28.6% to amikacin, 23.4% to ciprofloxacin but all isolates were susceptible to imipenem. All *P. mirabilis* isolates expressed an AmpC-type beta-lactamase producing phenotype and full resistance to quinolones. Among staphylococci, all isolates were resistant to penicillin but they were susceptible to glycopeptides. Resistance to aminoglycosides was 76.3% for gentamicin and 91.4% for tobramycin. 66.6% of *S. aureus* and 96.5% of CoNS were oxacillin resistant. A high degree of resistance to penicillin (63.9%) and to high level aminoglycosides (70%) was reported for *Enterococcus* spp. Two vancomycin resistant *Enterococcus* isolates (Van A phenotype) were detected (1 *E. faecalis*, 1 *E. faecium*).

Conclusion: *A. baumannii* and *P. aeruginosa* were mainly responsible for ICU bacteremias in our hospital. Alarmingly high resistance rates underline the need for more judicious selection of antibiotics in order to reduce resurgence of multidrug-resistant isolates.

P1872

Distribution and susceptibility of urine and blood uropathogens in a long-term care facility

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Objectives: The Haim Shoham centre is a geriatric home care institute with a population of 850 inhabitants. Urinary tract infections (UTI) is the most frequent infection in geriatrics and the most common source of bacteraemia. Since antimicrobials are started empirically, the knowledge of microbiological patterns and susceptibility of uropathogens in urine and blood is mandatory in order to choose the most adequate treatment.

Methods & Results: From 01/2002 to 10/2003, 3161 urine cultures were sent; 2581 (81.6%) grew microorganisms; 93% gram-negative (*E. coli* 38%, ESBL+ 27.6%, *P. mirabilis* 21% and 183 (7%) gram positive cocci (*E. fecali* 6%). Out of 2265 blood cultures, 296 were positive (13%), 258 of them (87.1%) grew gram-negative bacteria (*E. coli* 18%, ESBL+ 15%), and 38 (12.8%) gram-positive cocci. As in previous studies, *E. coli*, *P. mirabilis* and *K. pneumonia* bacteraemia are more sensitive to antibiotic than the microorganisms growing in urine. Only *P. aeroginosa* bacteraemia in more resistant than this pathogen growing in urine.

Conclusions: The high levels of resistance of the uropathogens growing in urine and blood in our geriatric home care obligate a constant surveillance in order to offer the patients the most adequate treatment.

P1873

Antimicrobial susceptibility of invasive isolates of Enterobacteriaceae collected in Austria

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Objective: To investigate the epidemiology of antimicrobial resistance of Enterobacteriaceae isolated from blood cultures in Austria.

Methods: 538 strains of Enterobacteriaceae (341 *E. coli*, 74 *Klebsiella* spp., 40 *Enterobacter* spp., 40 *Proteus* spp., 43 strains of other species) isolated in clinical laboratories from 7 different regions of Austria between December 2002 and august 2003 were tested for susceptibility to ampicillin/sulbactam (A/S),

piperacillin/tazobactam (P/T), cefazolin (CZ), cefoxitin (FOX), cefuroxim (CXM), cefotaxim (CTX), ceftazidim (CAZ), cefepim (FEP), gentamicin (G), ciprofloxacin (CI), fosfomycin (FOS) and meropenem (MEM) using an agar dilution method. The strains with CAZ or CTX MIC >1 mg/L were further investigated for the production of ESBL using 3 E-test strips: TZ/TZL, CT/CTL, PM/PML. The breakpoints used for interpretation of FOS results were (16 mg/L for susceptibility and >128 mg/L for resistance. For the other antibiotics we used the breakpoints recommended by NCCLS.

Results: Overall, the rates of susceptibility were: A/S 77%, P/T 93%, CZ 67%, FOX 84%, CXM 86%, CTX 95%, CAZ 93%, FEP 98%, G 96%, CI 88%, FOS 93% and MEM 100%, lower in Tirol, Salzburg and Vienna than in other regions of the country. For CI higher resistance rates were encountered in Tirol (19%), Vienna (17%) and Salzburg (16%), for FOS in the Steiermark 17% and for G in Vienna (7.4%). The CI, FOS and G resistance were higher in *Proteus* spp., 18%, compared to *E. coli*: 13%, 2.6% and 2.3%, respectively. The rates of resistance to β-lactams were higher in the ICUs were *Enterobacter* and *Klebsiella* spp. were more frequently recovered, together with a higher rate of AmpC derepressed mutants (13%) and ESBL strains (6.6%), whereas the rates of resistance to CI (25%) and G were higher in the Urology departments.

Conclusions: Although the overall antimicrobial resistance rates were moderate (with the exception of CZ and A/S), the emergence of CI resistance in invasive isolates of Enterobacteriaceae and the association with potent ESBLs in some strains is of great concern.

P1874

Epidemiology and resistance pattern of bacteraemia pathogens in medical patients over an 8-year period

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Objective: To estimate difference regarding epidemiology, outcome and resistance (R) to antimicrobials of medical patients with bacteraemia (B), over 8 years

Methods: Prospective demographic, clinical and microbiology, as well as hospital stay and outcome, data entry of medical patients with documented B. Time Jan 1997 to Oct 2004. Data entry and analysis in IBM compatible PC using EPI5-Info (CDC, 1993) programme. Sensitivity as by Kirby–Bauer, statistics by Yates corrected (2

Results: Documented B patients were 357 (M: 46-F: 54%) m. age 69.7 years. Chronic disease present in 79.8%, and B was nosocomial in 26% of patients. More frequent pathogens were *E. coli* (39.6) and *S. aureus* (15.1%), most common origin of B being the urinary tract (48%) Main differences between first and second half of the studied period was the considerable rise of *Klebsiella* sp and *Acinetobacter* Bs and relative rise of Enterococcal B and *Candidaemias*. Regarding R data of Gram(pathogens, significance was most prominent was R to 3rd generation cephalosporins [rising from 6.9 to 22.1%, $p = 0.0057$] and ciprofloxacin [8.7–21.3%, $p = 0.004$], and several multiresistant strains. The annual rise of Gram(cocci percentage is noted, with constant MRSA rates (~45%), but no glycopeptide R. Mean hospital stay was 13.5 days and mortality during stay was 17.4%, though not always directly attributed to B.

Conclusions: The constant variability of B pathogens, the appearance of less expected ones, and mainly R profile changes deem continuous surveillance and awareness, to ensure the optimal empirical antimicrobial choice based on the most recent data of the given milieux.

P1875

Epidemiology and resistance pattern of urinary pathogens in medical patients over a 6-year period

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Objective: To estimate difference regarding epidemiology, outcome and resistance (R) to antimicrobials of medical patients with documented urinary tract infection (UTI), over a 8 years.

Methods: Prospective demographic, clinical and microbiology, as well as hospital stay and outcome, data entry of medical patients with documented UTI Time Jan 1997 to Oct 2004. Data entry and analysis in IBM compatible PC using EPI5-Info (CDC, 1993) programme. Sensitivity as by Kirby–Bauer, statistics by Yates corrected chi-square

Results: Documented UTI, was recorded in 501 patients (M: 37.7-F: 62.3%) m. age 73.1 years. Chronic disease present in 85%, and UTI was nosocomial in 34%. More frequent pathogens were *E. coli* (50.6%), *Pseudomomonas* sp (8%) *Klebsiella* sp 7.5, and *S. faecalis* (6.4%). Main difference between first and second half of the studied period was the rise of *Pseudomonas* from 5.8 to 13.2% and *Proteus* sp [7.4–11.8%], both $p < 0.001$, while other pathogens fluctuated . Regarding Gram(pathogens R, significant were the increase to cotrimoxazole [31–48%, $p < 0.01$] and gentamicin [13.6–32.2%, $p = 0.004$]. Also of note was the rise of ciprofloxacin R, [19–29.6%, ns] even among community acquired *E. coli* . Mean hospital stay was 10.6 days and mortality during stay was 10.4%.

Conclusions: The constant variability of UTI pathogens, others rising, others decreasing, the appearance of unexpected ones, and mainly R profile changes deem continuous surveillance and awareness, to ensure the optimal empirical antimicrobial choice based on the most recent data of the given milieux. Designing and implementing local guidelines for hospital and community setting is obviously warranted

P1876

Trends in antimicrobial resistance in urinary isolates of *E. coli* from patients hospitalised at a teaching hospital Maribor, Slovenia

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Objective: To assess the prevalence and trends in antimicrobial resistance in urinary isolates of *E. coli*.

Methods: We studied resistance rates to different antimicrobial agents in urinary isolates of *E. coli* from patients hospitalised at Teaching Hospital Maribor, Slovenia in years 1998, 2000 and 2003. The isolation was performed by standard methods . Susceptibility was determined by disc diffusion test according to the NCCLS.

Results: Results are collected in Tabel 1.

Conclusion: *E. coli* remains the most common uropathogen representing between 39% to 40.3% of all urine isolates.We have noticed significant increase in resistance rates in *E. coli* strains to some commonly used antibiotics in the last six years. Susceptibility to amoxicillin–clavulanate, cephalosporins of second and third generation and aminoglycosides remained stable, although the resistance rates to trimethoprim–sulfamethoxazole and ciprofloxacin are increasing very rapidly. We believe that surveillance of antimicrobial resistance is very important for giving recommendations about empirical antibiotic treatment.

Table 1 Resistance rates of *E. coli* isolates to different antimicrobial agents in the years 1998, 2000 and 2003

Year	1998,0	2000,0	2003,0	*P value
Total number of Isolates	2563	2327	2456	
Percentage of *E.coli* (%)	39,1	39,4	40.3	
Resistance rates of *E.coli* (%)	%	%	%	
Amoxicillin/ampicillin	39,5	38,4	43,1	0,0230
Amoxicillin clavulanate	15,4	10,5	14,0	NS
Cephalotin	7,8	5,7	11,9	0,0046
Cefaclor	2,8	2,2	2,7	NS
Ceftriaxon	ND	0.8	0.8	NS
Ciprofloxacin	4,9	6,9	11,9	0.0001
Gentamicin	2,3	2,2	3,5	NS
Cotrimoxazole	15,0	18,4	22,0	0,0001

*P value of chiquadrat test, NS not statistically significant. ND No Data.

P1877

Bacterial spectrum and antibiotic resistance of uropathogens in hospitalised urological patients with urinary tract infections (1994–2004) and consequences for the empiric antibiotic therapy

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Objectives: Surveillance of the bacterial spectrum and antibiotic resistance of uropathogens is important for the right antibiotic choice. Uropathogens causing complicated urinary tract infections (UTI) in the years 1994–2004 were surveilled.
Methods: In the years 1994–2004 all uropathogens of hospitalized urological patients were identified and the susceptibility was tested against 14 antibiotic substances (trimethoprim (TMP)/ sulfamethoxazole (SMZ), ciprofloxacin, ampicillin, mezlocillin, ampicillin/ sulbactam, piperacillin/ tacobactam, cefuroxime, cefpodoxime, cefotaxime, ceftazidime, gentamicin, penicillin, oxacillin and vancomycin). Since 2002 levofloxacin, gatifloxacin, piperacillin/ sulbactam and linezolid were tested additionally.
Results: 1. There was no general trend in the emergence of resistance, except with TMP/SMZ and ciprofloxacin in *E. coli*.
2. Vancomycin or linezolid intermediate or resistant staphylococci or enterococci were not observed.
3. The lowest rate of resistance was observed with piperacillin/ tazobactam; carbapenems however were not tested regularly.
Conclusion: If the uropathogens are stratified into Gram positive and negative bacteria, for oral application ciprofloxacin or levofloxacin showed the lowest resistant rates for Gram negative, ampicillin/sulbactam, gatifloxacin or linezolid for Gram positive uropathogens. After further differentiation of uropathogens with quick and simple methods, i.e. catalase, coagulase, oxidase testing, the empiric antibiotic therapy can be done more taylored. In order to be able to do so, the urologist has to become involved in the analytic processing of the urine probe.

P1878

Resistance to third generation cephalosporins in *Klebsiella pneumoniae*: a five years multicentric Tunisian study (1999–2003)

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Objective: The massive use of third generation cephalosporins (3rdGC) was subsequently followed by a marked increase in their resistance incidence. *Klebsiella pneumoniae* is the most common Gram negative bacteria exhibiting this resistance pattern. In order to assess the impact of resistance to 3rdGC, a multicentric study was carried out over a five years period (1999–2003), in 4 teaching hospitals.
Methods: Isolates were identified by Api 20E system and their antimicrobial susceptibility was determined by the disc- diffusion method in Mueller–Hinton agar with regular quality control. Data analysis was done using the Whonet 4 software
Results: Over the 6378 strains of *K. pneumoniae* collected, 34.1% were resistant to 3rd GC. Their annual rate has increased from 26.4% in 1999 to 38.9% in 2003 without difference between hospitals except for paediatric hospital in 1999 (65.2%). These isolates were recovered mainly from paediatrics (38.2%), Medicine (18.2%), urology (12.4%), intensive care units (11.7%) and surgery (10.2%). They were more frequently isolated from urines (49.7%), blood cultures (23.3%), and pus (13.3%). Associated resistance rates were as follows: 18.4% to cefoxitin, 88.3% to gentamicin, 62.5% to amikacin, 43.4% to chloramphenicol, 80.3% to trimethoprim–sulfamethoxazol and 40.6% to ciprofloxacin. All isolates were susceptible to imipenem.
Conclusion: The alarming rate of *K. pneumoniae* resistant to 3rdGC needs the implementation of infection control measures, hand washing and rational use of antibiotics.

P1879

Antimicrobial resistance, epidemiology, and outcome of *Clostridium difficile* associated diarrhoea

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Objectives: To determine susceptibility patterns of clinical *C. difficile* isolates obtained from hospitalized patients in a tertiary medical centre in Israel, and to delineate epidemiological characteristics and predictors of mortality in patients with *C. difficile* associated diarrhoea CDAD.
Methods: The enzyme immunoassay (EIAs) TOX A/B was used for toxin detection. Isolation attempts of *C. difficile* were performed only on *C. difficile* toxin positive stool samples. All isolates underwent screening for antimicrobial susceptibility to metronidazole (5 μg), vancomycin (30 μg), rifampicin (5 μg), fucidic acid (10 μg), doxycycline (30 μg), and linezolid (30 μg). Minimal inhibitory concentration (MIC) was determined by the E-test if disc diffusion was in the inhibition zone of (30 mm. Bacterial typing was performed on isolates resistant to any of the studied drugs, and on four randomly selected pan-sensitive isolates were studied for clonal relatedness by the random amplification of polymorphic DNA (RAPD)-PCR amplification assay.
Results: Resistance to metronidazole was found in 2% (1/49 isolates) of isolates (MIC $\geq$ 256 μg/mL); linezolid in 2% (1/48 isolates) (MIC = 24 μg/mL), and one isolate had a combination of resistance to fucidic acid (by disc diffusion test) and rifampicin (MIC > 32 μg/mL). All isolates were sensitive to doxycycline and vancomycin. Molecular typing method showed an absence of clonality among the resistant isolates, while pan-sensitive isolates were monoclonal. On multivariate analysis, predictors of mortality were previous treatment with fluoroquinolones, hypoalbuminemia, dementia, renal failure, and admission from a nursing home.
Conclusions: Our study demonstrates that resistance of *C. difficile* to metronidazole and other antimicrobials including linezolid exists in our institution. This finding should promote exploration of this problem in Israel, clarify the impact of resistance on clinical course and outcome, delineate risk factors and mechanisms for developing resistance, and search for new alternative therapies and prevention of CDAD.

P1880

Antibiotic resistance of 69 *Klebsiella* strains isolated from a paediatric clinic over three years

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Objective: Blood cultures taken from patients of Paediatric clinics of Health Ministry Haseki Education and Research Hospital have been evaluated for 3 years.
Methods and Results: The growth rate in blood cultures were 303/1019, 357/1062, 321/1019, in years 2002, 2003, and 2004, respectively. The ratio of Gram positive bacteria to Gram negative bacteria were 230/46 in 2002, 296/48 in 2003, and 234/55 in 2004. Gram negative bacteria were 1/5 of the total grown bacteria. Ratio of *Klebsiella* spp. among the grown Gram negative bacteria was 16/46 in 2002, 21/48 in 2003, and 32/56 in 2004. When the distribution of *Klebsiella* spp. according to months is examined, it is seen that there is no accumulation in 2002, while 10 bacteria were accumulated in June and July in 2003, and 21 bacteria were accumulated in May, June and July in 2004. As a result of examination of susceptibilities of the strains to antibiotics; in 2002 88% of the strains were susceptible to amikacin, 62% to seftiakson, 94% to sefepime, 100% to imipenem. These rates were as 71%, 71%, 95%, 100% in 2003 respectively, and 53%, 46%, 81%, and 100% in 2004, respectively.
Conclusion: In 2004, *Klebsiella* strains resistant to imipenem were isolated from especially the ICU of our hospital. The paediatrics clinic is a ward where patients stay for a long time and where wide spectrum antibiotics are used and *Klebsiella* bacteria are being watched closely for carbapenem resistance and ESBL formation since they spread in the clinic and caused a hospital epidemic.

P1881

TARGET Surveillance, a part of the LIBRA initiative: susceptibility of *Enterobacter* spp. and *Klebsiella* spp. isolated during 2003

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Objectives: To assess the antibiotic (ABX) susceptibility of *Enterobacter* (E). sp and *Klebsiella* (K). sp circulating in France, Germany, Italy, Mexico, South Africa, Spain and the USA during 2003.
Methods: MICs for ampicillin (AMP), amoxycillin/clavulanate (AMX/C), ceftazidime (CTZ), ceftriaxone (CTX), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMI), nitrofurantoin (NIT), piperacillin (PIP), piperacillin/tazobactam (PIP/TAZ) and Trimethoprim/sulphamethoxazole (TM/SX) were determined by microbroth dilution.
Results: Percent ABX susceptibility (sus) of all K. sp and E. sp collected are shown in the Table (N, number of isolates collected). Against both pathogens, IMI and GEN were the most active parenteral agents and CIP the most active orally available ABX. For E. sp, lower sus was observed in Italy and Mexico: CTZ or CTX (50–60%), GEN (70–80%), PIP (~50%) or PIP/TAZ (~60%). TM/SX sus was also low in Mexico (~60%) and CIP slightly lower in Italy (82.2%). Lower sus to CTZ or CTX (~60%), CIP (71.6%), PIP (60.3%), PIP/TAZ (67.8%) or TM/SX (82.9%) was seen in France. Interestingly, NIT sus was low in France and Germany (13–20%) but higher in Mexico or Spain (~50%). Higher E. sp sus was observed in Germany (except NIT), Spain or the USA, with (90% sus to CIP, GEN, IMI or TM/SX. Higher CIP sus also occurred in South Africa ((95%). Generally K. sp sus was lower in Mexico and South Africa but isolates were still over 90% sus to IMI or CIP. Higher K. sp sus was observed in Spain and the USA ((90% for all ABX except AMP or PIP). However, K. sp showed low AMP or NIT sus worldwide.

	N	AMP	AMX/C	CTZ	CTX	CIP	GEN	IMI	NIT	PIP	PIP/TAZ	TM/SX
E.sp	2238	15.3	5.2	69.8	70.3	87.1	90.3	99.5	26.5	67.7	74.2	87.6
K.sp	2195	5.7	88.5	93.1	92.4	93.9	94.2	99.9	47.0	77.2	89.3	88.9

Conclusion: K. sp is generally more sus to ABX than E. sp. Of the ABX included within the study only CIP, GEN or IMI showed consistent activity against E. sp within each country investigated. Of these CIP would be the most suitable for step-down and/or community use against E. sp or K. sp infection.

Susceptibility testing methods

P1882

Antibiotic susceptibility tests on *P. aeruginosa* isolated in ICU using the Uro-Quick system

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Objective: *P. aeruginosa* is responsible of severe infections, especially in nosocomial settings. This species is also characterized by an easy evaluation toward resistance against antimicrobial agents. The management of infections sustained by *P. aeruginosa* in fact requires a treatment based on a combination of drugs. Because the therapy needs the use effective drugs, laboratory tests are challenged to support clinicians in a timely manner. In order to gain time in the evaluation of antimicrobial susceptibility in *P. aeruginosa*, Uro-Quick, an automatic instrument capable to test antibiotic resistance, was used to perform antibiotic susceptibility against this pathogen.

Material and methods: Appropriate concentrations of bacterial cultures were used to test different media containing various concentrations of KCl and Tween 80. Susceptibility tests were carried out exposing *P. aeruginosa* isolates to antibiotics in fixed concentrations. Ciprofloxacin (CIP), amikacin (AN), gentamycin (GM), imipenem (IPM), ceftazidime (CAZ), piperacillin/tazobactam (TZP) were used. All tests were carried out under aerobic environment.
Results: After evaluation of growth conditions in different media, *P. aeruginosa* was routinely cultured in MH broth containing KCl 2% and Tween 80, 0.2%. The growth rate of *P. aeruginosa* in this medium, using an inoculum of 106 CFU/ml, was found faster than in the usual MH without any other ingredients. The instrument printed out the results after 3–4 hours in comparison with 6 hours required under the standard conditions.Susceptibility tests, read after 4 hours of incuba-

tion, demonstrated an agreement >95% (CIP 96%, AN 96%, GM 100%, IPM 92%, CAZ 92%, TZP 92%) between Uro-Quick and Kirby–Bauer method.
Conclusions: The Uro-Quick system may be very useful for the evaluation of antimicrobic susceptibility in *P. aeruginosa* because of its rapid achievement of the results.

P1883

Evaluation of MicroScan Dried Overnight Panels compared to a NCCLS reference panel for daptomycin susceptibility testing with a challenge set of organisms

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Objectives: The accuracy and reliability of MicroScan Dried Overnight Gram-positive panels compared to the NCCLS frozen reference broth microdilution method for susceptibility testing of daptomycin were assessed during a multi-site evaluation.
Methods: Accuracy was evaluated at one site with 75 challenge isolates (34 *S. aureus*, 11 coagulase-negative *Staphylococci*, 19 *Enterococcus* spp., and 11 group B beta-hemolytic *Streptococci*), selected to provide a range of on-scale results. The inoculum was prepared with the turbidity and Prompt methods of inoculation and the panels were tested with the WalkAway System, the autoSCAN-4 instrument, and manual readings. Reproducibility was evaluated at three sites using 10 gram-positive organisms, including the recommended NCCLS quality control strains. The reproducibility strains were tested in triplicate for three days at each site with both inoculation methods. Results obtained with the WalkAway System and the autoSCAN-4 were compared to the mode and median of the manual panel readings.
Results: Essential agreement for challenge isolates tested with the turbidity inoculation method was 98.7% (74/75) for all reading methods when compared to the reference panel. Essential agreement with the Prompt inoculation method was 94.7% (71/75) for the WalkAway, 96% (72/75) for the autoSCAN-4, and 90.7% (68/75) for the manual readings. Categorical agreement was >95% for all combinations. Inter-laboratory reproducibility (manual readings) was 100% for both inoculation methods. Reproducibility was 100% (275/275) for both the WalkAway and autoSCAN-4 with the Prompt inoculation method. For the turbidity inoculation method, reproducibility was 98.5% (271/275) for the WalkAway and 99.6% (274/275) for the autoSCAN-4.
Conclusion: MicroScan Dried Overnight Gram-Positive panels used with WalkAway, autoSCAN-4 instrumentation and manual readings and with either turbidity or Prompt inoculation methods correlated well with the NCCLS reference broth microdilution method for susceptibility testing of daptomycin.

P1884

Evaluation of the MicroScan Dried Overnight Panel and Instrumentation for antimicrobial susceptibility testing of mupirocin

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Objectives: The MicroScan System (Dade Behring Inc.) provides automated, semiautomated or manual antimicrobial susceptibility testing for the clinical laboratory. Mupirocin is a topical antimicrobial agent active against staphylococci. Gram-Positive panels containing mupirocin were introduced as part of LabPro V1.55. Panels were evaluated in comparison to an NCCLS reference panel with fresh and stock *Staphylococci*. The performance of automated MicroScan instrumentation (WalkAway, autoSCAN-4) in reading panels was also assessed.
Methods: A total of 288 *Staphylococci* were tested for mupirocin MIC determination using MicroScan Dried panels and reference NCCLS broth microdilution. Mupirocin concentrations tested were 1–512 μg/ml. Agreement was assessed using both manufacturer's skin and nasal interpretive breakpoints. In addition, a total of 126 *Staphylococci* were tested on multiple MicroScan instruments to evaluate automated performance in comparison to manual read of the same panels. Both susceptible and resistant organisms were tested. There were 212 data points established for these staphylococci for the WalkAways and 534 data points established for the autoSCAN-4s. Both turbidity and Prompt inoculation methods were used for all studies.
Results: The essential agreement (± one doubling dilution) between the MicroScan panel and the reference panel was 100%. There were no major or very major errors using either interpretive breakpoint. For WalkAway automated reads, the essential agreement between instrument and manual read was 99.5%. There were no major or very major errors using nasal breakpoints; there was one (1/56,1.8%) very major error with a *S. aureus* using skin breakpoints. For autoSCAN-4 reads, essential agreement between instrument and manual read was 98.7%. There was one very major error (1/67, 1.5%) for a *S. aureus* using skin breakpoints and a second very major error (1/44, 2.3%) for a *S. epidermidis* using nasal breakpoints.
Conclusion: This study showed that the new MicroScan Dried Overnight gram-positive panels containing mupirocin give excellent automated performance and excellent correlation with an NCCLS broth microdilution reference panel.

P1885

Comparison of Uro-Quick and Kirby–Bauer methods to detect ESBL-producing strains

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Objectives: To evaluate the Uro-Quick system for detection of extended-spectrum beta-lactamase (ESBL) in nosocomial strains that exhibit ceftazidime resistance phenotype, and/or in isolates requiring confirmatory tests for the identification of strains producing these enzymes.
Methods: A total of 160 strains collected from nosocomial patients were tested for antibiotic susceptibility by the Uro-Quick system. About 106 cell/ml were used to seed 2.5 ml of broth in vials containing ceftazidime (20 mg/l) and ceftazidime (20 mg/l) plus clavulanic acid (10 mg/l). After incubation the results were plotted as growth curves. All result were confirmed by Kirby–Bauer method. Control strains included *E. coli* ATCC 25922, and a TEM-4 producer.
Results: Using an inoculum of 106 cell/ml, the instrument was capable to print out the result after 5 hours of incubation. Among the pathogens studied, 34 strain resulted resistant to ceftazidime (presence of growth) and showed susceptibility to the combination of this cephalosporin with clavulanic acid (absence of growth). These results were then compared with those obtained with the Kirby–Bauer method, and it was found that among these 34 ceftazidime-resistant clones all were ESBL-producers, even if 5 showed an intermediate, and 6 a susceptible antibiotic phenotype by Kirby–Bauer method.
Discussion: Present findings suggest that Uro-Quick represents a useful technology to detect ESBL-producing strains especially those that required further confirmatory tests for their identification. The period of time needed to achieve the results, in some cases less than 5 hours, is a sure advantageous over the usual methodologies.

P1886

Evaluation of the essential equivalency of the antimicrobial susceptibility test GN09 Card on the bioMerieux VITEK 2 and the VITEK 2 Compact System

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Objective: The objective of this trial was to compare and confirm equivalency between the new bioMérieux VITEK® 2 Compact System and VITEK® 2 using the VITEK® 2 Gram Negative Antimicrobial Susceptiblity Test (AST) GN09 card. The three components of this study included reproducibility, challenge and Quality Control (QC) testing.
Methods: Thirty-two (32) reproducibility strains were tested for three days, and twenty replicates of QC were performed using *E. coli* ATCC 25922, *E. coli* 35218 and *P. aeruginosa* ATCC 27853. 135 challenge strains were also included in this study. These strains encompassed a range of both fermenting and non-fermenting Gram-negative bacilli and were chosen to cover the range of results provided by the systems in order to detect a significant process shift should one exist. Two AST GN-09 cards were inoculated from the same inoculum and one card was put onto each automated system.
Results: The challenge set of organism yielded good results. There were no dilution differences between the 2 systems for 94.2% of results. Only 5.3% showed one dilution difference. Essential agreement for all strains was 99.5%. The essential agreement for on-scale results was 98.7%, with 80.7% of results showing no dilution difference. Reproducibility tests showed100% essential agreement. The QC in-range results for VITEK® 2 were 98.9% and 99.0% for the VITEK® 2 Compact.
Conclusions: The essential equivalency of bioMérieux VITEK® 2 and the new VITEK® 2 Compact were evaluated with the GN09 antimicrobial susceptibility card. The studies performed challenged the range of antimicrobial agent – organism combinations offered for laboratory testing of aerobic gram-negative bacilli. For reproducibility and challenge studies, observed performance exceeded the minimum level of acceptable performance. All acceptance criteria were met when data were restricted to on scale results. These data provide convincing evidence that VITEK® 2 AST results will be essentially equivalent regardless of the system used for testing.

P1887

Rapid antimicrobial sensitivity testing: direct from positive blood cultures

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Objectives: This study describes a new approach which allows a pathogens susceptibility pattern to be determined in 4–5 hours direct from positive blood cultures. The test is based on using adenylate kinase (AK) as a sensitive and quantitative marker of cell biomass. AK catalyses the equilibrium reaction 2ADP to ATP (AMP and can be used as a cell marker by monitoring the ATP produced using firefly luciferase. The AK technique is >1000,000 fold more sensitive than optical techniques (e.g. turbimetric, colorimetric) used in current commercial systems and shows significantly better correlation with organism numbers. This allows changes in microbial populations to be detected very early in the growth curve, many generations before any signal can be measured by optical methods.
Methods: The study was divided into 2 phases. The first phase examined techniques to extract bacteria from the blood culture specimen matrix to prevent blood culture components interfering with the antibiotic sensitivity test (AST)results. The second phase compared AST results achieved using the 'Rapid AK' test to AST results determined using traditional approaches, for the 12 organisms most commonly associated with blood infection. The study looked at both qualitative (breakpoint) and quantitative (NCCLS MIC) methods of AST.
Results: After examining a range of extraction approaches a modified lysis centrifugation technique was shown to reliably extract common blood isolates at numbers consistent with those present in positive blood cultures (10^5 orgs/ml). There was no determinable effect on organism growth rates or AST results using this technique. The rapid AK AST allowed the correct determination of the antibiotic susceptibility for all organism/antibiotic combinations tested, using qualitative or quantitative approaches, direct from blood cultures within 5 hours.
Conclusion: The results of this study suggest that the 'rapid AK assay' can be used to accurately and rapidly determine the antimicrobial susceptibility of common clinical pathogens direct from positive blood cultures. This is a significant improvement over current methods which generally require overnight incubation. Further work is progressing to further reduce timescales and to examine a wider range of clinically important organisms/resistance mechanisms.

P1888

Accuracy of three automated systems for susceptibility testing selected Gram-negative bacilli against five broad-spectrum beta-lactam agents

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Objectives: To evaluate the accuracy of three automated systems; MicroScan WalkAway (MSWA), Vitek 2 (VT2) and Vitek Legacy (VTL), for susceptibility (S) testing *P. aeruginosa* (PSA) and various Enterobacteriaceae (ENT) species against aztreonam (AZT), cefepime (CPM), ceftazidime (CAZ), imipenem (IMP) and piperacillin/tazobactam (P/T).
Methods: Recent clinical strains (100 PSA and 20 ENT) from North American hospitals were selected to over-represent isolates with CPM and P/T MIC values within ±1 log 2 dilution of the current NCCLS S and resistant (R) breakpoints for the studied compounds. Categorical results from automated systems were compared to the consensus of 3 reference/standardized methods: broth microdilution, E-test (AB BIODISK, Solna, Sweden) and disk diffusion. Categorial disagreements were classified as: in very major (VM, false-S), major error (MA, false-R) and minor errors (MI; involving the intermediate category).
Results: The consensus testing S/R rates (%) among PSA strains were 45/55 for P/T, 46/40 for CAZ, 48/35 for CPM, 69/27 for IMP and 36/35 for AZT. A summary of the categorial disagreements for PSA is shown in the Table. All three systems showed a high, unacceptable rate of VM for P/T (21–32%). For other drugs VM rates ranged from 0 to only 4% (IMP tested on VT2). MA rates were acceptable for comparisons (0–3%) and MI rates were generally elevated (5–33%), reflecting the high proportion of consensus results within the intermediate category or skewed, erroneous results for CPM (VT2 and VTL) and AZT (all systems). Among ENT (20 strains), VM was 5% for AZT and 0% for P/T, CAZ, CPM and IMP (100% S) on all 3 systems, and MA was detected only on MSWA (0–15%). MI rates were higher for P/T [20 (VT2) to 35% (MSWA and VTL)] and CPM [15 (VTL) to 35% (MSWA)].

	Error rates (%)				
System/Error type	AZT	CPM	CAZ	IMP	PIT
MSWA					
VM	0	0	0	1	21
MA	0	1	1	1	1
MI	33	14	11	11	-
VTL					
VM	3	0	1	0	26
MA	0	0	1	0	0
MI	32	22	12	5	-
VT2					
VM	0	2	2	4	32
MA	1	0	3	1	0
MI	28	23	12	12	-

Conclusions: The results of this study demonstrates that the automated systems (MSWA, VT2 and VTL) generally failed to accurately detect P/T-R among PSA. The criteria used to select the strains (MIC values close to breakpoints or within R range) increased the sensitivity of detecting significant categorical disagreements, dominated by MI. Re-evaluation of the P/T testing for PSA would be prudent for these systems to minimize adverse therapeutic outcomes worldwide.

P1889

Detection of carbapenemase production in the *Bacteroides fragilis* group using the MBL E-test strip: correlation with broth microdilution testing

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Objective: Resistance to beta-lactam agents among bacteria is often due to beta-lactamase (BLa) enzymes. The majority of the *Bacteroides fragilis* group (Bfg) produce BLa and are easily detected using the nitrocephin assay. MBL are also produced by a small per cent of Bfg and renders them resistant to all beta-lactam agents including carbapenems. The nitrocephin assay does not distinguish between substrate profiles of these various enzymes which can have therapeutic implications. The AB Biodisk MBL E-test strip provides a simple test that allows the detection of MBL enzymes through the simultaneous testing of imipenem(IMI)/imipenem-EDTA(IMI-E)on a single test strip. This study was designed to test the correlation of MBL E-test results with results from Broth Microdilution (BMD) testing of the Bfg.
Methods: A challenge group of 100 Bfg isolates were tested. MBL E-test strips were placed on inoculated prereduced *Brucella* blood agar plates according to the manufacturers recommendations with simultaneous inoculation of BMD plates containing two-fold dilutions of IMI and meropenem(MERO) in supplemented *Brucella* broth. All plates were incubated anaerobically at 35°C for 48 h and read. The ellipse MIC endpoint was determined for the IMI and IMI-E potions of the MBL strips. A positive result for production of MBL was an MIC 4-fold or more lower for the IMI-E portion of the strip than for the IMI portion. For BMD the MIC was the lowest concentration of IMI or MERO that inhibited the visible growth of the test isolate. BLA was also determined using the nitrocephin assay. QC was performed using *B. fragilis* ATCC25285 and *B. fragilis* TAL3636.
Results: All isolates produced BLa in the nitro cephin assay. Based on BMD MICs 19 isolates were resistant(R) to IMI and MERO; 2 were intermediate(I); and 79 were susceptible(S). All 19-R isolates were positive by MBL E-test assays with MIC results of 16/<1 to >256/<1. For the 2-I isolates the MBL tests were negative with results of <4/<1. All 79-S isolates were MBL negative with all results <4/<1. The MIC endpoints for the MBL strips were easily read with the exception of the 2-I isolates which had colonies into the inhibitory zone and repeat testing gave the same results. Overall the two tests correlated 98% of the time. For S and R isolates the correlation was 100%. The I isolates had discrepant results suggesting elevated MICs due to non-BLa mechanisms.
Conclusion: The performance of the MBL E-test correlated very well with NCCLS BMD in the detection of Bfg isolates that produced MBL.

P1890

Evaluation of a microdilution sensitivity test which uses 384 Well plates

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Objectives: Antibiotic susceptibility tests are the most frequently used tests in clinical microbiology. Due to economic pressure fully or partly automated test systems are developed in order to reduce personal and reagents costs. In this study, we compare the MICRONAUT system using a 384 well microtiter plate with a conventional microdilution method according to DIN guidelines.
Methods: The MICRONAUT system uses vacuum dried antibiotics in a 384-well-plate. The inoculum is applied with an automatic dispenser with a final inoculum of 1×10^5 cells/μL in 50 μL cation adjusted Mueller–Hinton medium with the addition of 0.025% phytagel. As the comparator we used the microdilution method according to DIN 58940-8. Evaluation was performed by the comparison of (i) the performance of quality control strains (*E.coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212), and (ii). the natural sensitivity of clinical isolates of common pathogens (*E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*). For each species-antibiotic-combination meeting the conditions for comparison (at least five-dilution-series with a mode at the third dilution of the naturally sensitive population) two figures are calculated to determine the degree of similarity of the MIC-distributions: the mode and the percentage of strains within the range of ± one dilution step of the mode. Results:The results for the QC-strains match to 100% for 23 strain–antibiotic pairs. For 16 pairs there is a difference of 1 dilution step for the mode MIC, and only once (*S. aureus* and Penicillin) the mode MIC varied for 2 dilution steps. The results for the MIC-distributions of the natural sensitive population show in 10 out of 15 cases an excellent match with regard to the peak and the width of the MIC-distributions. For imipenem and gentamicin with *P. aeruginosa*, for vancomycin, moxifloxacin and meropenem with *E. faecalis* differences of the mode are one dilution step only. The percentage of values within the range of ± one dilution step of the mode for the above mentioned exceptions is nearly identical.
Conclusions: In general, both methods show nearly identical results. Differences in mode MICs rarely exceed one dilution step, although not identical batches of the test medium were used for both methods. This way of evaluation seems more reliable than the use of already evaluated results with S, I and R categories describing major and very major errors.

P1891

Susceptibility patterns for amoxicillin/clavulanate tests mimicking newly indicated formulations and pharmacokinetic relationships: does the 2:1 ratio MIC accurately reflect activity against beta-lactamase-producing *H. influenzae* and *M. catarrhalis*

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Background: Amoxicillin/clavulanate (A/C) has recently undergone formulation changes (XR and ES-600) that represent

14:1 and 16:1 ratios of A/C. These ratios greatly differ from the 2:1 ratio used in initial tablets or for in vitro susceptibility (S) testing, and produce a serum concentration ratio of approximately 9:1. The effects of these altered ratios on the S test was assessed.
Methods: Activity of A/C was determined by NCCLS broth microdilution methods (M7-A6; 2003) against *H. influenzae* [HI; 19 strains, 12 beta-lactamase-positive (BL+)] and *M. catarrhalis* (MCAT; 23 strains, 16 BL+ including BRO-1 and -2). A/C was tested in 8 combinations reflecting formulation and serum PK ratios (4, 5, 7, 9, 14 and 16:1; 0.5 and 2 mg/L fixed C conc.) and compared with 2:1 MIC results. Mueller–Hinton broth was modified to HTM for testing HI.
Results: The reference A/C (2:1) MICs for HI BL+ and BL− strains ranged from 0.5–4 and 0.25–2 mg/L, respectively. BL+ MCAT A/C MICs (MIC90, 0.25 mg/L) were routinely ≥16-fold higher than BL- strains showing incomplete enzyme inhibition by C. All A/C MIC ratio test results were unchanged for BL− isolates compared to 2:1 ratio MICs. However, trends toward a 2-fold higher A/C MIC were observed for all ratio tests of >5:1 and >4:1 for BL+ HI and MCAT, respectively. Both C fixed concentration MIC tests were equal to or lower than 2:1 MICs, however the Cmax for C was only 1.5–2.2 mg/L (1.7–2.0, new formulations) questioning the PK/PD validity of the C level at the current NCCLS breakpoint (S at ≤4/2 mg/L).
Conclusions: The A/C MIC test using the 2:1 ratio was established prior to contemporary PK/PD calculations and before current altered formulations significantly modified drug ratios. At ratios of ≥4:1 the inhibition of BL+ strains was less efficient resulting in a 2-fold greater A/C MIC and reducing the probability of favorable clinical responses. Re-evaluation of A/C MIC testing should be considered by S test standard organizations.

P1892

Validity of antimicrobial susceptibility results from 29 Mediterranean laboratories in the ARMed Project

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Objective: The Antibiotic Resistance Surveillance & Control in the Mediterranean Region (ARMed) project is a ten-country multi-centre surveillance network coordinated by the Infection Control Unit of St. Luke's Hospital, Malta and funded by the European Commission under the INCO-MED FP5 programme. In order to validate the results of the ARMed-EARSS antimicrobial susceptibility component of the project, the 29 laboratories from 6 Euro-Mediterranean countries, participate in a yearly external quality assessment (EQA) exercise.
Methods: This EQA exercise was organised through the link with EARSS (European Antimicrobial Resistance Surveillance System) in collaboration with UK NEQAS (United Kingdom National External Quality Assessment Scheme) for Microbiology, Centre National de Référence des Antibiotiques (CRAB) and the members of the EARSS EQA committee. The strains were distributed to the participating laboratories by UK-NEQAS through the ARMed co-ordinators and the laboratories are asked to report clinical susceptibility categorisation (S, I, R). The results are analysed and considered 'concordant' if the reported categorisation agreed with the designated interpretation of the reference laboratories.
Results: The overall response rate of the EQA in the first year of the ARMed network was 89%. These results showed that there is room for improvement in routine susceptibility testing. For *Streptococcus pneumoniae*, 23% of laboratories failed to detect penicillin non-susceptibility. Moreover, 31% of laboratories missed detection of an MRSA that caused epidemics in Europe. This strain is difficult to detect since the resistance phenotype is heterogeneously expressed and maybe this property even contributes to its spread. For the fully susceptible *Staphylococcus aureus*, the overall concordance was 100% for species identification and for most of the antibiotics tested. The ESBL production of the *Escherichia coli* strain was correctly identified by 90% of the laboratories.
Conclusion: This ARMed-EARSS EQA exercise showed that, overall, laboratories of the southern and eastern Mediterranean counties are capable of delivering susceptibility data of good quality and that can be compared between different laboratories and countries. The exercise has also allowed a number of participating laboratories to identify aspects of routine culture and sensitivity that need improvement can improve.

P1893

Evaluation of antimicrobial susceptibility of bacteria containing plasmid mediated qnrA and FOX-5 beta-lactamase by four automatic systems

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Objectives: Resistance to quinolones may be caused by plasmids expressing qnrA. These plasmids usually also code for beta-lactamases, including FOX-5. The accuracy of automatic systems for susceptibility testing of organisms expressing qnrA has not been assessed yet. The purpose of this study was to evaluate the performance of four automated instruments for susceptibility testing to fluoroquinolones and beta-lactams of four clinical isolates of *Klebsiella pneumoniae* (UAB1, N5, 1960 and 1132) producing both QnrA and FOX-5 and their respective four *Escherichia coli* transconjugants. No transconjugants containing qnrA from strain 1132 are available.
Methods: The automatic systems BD Phoenix, MicroScan WalkAway, Vitek-2 and Wider were used, according to manufacturer's instructions. MICs of ciprofloxacin (CIP), nalidixic acid (NAL), norfloxacin (NOR), ofloxacin (OFL), cefoxitin (FOX), ceftazidime (CAZ), cefotaxime (CTX) and ampicillin (AMP) were determined by microdilution according to NCCLS guidelines. MICs obtained either by reference microdilution or by the automatic systems were translated into clinical categories according to the NCCLS.
Results: Considering quinolone testing, very major errors (VME, false susceptibility) were observed for NAL in Phoenix for N5 and transconjugants derived from UAB1, N5 and 1960, and in Wider for the same strains and transconjugants and UAB1. VME were also noted for OFL and NOR in Vitek-2 for strain UAB1. Major errors (ME, false resistance) were only observed for CIP and both Phoenix and Wider for 1960. Minor errors (intermediate with one method and resistant or susceptible with the other method) were noted with all 4 systems for at least one quinolone/strain combination. When testing beta-lactams, VME were only found for CAZ when testing the transconjugant from UAB1 with Phoenix or Wider. One ME for CTX was observed when testing N5 with Phoenix. Minor errors were noted with all 4 systems for CAZ (15 out of the 32 performed tests) and with CTX (3 out of 32 tests). No errors were found for FOX or AMP with any system.
Conclusion: Automated systems are not completely reliable for establishing clinical categories of quinolones against *K. pneumoniae* strains or transconjugants derived from them producing QnrA. While these systems are reliable for detecting FOX resistance usually associated to QnrA production, there are also problems for detecting resistance to CAZ or CTX in these strains.

P1894

Direct susceptibility testing of Gram-negative rods from positive BacT/Alert blood cultures by using disc diffusion method

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Objectives: To evaluate the accuracy of direct antimicrobial susceptibility testing of aerobic gram-negative rods (GNRs) from positive BacT/Alert blood cultures by using disk diffusion method compared to standardized testing with isolated colonies.
Methods: Our study was carried out over a 10-months period (1–10/2004) and included 50 positive BacT/Alert blood cultures showing gram-negative rod-like morphology in Gram smears and yielding monomicrobial aerobic GNRs in subculture. A 8-ml sample from the positive blood culture bottle was aspirated into a serum separator tube and centrifuged at 3,000 × g for 10 min. The supernatant was discharged and the bacterial pellet was harvested from the surface of the gel with a cotton swab to make a suspension of McFarland 1.0 standard. Then, susceptibility tests to 15 antibiotics were done by disk diffusion method (NCCLS guidelines). Identification to species level and susceptibility testing with isolated colonies were also performed for comparison. The results were analysed for categorical agreement (discrepancies were categorized as minor, major and very major errors).
Results: Of the 50 GNRs isolated, 45 (90%) corresponded to the family Enterobacteriaceae and 5 (10%) were non fermenters. A total of 750 antimicrobial-organism combinations were interpreted. Thirty six of 50 (72%) gram-negative isolates showed complete categorical agreement. Analysis of errors by antimicrobial agent yielded 16 (2.1%) minor, 1 (0.13%) major, and 1 (0.13%) very major errors. An overall categorical agreement of 97.6% for the drugs tested was observed and discrepancies were occurred with cephalothin (6, 0.8%), ampicillin (4, 0.53%), amoxicillin–clavulanic acid (3, 0.4%), ampicillin–sulbactam (3, 0.4%), and piperacillin (2, 0.27%).
Conclusions: The direct susceptibility testing of aerobic GNRs isolated from positive BacT/Alert blood cultures by using disk diffusion method with a centrifugation step performed well since high categorical agreement and low error rates were noted. The direct method cannot replace the standard procedure, but given the benefits on patient care the laboratories should evaluate the possibility of applying this method for routine use.

P1895

Direct susceptibility testing of positive BacT/Alert blood cultures with and without charcoal by using MicroScan panels

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Objectives: The rapid and reliable detection of bloodstream infections, including bacterial identification and antimicrobial susceptibility testing is one of the most important tasks of clinical microbiologists. We performed a prospective study in which MicroScan panels were directly inoculated with different BacT/Alert blood culture broth (with and without charcoal), and susceptibility and identification results were compared with those obtained by standard inoculation method.
Methods: A total of 110 positive aerobic blood cultures collected from patients suspected of having bacteriemia were studied. Forty-four were standard aerobic media (SA) and 66 with charcoal aerobic (FA) and paediatric (FP) media. Specimens that yield more than one microorganism in subculture control plates were discharged. MicroScan Combo Negative 1S and Positive 2SA (DADE BEHRING, California, USA) were used for gram-negative and gram-positive isolates, respectively.
Results: Forty isolates were gram positive cocci and 70 isolates were gram negative bacilli. Seven (17.5%) gram positive cocci strains were misidentified by the direct method, all of them being coagulase negative. All gram negatives rods (n = 58) belonging to Enterobacteriaceae family were correctly identified by the direct method (DM). The DM yielded 3 (0.25%) very major, 6 (0.5%) major, and 34 (2.8%) minor errors for gram-negative rods and 14 (2.2%) very major, 10 (1.6%) major, and 11(1.7%) minor errors for gram-positive cocci. When we compared categorical agreements for blood culture media with and without charcoal, no differences were found for gram positive cocci (94.7% vs 94.4%), but statistical differences ($p < 0.05$) were detected for gram negative bacteria (overall errors rate were of 4.3 and 2.2% for blood culture medium with and without charcoal, respectively).
Conclusion: We found that direct inoculation of microScan panels provide an acceptable bacterial identification and susceptibility testing, in comparison with standard method, when gram negatives bacilli were observed by gram-stain of blood culture, and subculture plates show monomicrobial culture. As we have detected significant differences between culture medium with or without charcoal, the latest should be avoided whenever possible. This method improves significantly the turnaround time of blood cultures reports.

P1896

A novel gradient technique for combination testing: configuration and applications

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Objectives: Combination therapy regimens of serious infections are seldom guided by in vitro testing due to the lack of simple yet reliable quantitative technique. Currently used methods such as checkerboard titrations (CT) are non-standardised and imprecise while kill curves are cumbersome to set up and interpretations remain arbitrary. A novel gradient technique, Xact™ (AB BIODISK) for quantification of drug interactions is described using a trim/sulpha (TR+SU) synergy model and other drug combinations/applications are also addressed.
Method: Xact consists of a 50 mm or 75 mm square carrier with a gradient quadrant of two or more test drugs in perpendicular alignment that gives a predefined concentration pattern with 225 or 484 unique drug ratios. Xact gradients for TR + SU across 0.016–256 and 0.001–2048 μg/mL were compared to CT using 2 *E. coli*, 1 *E. cloacae*, 1 *S. aureus* and 1 *S. epidermidis* tested in 10 replicates. The carrier was placed onto an inoculated agar surface and left for 1 hour to transfer the gradient imprint to the agar after which the carrier was removed. After overnight incubation, FICIs (Fractional Inhibitory Concentration Indices) were read where the inhibition isobologram intersected the predefined FICI grid. Xact was used with other drug/organism models to evaluate potential applications for yeast, rapid growing *Mycobacteria* (RGM) and for bactericidal interactions by relica-plating of the inhibition isobolograms.
Results: Maximum synergy was seen at MIC:MIC ratios of TR+SU for all strains with excellent reproducibility of FICIs (n = 50, SD. of <25% of the mean FICI value at 95% CI.) and good agreement with CT. Models for yeast and RGM showed various interactions with the antifungal and antimycobacterial

combinations studied. Xact could be used to demonstrate bactericidal interactions using the replica plating method.
Conclusion: The novel gradient technique was equivalent to CT as assessed with a TR + SU synergy model. It is simple to use to quantify interactions over a wide range of drug ratios. It could also be used for yeast and RGM testing and was able to demonstrate bactericidal interactions. Xact deserves further investigation as a potential tool for combination testing.

Surveillance of nosocomial infections

P1897

Surveillance of nosocomial infection: extension, organisation and methods in European countries and regions

J. Fabry, I. Russell, A. Savey, C. Suetens *and the coordinators of the Surveillance Networks*

In the context of European harmonisation of communicable diseases surveillance, EU countries and Norway were surveyed to assess the status of surveillance of nosocomial infections (NI) nationally and/or regionally. The questionnaire was completed by the network's coordinators. Twenty-six networks were created in 17 countries (or regions) between 1992 and 2003, and were still functioning in September 2004. Six countries targeted Surgical Site Infections (SSI) only, one infections in ICU patients only and ten in both. Nineteen networks in 14 countries use a HELICS-compatible protocol and already contribute to the HELICS European database. Other countries or regions are initiating incidence or prevalence pilot surveys, with the objective of implementing a stable HELICS-associated network. Usually a national Public Health Institute runs the programme, sometimes in connection with other bodies. Around 2000 European hospitals participate (usually voluntarily) in a structured surveillance network, yielding an estimated coverage of 30%. Half of them receive funding from their Ministry of Health, with 8 receiving additional funding from a health agency, hospital organisation or from hospitals themselves. The funding covers expenses of information technology and variable levels of coordination staff in the networks. Except in Germany, the data collection in ICU networks is patient-based. Only 6 out of 16 SSI networks organise post-discharge surveillance (PDS). Surveillance is continuous in a few cases, with others being discontinuous. Data is entered locally in all networks, except in 4 where it is entered at the coordinating centre. Control of data quality is always performed but with different means and scope. Surveillance of NI is expanding in Europe and the HELICS programme has allowed the progressive harmonisation of data. However, persisting discrepancies should be addressed: (1) resources required for coordination and data collection should be defined more precisely, (2) the need for training materials and exchange of experience between networks, (3) collaborative, multi-centre research should address the unresolved problems of PDS and (4) monitoring of the validity of data.

P1898

The HELICS Database Management Software project: electronic exchange of nosocomial infection surveillance data across Europe

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Objectives: As defined in the HELICS Database Management Software (HDBMS) project, software was developed to enable input, analysis and export of Nosocomial Infection (NI) surveillance data at the hospital level and to collect, validate and prepare data at National Coordinating Centers (NCC), the HELICS Coordinating Center (HCC) and the Collaborating Centers (COCs).
Methods: Following the HELICS data-flow organization and data specification rules contained in the Operating Manual and the ICU and SSI protocols, development relied on a joint effort of the HCC and COCs in Lyon, Brussels and Berlin. Development using available IT-resources within COCs will also facilitate helpdesking for NCCs and future updates.
Results: In support of hospitals participating in HELICS surveillance, the HELICSwin software was developed. This Microsoft Access-based application enables manual input of data of surveillance of ICU-acquired infections and surgical site infections, as well as analysis and export of data for send-up to the NCC. A separate module supports the automatic import of surveillance data from Microsoft Excel format into HELICSwin.In support of NCCs organizing HELICS surveillance, dedicated STATA-tools were created which enable processing of incoming HELICSwin export data, the validation and analysis of these data as well as preparation for send-up to the HCC in Lyon. In addition, the HELICSval software enables NCCs and the HCC to control, validate and prepare HELICS data. This Microsoft .NET based application processes NCC data in ASCII format to validate – giving detailed feedback if these do not conform data to specifications – and convert these to the XML-format (Extensible Markup Language) for send-up to the HCC. HELICSval also allows the HCC to append incoming XML data into a Microsoft SQL Server Relational Database, which was set up for collected HELICS data to be stored in a secured and authorized manner, thus constituting the European database of NI Surveillance data. Furthermore, to guarantee data confidentiality and authenticity, the HELICS Data Exchange Specification was set up, describing the procedure of data flow between the NCC and HCC as well as between the HCC and the COC. The GnuPG application was chosen for encryption and signature of data files to be exchanged.
Conclusion: In guaranteeing HELICS data input, validation, analysis and exchange at the hospital, network and European level, the HDBMS will contribute considerably to achieving the objectives of the HELICS project.

P1899

Surgical site infections in the Helics network: results from 11 European surveillance networks

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Objective: Surveillance of surgical site infections (SSI) is a priority in many countries. However, comparability of results from different national or regional networks is uncertain. An objective of the Helics cooperation is to enhance comparability of SSI surveillance for selected surgical procedures using uniform data collection protocols.

Methods: A protocol was agreed between the partners in 2003. Data collected by the networks from 2000 through 2003 were translated into this common protocol. Infection rates were expressed as the proportion of surgical procedures that led to SSI. In addition, the incidence was expressed as incidence density: the number of in-hospital SSI per 1000 patient-days prior to infection. This takes account of the variation in follow-up period associated with different approaches to, or absence of, post-discharge surveillance. All incidences were calculated by surgical procedure and by NNIS risk index group. Compatibility of data with the protocol and differences between countries were explored.
Results: Data on surgical procedures (n = 169,575) from Belgium, Finland, France, Germany, the Netherlands, Spain, Poland and the UK (England, Northern Ireland, Scotland and Wales) were received. Compatibility with the protocol was high, over 90% for mandatory or required variables (such as components of the NNIS risk index). Most countries had a high completeness of data for the components of the basic NNIS risk index (wound contamination class, ASA classification and duration of operation) while the reporting of endoscopy data was less homogeneous. Overall incidence was 6.4 SSI (detected in-hospital) per 1000 patient days for colon surgery, 3.6/1000 for caesarean section, 2.8/1000 for cholecystectomy, 2.7/1000 for CABG, 1.7/1000 for hip prosthesis and 0.6/1000 for laminectomy. For most surgical procedures the SSI incidence increased with increasing NNIS risk index levels, except for CABG. Inter-country differences related to variation in the types of procedures undertaken (e.g. in hip prosthesis), scoring of the NNIS risk factors and the reporting of superficial SSI's were observed.
Conclusion It is feasible to pool data on SSI from different European countries and strict adherence to common protocols makes comparisons meaningful. The data has also highlighted interesting differences between countries, however, some of these are likely to be related to healthcare provision and caution should be used when comparing results between countries.

P1900

Results from the minimal dataset of the European surveillance of ICU-acquired infections (HELICS-ICU), 2000–2004

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Objective: Data from 8 national or regional networks for ICU-acquired infection surveillance (Austria, Belgium, Germany, Spain, France-SE, Luxemburg, The Netherlands and Portugal) were pooled in order to assess the compatibility with the HELICS-ICU (HI) protocol (http://helics.univ-lyon1.fr), to analyze indicators issued by the minimal data set (unit-based ''level 1'' surveillance) and to explore inter-country differences.
Methods: Seven patient-based and 1 unit-based (Germany) surveillance networks contributed data on 243,817 patients, 8479 episodes of ICU-acquired pneumonia (PN) and 4029 bloodstream infection (BSI) episodes from 617 ICUs between 2000 and June 2004. Since the HI protocol excludes patients with a length of stay (LOS) of <3 days in the ICU, data from Germany (including patients with LOS of <3 days) were excluded for the calculation of indicators, but included for the description of infections.
Results: The overall percentage of non-missing values for minimal HI data was 88%. Of 82,996 patients staying more than 2 days in the ICU, 6.8% acquired a PN and 3.1% developed a BSI in the ICU. The crude incidence was correlated with LOS in the ICU (Spearman correlation coefficient PN 0.73; BSI 0.74) and the percentage of intubated patients, a proxy of severity of case-mix (Spearman PN 0.54; BSI 0.47). The overall incidence density was 8.4 PN episodes per 1000 patient-days (pd) (distribution ICUs P25 3.4; P50 7.3; P75 11.9) and 3.9 BSI episodes/1000 pd (P25 1.9; P50 3.5; P75 6.0). The incidence density varied from 5.3 PN and 2.6 BSI/1000 pd in ICUs with < 30% intubation to 10.8 PN and 4.7 BSI per 1000 pd in ICUs "d 60% intubation. The most frequently reported micro-organisms were *P. aeruginosa* (18%), *S. aureus* (16%), *Candida* sp. (8%), *Klebsiella* sp. (7%) and *Enterobacter* sp. (7%) in PN and coagulase-negative *Staphylococci* (25%), *S. aureus* (13%), *Enterococcus* sp. (12%), *P. aeruginosa* (9%) and *Candida* sp. (8%) in BSI. Prospective data (2003–2004) showed that the diagnosis of PN was confirmed by quantitative culture in 85% in FR, 62% in ES and 22% in BE.
Conclusion: Although underlying differences in diagnostic practices persist, the compatibility with the HI protocol was already quite high (while further adaptations are made) and allowed for drawing first EU reference figures. Level 1 HI data provide sufficient indicators for continuous follow-up of infection rates within the ICU and limited risk-adjusted inter-ICU comparisons with a low workload.

P1901

Nosocomial infections survey in a university hospital: results of the last 5 years

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Objectives: Nosocomial infections develop in at least 5% of patients admitted to hospitals. The first aim of surveillance is to determine endemic rates of infection. In this study nosocomial infections surveillance results of last 5 years in a university hospital were evaluated.
Methods: The study was performed using a computer based surveillance program. 50155 patients were survived from January 2000 to October 2004. Infection control nurse and infectious diseases specialist analysed the patients by active surveillance method. The diagnosis of nosocomial infections was established according to Center for the Disease Control and Prevention criteria. The data were collected using a computer program (NosoLine-Hospital Infections Society of Turkey).
Results: A total of 3226 nosocomial infection episodes were detected in 5 years period. The rate of nosocomial infections was detected as 6.07% for 2000, 6.01% for 2001, 8.08% for 2002, 6.86% for 2003 and 5.84% for 2004 in Osmangazi University Hospital. The most common nosocomial infections were bacteraemia (28.74%), surgical site infections (21.95%), urinary tract infections (21.79%) and respiratory tract infections (13.01%). The most common microorganisms isolated from nosocomial infections and their isolation rates for each year respectively were found as *Acinetobacter* spp (20.53%, 22.87%, 18.5%, 21.77%, 17.5%), *Pseudomonas* spp. (21.3%, 16.8%, 11.47%, 11.37%, 12.94%), *Staphylococcus aureus* (19.75%, 22.22%, 23.72%, 17.99%, 13.21%). *Candida* spp isolation rates for each year from 2000 to 2004 were found as *C. albicans* (1.1%, 0.26%, 8.93%, 6%, 10.09%) and non-albicans *Candida* spp (1.26%, 0.39%, 3.16%, 5.38%, 6.07%). The most common probable risk factors were found as transfusion (68%), urinary catheterization (64.9%), vascular catheters (55.8%) H_2 blocker 55.2%, nasogastric tubes 40.4%, various operation drains (27.8%), and mechanical ventilation (27.1%).
Conclusion: The surveillance results of nosocomial infections in our hospital pointing out the most common three bacteria as well as increasing problem of *Candida* infections.

P1902

Prevalence of nosocomial infections in Italy: results of the INF NOS Project in 2003

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Introduction: Hospital acquired Infections (HAI) represent an important cause of morbidity and mortality in patients admitted to hospitals. Surveillance is the main tool for driving programmes for the control of HAIs. The INF NOS Project, which started in 2001, consists of repeated yearly prevalence surveys of HAIs and associated risk factors in a sample of Italian hospitals.We report here the results for the year 2003.
Methods: Intensive Care Units (ICU), surgical and medical wards of the participating hospitals were enrolled. All adult patients present in the participating wards on the day of the survey were screened for presence of a HAI and/or of risk factors for HAI. CDC criteria and definitions of HAI were adopted.
Results: In October–November 2003, 41 Italian hospitals participating in the INF NOS Project surveyed 2813 patients. Male patients were 53.7%, the mean age was 64.2 years (SD 18 years), the mean hospital stay was 7.8 days. Co-morbidities were detected in 73.7% of patients; the most frequently encountered being cardiovascular diseases (42.1%), cancer (20.6%), chronic pulmonary diseases (18.6%) and diabetes (15. Invasive procedures were described in 46.1% of patients, 98.4% in ICUs and 23.7% in medical wards. The HAI-associated invasive procedures were surgery (30.9%), urinary catheter (25.6%) or central venous catheter (12.1%). Overall observed prevalence of HAI was 6.4% (95% CI 5.1–6.8) involving 5.7% (95% CI 4.8–6.6) of the patients surveyed. The prevalence of infected patients in ICU, surgical and medical wards was 43.4% (95%CI 28–42), 2.5% (95%CI 1.7–3.5) and 4.6% (3.4–5.7), respectively. The most frequent HAIs detected were pneumonia (22.9%), urinary tract infections (17.9%) and sepsis (16.8%), *Staphylococcus* spp (16.3%), MRSA (10.7%), *Pseudomonas aeruginosa* (15.7%) and Enterobacteriaceae (15.1%) were the main isolated pathogens in HAI.
Conclusions: The INF NOS Project represents a shared tool for HAI surveillance, allowing comparisons between participating hospitals. The 2003 INF NOS survey results confirm rates of HAIs comparable with those of other studies with the exception of a higher rate of lower respiratory tract infections, the latter result due to a likely overrepresentation of ICUs in the INF NOS sample.

P1903

The prevalence of hospital-acquired infections in a Danish county

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Objectives: To estimate the prevalence of hospital-acquired infections (HAI) and the prescription of antibiotics at the hospitals in the County of Aarhus, and to compare the results with previous surveys.
Methods: Only patients from departments open seven days a week were included. The prevalence-survey took place in September 2003 and comprised 1510 patients. Teams consisting of a clinical microbiologist and an infection control nurse/worker visited the departments on a particular day. They registered for all patients hospital-acquired and community-acquired infections. Data on antibiotic treatment and microbiological findings were furthermore registered.
Results: The overall prevalence of HAI in hospitals in Aarhus County was 10.0% and ranged for the various medical specialities from 7 to 28%. The relative distribution of the most frequent HAI was urinary tract infection (33%), postoperative wound infection (19%), pneumonia (12%), skin infection (10%), and blood-stream infection (9%). Blood-stream infection and pneumonia were more prevalent in ICU patients. A total of 456 patients (30.2%) received antibiotics at the prevalence-day. Antibiotics were given prophylactic in 64 (14%) of the patients receiving antibiotics. The relative distribution of prescriptions of the various groups of antimicrobial agents was penicillins (42%), cephalosporins (17%), metronidazoles (9%), quinolones (8%), aminoglycosides (8%), sulphonamides/trimethoprim (5%), antimycotics (4%), and glycopeptides (2%). Beta-lactam drugs accounted for more than half the prescriptions of antimicrobial prophylaxis.
Conclusion: The prevalence of HAI in 2003 was similar to that of a previous prevalence-survey in 1999. Fewer infections, however, were seen in intern medicine and more in abdominal surgery (almost a doubling of HAI) in 2003.Compared to a prevalence study in a single hospital in Aarhus County in 1994, the prevalence of antibiotic prescriptions in that hospital has increased. The relative use of the various groups of drugs in the hospital has also changed with a substantial increase in prescriptions of cephalosporins and quinolones and a decrease in use of sulphonamides and in antimicrobial prophylaxis in general.

P1904

Incidence study of nosocomial infections in ten intensive care units in Greece

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Objectives: To determine the incidence of nosocomial infections, the device utilization ratios and the device-associated infection rates in the intensive care unit setting.
Methods: A prospective study for the surveillance of nosocomial infections was conducted among ICU patients in seven general hospitals in Greece. Intensive care units from three hospitals in Athens, one in Heraklion, one in Thessaloniki, one in Alexandroupolis and one in Ioannina participated in the study. Each ICU was surveilled for a minimum period of six months. Data collection included patient demographics, site of infection, date of onset of infection, ventilator, central venous catheter and urinary catheter use, total length of stay in the ICU and outcome. Device utilization ratios were defined as the number of device-days/ number of patient days. Infection rates are also presented as incidence densities, by using as a denominator urinary catheterization, central vascular catheterization or ventilation days.
Results: The overall incidence of nosocomial infections was 34/1000 patient-days. Bloodstream infections were the most common nosocomial infections (14/1000 patient-days), followed by pneumonias (11.5/1000 patient-days), urinary tract infections (4.8/1000 patient-days), surgical site infections (1.5/1000 patient-days) and other sites of infection (2.3/1000 patient-days). The central line utilization ratio was 0.93, the ventilator utilization ratio was 0.83 and the urinary catheter utilization ratio was 0.97. The central line-associated bloodstream infection rate was 15/1000 central line-days. The ventilator-associated pneumonia rate was 13.8/1000 ventilator-days and the urinary catheter-associated UTI rate was 4.9/ 1000 catheter days.
Conclusion: Estimation of site-specific infection and device utilization ratios is a useful tool for comparison and improvement of quality within ICU's. Incidence studies to calculate infection rates and device utilization ratios in the ICU's have not been previously performed in Greece. This study is the first effort to calculate infection and device utilization ratios in ten ICU's in our country

P1905

Hospital infections in a Brazilian neonatal intensive care unit

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Objectives: To describe the incidence and etiology of nosocomial infections in the neonatal intensive care unit of Santa Casa Complexo Hospitalar, Brazil.
Methods: Prospective study conducted during the period comprising October 2003–October 2004 in a large Brazilian neonatal intensive care unit. During this period, active surveillance for nosocomial infections were performed by infection control nurses, using the criteria proposed by the Centers for Disease Control and Prevention (CDC) and National Nosocomial Infections Surveillance (NNIS) methodology.
Results: During the period of study, a total of 7,774 patient day were followed by active surveillance. Among those, 655 patients admitted to the neonatal intensive care unit, 124 developed nosocomial infections (18.9%). Table 1 shows the topography of these infections. The etiology of the nosocomial infection was obtained in 41cases, as shown in table 2.

Table 1. Topography of nosocomial infection in the ICU of Santa casa complexo Hospitalar, Brasil

Major specific site	n	(%)	Rate of noso comial infections/ 1000 patients per day
Bloodstream infection	66	53.2	8.5
Gastrointestinal system infection	14	11.3	1.8
Pneumonia	9	7.3	1.2
Central nervous system infection	8	6.4	1.0
Surgical site infection	7	5.6	0.9
Cardiovascular system infection	5	4.0	0.6
Skin and soft tissue infection	5	4.0	0.6
Lower respiratory tract infection, other than pneumonia	4	3.2	0.5
Eye, ear, nose, throat, or mouth infection	4	3.2	0.5
Urinary tract infection	1	0.8	0.1
Systemic infection	1	0.8	0.1
Total	124	100.0	15.9

Table 2. Etiology of nosocomial infection in the ICU of Santa casa complexo Hospitalar, Brazil

Organism	n	(%)	Rate of nosocomial infections/1000 patient day
Staphylococcus epidermidis	21	51.2	2.6
Candida spp.	6	14.6	0.8
Klebsiella oxytoca	3	7.3	0.4
Klebsiella pneumoniae	4	9.8	0.5
Escherichia coli	2	4.9	0.3
Acinetobacter baumanni	1	2.4	0.1
Enterobacter cloacae	1	2.4	0.1
Enterococcus spp.	1	2.4	0.1
MRSA *(Staphylococcus aureus)*	1	2.4	0.1
Pseudamonas aeruginosa	1	2.4	0.1
Total	41	100.0	5.3

Conclusions: This study has documented the incidence of nosocomial infections in a single large Brazilian neonatal intensive care unit, and the main sites and etiologies of nosocomial infections in these patients. Most of these infections were caused by gram-positive strains (56.1%), followed by gram-negative strains (29.3%), and fungi (14,6%). *Staphylococcus epidermidis* was the most prevalent organism (51.2%). Since the epidemiology of nosocomial infections shows large variations in different institutions, it is very important to characterize local epidemiology.

P1906

Nosocomial infections in neurology intensive care unit of a university hospital

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Objectives: To determine the types of nosocomial infections and the frequency and resistance rates of pathogens isolated from neurology ICU in a university hospital.
Materials and Methods: Between March 2002 and April 2004, type of nosocomial infection and risk factors for patients were determined in neurology ICU patients prospectively. The identification and susceptibility to antibiotics has been performed Vitek 2 as described by the NCCLS.
Results: Ninety-six nosocomial infections were diagnosed in 89 patients. Of the patients 53(60%) were male. Mean age was 59 years. Isolates from ICU patients were recovered from urinary tract infection 42(47%), primary bacteraemia 25 (28%), nosocomial pneumonia 11(12.3%), secondary bacteraemia 7(7.9%), decubitus infection 2(3%) and meningitis 1(1.5%). 42 patients with nosocomial urinary infection had 53 episodes. Five of them were bacteremic. Candidemia was determined in 7(7.9%) of them. Nosocomial urinary infection was the most frequently observed infection type in neurology ICU. Primary bacteraemia was the second reason of infection in ICU. 62.5% of the pathogens were gram-negative, 26.5% were gram-positive bacteria and 11% were *Candida* species. The most frequently isolated gram-negative bacteria was *E. coli* (24%), followed by *Pseudomonas* spp. (18%), *Acinetobacter* spp. (12.5%) and *K. pneumoniae* (17.3%). In nosocomial urinary infections, *E. coli* (35.7%), *P.aeruginosa* (26.7%), *Candida* spp (14.2%) and *A. baumannii* (8.9%) were the most frequently isolated pathogens. Coagulase-negative *Staphylococci* (21.7%) and *S. aureus* (34.7%) were the most common pathogens isolated in primary bacteraemia. The rest of the pathogens were *Acinetobacter* spp. (21.7%) and *Candida* spp (8.6%) Resistance to antimicrobials in *E. coli* strains were as follows: ampicillin 94%, quinolone 81%, piperacillin 81% and cefepime 5%. High resistance rate was determined in *Acinetobacter* spp to quinolones (60%) and aminoglycosides (40%). The most effective antibiotics against *Acinetobacter* spp were cefepime (80%) and imipenem (90%). Resistance to antimicrobials in *Pseudomonas* spp were as follows: quinolones (23%), aminoglycosides (23%), ceftazidime (31%), carbapenems (8%).70% of the *S. aureus* and CNS strains isolated in our study were found to be methicillin-resistant.

Number of Pathogens is olated from Neurology ICU

Microorganism	Number (percent)
E. coli	20 (%24)
P. aeruginosa	15 (% 18)
A. baumannii	10 (%12.5)
S. aureus	10 (%12.5)
Candia spp.	9 (%11)
Coagulase negative Staphylococcus	6 (%7)
Enterococcus spp	6 (%7)
K. pneumoniae	5 (%6)
S. maltophilia	1 (%1)
Burkholderia cepacia	1 (%1)

P1907

Hospital infection surveillance in a neurosurgical intensive care unit

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Objectives: Hospital infection surveillance by the infection control team (ICT) was carried out in the Neurosurgical intensive care unit (Neurosurgical-ICU) of the teaching hospital Policlinico Umberto I in Rome.
Methods: Starting from 1.1.2002, all patients admitted in the Neurosurgical-ICU for ≥48 hours were included in the study. According to CDC case definitions we considered the following site-specific infections: blood stream infections (BSI), pneumonia (PNE), urinary tract infections (UTI), surgical site infections (SSI) and prosthesis infections (PI). Furthermore risk factors (i.e. age, sex, ASA), invasive procedures (i.e. endotracheal intubation,

vascular and urinary catheterisation), microbiological isolates and their antibiotic susceptibility were screened.
Results: An overall of 318 patients (156M and 162F) admitted in the Neurosurgical-ICU were investigated. The mean age was 53.4 ± 17.6 years (range 1–91), the length of stay in the Neurosurgical-ICU 26.1 ± 30.0 days (range 2–284), the ASA score was 2.86 ± 1.47, the Infection Risk Index (IRI) 1.40 ± 0.73. Crude mortality was 16.4%. Overall, 61 (19.2%) patients developed 111 Neurosurgical-ICU acquired infections, 34 BSI, 34 PNE, 25 UTI, 10 SSI and 8 PI during the study period. The general wound infection rate was 5.3% (6.1% for craniotomy). Also infection rates associated to invasive procedures were considered. CVC-associated BSI rate 7.2/1000, Ventilator-associated PNE rate (9.0/1000), Urinary catheter-associated UTI rate (4.2/1000). Wound infection was significantly ($p < 0.01$) associated to external ventricular drainage. Overall mortality associated with infection was RR 1.87; 95% CI 1.11–3.14; $p < 0.02$. During the study period, we observed a general infection rate decrease from 19.5% in 2002 to 13.1% in 2004 (particularly SSI). Among the infected patients, the most common microorganisms isolated were *Pseudomonas* spp. (27.6%), *Klebsiella* spp. (10.2%), *E. coli* (7.7%), *Candida* spp. (12.2%), MRSA (9.0%), CNS-MR (6.4%), *Enterococcus* spp. (5.8%) and others (21.1%).
Conclusions: Although the overall wound infection rate was high (5.3%) and risk associated with external ventricular drainage ($p < 0.01$), however the study showed that following the ICT activity an infection reduction was achieved. On the contrary, infection rates associated to invasive procedures were not high. Surprisingly most microorganisms responsible for infection were Gram(rather than Gram+.

P1908

Which isolates from ICU patients are most likely to cause nosocomial infections? Data from two surveillance systems in ICUs: SARI (Surveillance of antimicrobial use and antimicrobial resistance) and KISS (German hospital infection surveillance system)

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Objectives: To analyze the percentage of pathogens recorded in the SARI data base that are responsible for causing nosocomial infections and to analyze whether there are differences between different pathogens.
Methods: All SARI-ICUs also take part in KISS. KISS prospectively collects data on device-associated primary bloodstream infections, urinary tract infections, and pneumonia. Up to 4 pathogens can be recorded for each infection. SARI-ICUs collect data on the 13 most important pathogens responsible for nosocomial infections. Isolates are non duplicate and are not differentiated according to whether they have caused infection or colonisation. The analysis covered the period 02/2000–12/2003. Data was stratified by pathogen and type of infection.
Results: From 02/2000–12/2003, 36 ICUs collected data on 448,275 patient days and 41,989 pathogens and reported these to SARI. In KISS, 4,669 pathogens associated with nosocomial infection were reported. Approximately, 10% of all the pathogens isolated and reported to SARI caused one of the three commonest nosocomial infections. MRSA was responsible for 10.9%, MSSA for 8.7%, *P. aeruginosa* for 11.7% and *E. cloacae* for 14.8% of the infections. *S. maltophilia* was only responsible for 5.8% of the cases of nosocomial infection, while at 27.4%, *Klebsiella* was responsible for the highest infection rate.
Conclusion: Nosocomial infections caused by pathogens isolated from ICUs patients ranged from 5.8% (*S. maltophilia*) to 27.4% (*K. pneumoniae*). MRSA was responsible for nosocomial infections in 10.9% of the cases.

P1909

Epidemiology and outcome of community-acquired and nosocomial bloodstream infections at a Veterans Affairs medical centre, San Juan, Puerto Rico

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Background: Bloodstream infections (BSI) remain an important cause of morbidity and mortality. The true incidence of nosocomial BSI is unknown but has been reported as low as 15% and as high as 36% in bone-marrow transplant units. Overall mortality from BSI is estimated at 22–29%.
Objectives: 1) To prospectively determine the epidemiology and outcome of BSI at the San Juan VAMC; 2) To determine the variables most strongly associated to mortality in BSI; and 3) To recommend strategies for decreasing mortality associated to BSI.
Methods: A prospective review of all BSI is being performed at the San Juan VAMC. All positive blood culture (B/C) results are collected on a daily basis. The patient's charts are reviewed collecting data prospectively which includes: demographic data, date of admission, date of B/C collection, date of 1st positive B/C, clinical conditions such as temperature, blood pressure, WBC count and presence of central venous catheter. Blood cultures are followed until final organism identification. Outcome of patients with positive B/C results are followed until the patient is discharged from the hospital, the patient's death or up to 30 days after the date when the B/C was collected, which ever occurs first. Determinations include: contaminant rate, distribution of bacteremias (community vs. nosocomial), attack rate, outcome, crude mortality and attributable mortality. Statistical analysis will be performed.
Results: A total of 66 episodes of bacteraemia were documented in 59 patients admitted to the hospital. In 14 of the 59 patients the bacteraemia was considered as a contaminant and for the remaining 45 it was considered a true bacteraemia. In the 45 patients with true bacteraemia, 51 episodes were documented (53% community-acquired and 47% nosocomial). See Table I for data collected.

Table 1

Data	Result
Mean age	75 years
Contaminant Rate of Cultures	2% (absolute positivity rate 17%)
Community-Acquired BSI	53%
Nosocomial BSI	47% (absolute rate 3.2%)
Most Common Pathogen	MRSA Community Acquired (30%) Nosocomial (38%)
Most common community gram negative organism	*E. coli* 22%
2nd most common nosocomial organism	*K. peumoniae* 16%
3rd most common nosocomial organism	*C. parapsilopsis* (12%)
Mean time of bacteremia (from date of admission)	20.5 days* 75% occurred at >12 days of admission
Associated to central line	26%
Crude mortality in nosocomial BSI	39%
Crude mortality in community-acquired BSI	21%

Conclusions: In spite of adequate availability of antibiotics, BSI is still an important cause of morbidity and mortality. MRSA has become an important etiology in both nosocomial and community BSI. Although there is few studies available that review community-onset BSI, our data demonstrate that this is an important group with a significant associated mortality.

P1910

Nosocomial infections among earthquake victims in a backup university hospital after 1999 Marmara earthquake, Turkey

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Objectives: To analyze the epidemiology and risk factors for nosocomial infections in earthquake related trauma patients after the 1999 Marmara Earthquake.
Methods: Data regarding nosocomial infections and presumed risk factors were prospectively collected by a form prepared one day after the earthquake. All isolates were stored at −70°C freezer for further epidemiologic analysis. Pulsed field gel electrophoresis (PFGE) was used for molecular epidemiology of selected pathogens. Univariate and multivariate analysis were performed to identify risk factors for nosocomial infections.
Results: From a total of 476 patients referred to our hospital, 128 needed to be hospitalized 3 or more days and were included in the study. Eighty six (67%) of these patients developed crush syndrome. Prophylactic or preemptive sulbactam/ampicillin was given to 96 patients for a median 10 (1–72) days. At least one nosocomial infection was detected in 52 (41%) of these patients. The most frequent nosocomial infection was wound infection (%39), following blood-stream infections (16%), urinary tract infections (13%), pneumonia (12%), and others (19%). *Enterobacter* spp. was the most frequently isolated microorganism (24%), following *P. aeruginosa* (23%), MRSA (15%), *C. albicans* (12%), *A. baumannii* (6%), *Enterococci* (4%), *C. freundii* (4%), *E. coli* (3%), *S. maltophilia* (3%) and *Clostridium septicum* (1%). PFGE revealed that 4 clones of *Enterobacter* spp., 3 clones of *P. aeruginosa* and 1 clone of *E. faecium* were responsible for nosocomial outbreaks and suggests cross-contamination between patients in different wards. On univariate analysis crush injury, fasciotomy, amputation, duration of hospitalization, duration of antibiotic usage, type of ward and care giving team, hemodialysis, percentage of crushed body area, time under rubble were found to be significant risk factors for nosocomial infections. After logistic regression analysis only two risk factors were identified: fasciotomy (OR: 3.6, CI95, 1.1–12.0) and duration of hospitalization (OR: 12.1, CI95, 1.7–85.6). Eight of 12 deaths were infection related.
Conclusion: Fasciotomy significantly increases nosocomial infections and should be considered carefully. Prophylactic antibiotic use in earthquake related trauma patients seems to provide little benefit, but increases superinfections.

P1911

Analysis of risk factors for surgical site infections in a tertiary care hospital in Turkey

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Objectives: To identify the risk factors for the development of surgical site infections (SSI).
Methods: Prospective surveillance was conducted. Patients, whom underwent surgery between May–Oct 2003 were included in the study and evaluated before the surgery and followed 30 days after surgery for the development of SSI. SSI was defined according to CDC criteria. Potential risk factors were analysed by univariate and multivariate analysis.
Results: 245 patients were followed during study period. Mean age was 49.6 and 153 (62.4%) were female. SSI developed in 44 (18%) of them. After the surgery, SSI developed at an average of 8.6th day. 32% of the infections were diagnosed after discharge. Of the SSI, 50% were superficial, 40.9% involved an organ or space and 9.1% were deep. According to operation classification, the incidence of SSI was 6.3% in clean, 28% in clean-contaminated, 38.4% in contaminated and 53.3% in dirty. Antibiotic prophylaxis was given to 83.2% of the patients. The most commonly used antibiotic was cefazoline (75%). Procedures involving colon, small bowel and rectosigmoid were associated with higher rates of SSI (36.4%). A positive culture was obtained from 22 patients. *Escherichia coli* was the most frequently isolated microorganism (39.1%), followed by *Pseudomonas aeruginosa* (19.6%) and methicilline resistant *Staphylococcus aureus* (13%). Of the 22 positive cultures, 9 (40.9%) were polymicrobial. Factors associated with SSI found by univariate analysis ($p < 0.05$) included; age, diabetes mellitus, preoperative infection, emergent surgery, prophylactic antibiotic use more than 24 hours before the operation, longer duration of antibiotic prophylaxis, preoperative longer hospital stay, wound classification, ASA (American Society of Anestiology) score, NNIS (National Nosocomial Infections Surveillance) risk index, transfusion of blood and blood products, total parenteral nutrition, >2 comorbidity, hyperglycemia, anemia, number and duration of surgical drains and type of surgery. Logistic regression analysis showed that preoperative high blood glucose levels (OR 2.68; 95% CI 1.4–5.1, $p = 0.003$), wound classification (OR 2.04; 95% CI 1.3–3.1, $p = 0.002$) and duration of surgical drain (OR 1.17; 95% CI 1.1–1.3, $p = 0.0001$) were the most significant predictors of SSI.
Conclusion: SSI infection rate was high. Poor control of diabetes mellitus before surgery, dirty wound operations and prolonged duration of surgical drains significantly increased the rate of SSI.

P1912

The effect of performance feedback on wound infection rate in abdominal hysterectomy

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Objective: To ascertain the effect of an infection control program, using performance feedback, on wound infection (WI) rate in abdominal hysterectomy.
Methods: All patients with abdominal hysterectomy attended in our Centre between 1999 and 2003 were prospectively followed for 1 month after operation to know WI rate. CDC definitions were used. A complete set of tests including age, underlying illnesses, cancer, diabetes mellitus, immunosuppression therapy, albumin, ASA risk, inpatient preoperative stay, date of intervention, hygiene and preoperative antimicrobial prophylaxis, type of surgical operation, duration of surgery, surgeon, WI, were collected in each case. After data collection for 1999 concluded, we communicated every year surgical WI rates to surgeons (service and surgeon-specific). A logistic-regression analysis was performed to compare the WI rates with the observed in 1999.
Results: We recruited 831 females. Mean age, 50.6 ± 10.7 years, range 22–90.25% patients were cancer cases, 5.3% diabetes mellitus, 0.5% immunosuppression therapy, 26.8% ASA 1, 57.5% ASA 2, 14.4% ASA 3 and 1.2% ASA 4. In 7 (0.8%) patients surgery was done urgently and in 824 programmed, 724 in the morning and 100 in the afternoon. There were no differences between the years of study in the surgery with urgency, and in the frequency of risk ASA 3 and 4; the surgery in the afternoon was more frequent in the years 2000 (23.9%) and 2001 (19.1%) than in 1999 (12%), 2002 (6.9%) and no patient in 2003. The mean of days of preoperative stay was 2.6 ± 3.05, range 1–28. The

	1999	2000	2001	2002	2003
Albumin	3.35 ± 1.4	3.64 ± 0.9	3.60 ± 0.7	3.86 ± 0.3	3.88 ± 0.3
Prophylaxis	53/150 (35.3%)	41/163 (25.2%)	62/162 (38.3%)	33/173 (19.1%)	53/183 (29%)
W. Infection	16/150 (10.7%)	14/163 (8.6%)	8/162 (4.9%)	17/173 (9.8%)	12/183 (6.6%)

types of surgical operations were total abdominal hysterectomy 752 (90.5%), subtotal hysterectomy 53 (6.4%) and Wertheim in 26 (3.1%). In the multivariate model the factors associated with surgical WI were albumin (OR 0.97; IC 95%: 0.94–0.99) and the antimicrobial prophylaxis (OR 0.13; IC 95%: 0.04–0.43). The mean values of albumin and the number of patients with perioperative antimicrobial prophylaxis fluctuated between years. The surgical wound infection rate improved from 10.7% (IC95% 5.8–15.6) in the year 1999 as far as 6.6% (–38.3%) in 2003.
Conclusions: The effect of performance feedback to individual surgeons can reduce the infection rates of surgical wound infection.

Environmental impact on nosocomial infections

P1913

Water infiltration and fungal contamination: an environmental and mycological investigation

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Background: A wide variety of filamentous fungi may cause disease in compromised individuals. However, as fungal spores are ubiquitous, they may also contaminate laboratory cultures. A scanty growth of *Myriodontium* sp. was observed on several culture plates inoculated with specimens of sputum from different patients in Yorkhill Hospital, Glasgow. All of the specimens had been processed in the C L 3 laboratory. Although it was agreed that this mould was a probable contaminant, its continuing presence was a cause for concern.
Objectives: To identify the source of fungal contamination, take remedial action and eradicate the contamination.
Methods: (i) One-hour settle plates containing Sabouraud dextrose agar were placed at several sites within the C L 3 laboratory and in the corridor directly outside. (ii) A visual inspection of the laboratory was performed. (iii) Swabs were taken of the probable source of contamination (identified by (ii)).
Results: *Myriodontium* sp. was detected on all settle plates except those placed in the corridor outside the laboratory. Very few other moulds were evident. From site to site within the laboratory there was a gradient in counts of *Myriodontium* sp. with a 4-fold increase between the door area and the bench in front of the window. Counts of *Myriodontium* sp. on the settle plates in the safety cabinet exceeded those at all sites except the window area (a distance of 1 metre away). The visual inspection revealed a brown/black, moist area (85 × 15 cm) on a wooden ledge below the window. (This area was not readily visible as it lay behind a vertical timber support at the back of a bench.) A faint narrow line running down the adjacent wall indicated water penetration from above. Further investigation revealed that a leak in the roof was permitting slow but frequent penetration of rainwater into the laboratory. Swabs from the brown/black deposit yielded a heavy growth of *Myriodontium* sp. with a light growth of *Trichoderma* sp. and *Aspergillus niger*. Remedial action was taken in the laboratory and on the roof.
Conclusions: Infiltration of water into the C L 3 laboratory permitted fungal growth in a warm, moist environment. Spores drawn into the safety cabinet under negative pressure probably contaminated plates as they were being inoculated.

P1914

An attempt to detect the trace of faecal materials in drinking water of dairy farms around Tehran, Iran

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Objectives: Ther has been numerous reports of outbreaks of water born diseases in humans and farm animals throughout the world even from developed countries.This phenomenon demonstrates that transmission of pathogens by drinking water remains a significant cause of illness.
Materials: Water samples of reservoir tanks which received water from deep wells were taken from 28 dairy farms around Tehran-Iran. To distinguish total count and water pollution samples were cultivated on Agar blood and MacConkeys media. Positive samples to enterobacteriace were followed by biochemical tests.
Results: Total counts were reported from 20 to 1200 bacteria per milliliter.18(64%),16(57%), 14(50%) 6(21%), 5(17%), and 1(3.5%) out of 28 samples were positive to *E. Coli*, *Aerobacter aeroginosa*, *E. coli+A.aeroginosa*, *Salmonella typhimurium*, *E.Coli+A. Aeroginosa+S. typhimurium* and *Shigella dysentria* as well as *E. Coli+ A. Aeroginosa+S. dysentria* respectively.
Discussion: Presence of coliform group specially *E. Coli* is used to determine water fecal contamination. According to this study most of reservoir tanks(64%) of water in dairy farms around Tehran have been polluted with human or animal fecal materials.Existence of *Salmonella typhimurium* approved contamination of water with animal feces but the risk of pollution with human fecal matters still exists because of pollution with *E. coli* and *Aerobacter aeroginosa* in 14 samples synchronously. Only in one sample pollution with human feces was approved certainly because of *Shigella dysentria*. Therefore treatment of drinking water of dairy farms in this area to stop the risk of water born diseases is a must.

P1915

Microbial contamination in dental unit waterlines

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Objectives: The dental unit waterline (DUWL) provides an ideal environment for microbial colonization and proliferation primarily due to the high surface/volume ratio in the tubing and the character of fluid dynamics in narrow, smooth-walled waterlines. It is known that DUWLs are contaminated by numerous species of microorganism. Two case reports are described where medically compromised patients have been infected with *Pseudomonas aeruginosa* originating from DUWL. A single fatal case of *L. dumoffii* pneumonia has been reported in a Californian dentist exposed to contaminated water from his practise. The aims of our study were to investigate microbial contamination of DUWLs in Turkey and to warn dentists to check their dental units (DUs) for public health care.
Methods: Water samples were collected from 12 private dental offices. These samples were taken from air-water syringes, high-speed handpiece, oral rinsing cup and reservoir. R2A Agar was used for Total Viable Counts(TVC), Sabouraud Dextrose Agar was used for fungus, Buffered Charcoal Yeast Extract Agar was

used for *Legionella*. Gram negative strains were identified by API20E, API20NE systems.
Results: We found that most of units exceed American Dental Association recommendation (Anonymous 1996) for DU water quality (range 200–50000 CFU/ml). In 12 units studied, TVCs were higher in the high-speed handpiece(91.6%) than the air-water syringe(8.3%). Microorganisms isolated from DU: *Pseudomonas fluorescens*, *Moraxella* spp, *Photobacterium damsela*, *Ochrobacterium anthropi*, *Pasteurella haemolytica*. Fungus were identified as *Aspergillus flavus*, *Penicillium expansum*. All water samples were negative for *Legionella* sp.
Conclusion: Numerous studies showed that DUWLs contain high densities of bacteria, mainly Gram negative, aerobic, heterotrophic bacterial species many of which have been implicated in human infection. We suggested that microbial contamination in DUs should be controlled to eliminate opportunistic pathogens and to provide water for dental treatment which meets public health standarts for ADA recommendation.

P1916

Antibiotic resistance of bacterial strains isolated from water samples and fish

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Objectives: The presence of resistant bacterial strains in surface fresh and marine waters, in drinking waters and edible cultured and free-catch fresh water and marine fish was investigated in order to assess potential risks for public health.
Methods: A total of 1580 samples (240 from drinking water, 50 from lake water, 400 from river water, 450 from marine water, 230 from free catch and 210 from cultured fish) were collected during a 4 year survey (2000–2003) from different point sources in the region of Northwestern Greece. All samples were processed following standard microbiological methods and susceptibility tests to antibiotics used in the routine medical practice were performed, using the disk diffusion and the MIC test (E-test).
Results: There were isolated 104 *E. coli* strains, 147 *S. aureus*, 106 *E. faecalis*, 55 *E. faecium* and 207 *Pseudomonas* spp. The strains from cultured fish exhibited remarkable multiresistance: 64.3% of *E. coli* strains from cultured fish were resistant to Ampiciline, 35.7% to Cefuroxime and 7.14% to Ceftazidime and Ciprofloxacine. On the contrary, only 5% of the strains from free-catch fish were resistant to Ceftazidime and 37.33% of the *E. coli* isolates from water samples were resistant to Ampiciline. None of the *S. aureus* strains isolated during this survey was resistant to Vancomycine and Teicoplanine. The most commonly isolated *Enterococci* species were *E. faecalis* (66.6%) and *E. faecium* (33.3%). However, there were no Vancomycin resistant *Enterococcus* spp., but there were isolated *E. faecium* strains resistant to Ampicillin (7%).
Conclusion: The presence of resistant bacterial strains in the aquatic environment and in the food chain originating from the aquatic environment is an important health issue and the potential impact on public health has to be addressed in relation to food safety concerns.

P1917

Pattern of extended-spectrum beta-lactamase producers in coastal seawater of Northern Portugal after a four-year period

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Objectives: Access the presence of extended-spectrum beta-lactamase (ESBL) producers, in coastal seawater of Oporto urban area in 2004 and compare with those obtained in 2000.
Methods: Coastal seawater samples were collected monthly, between February and July 2004. Isolates were selected by membrane filtration technique and the filters were placed on Mac Conkey agar and Mac Conkey agar with ceftazidime (5 mg/l) or cefotaxime (2 mg/l). Colonies of lactose fermenters were randomly selected and screened for ESBL production by the double disc synergy test. Identification of the selected strains was achieved by classic biochemical tools and ID 32 GN. Susceptibility to antimicrobial agents was determined according to the NCCLS guidelines. Beta-lactamases were characterized by isoelectric focusing.
Results: ESBL producers were found in marine coastal waters of Oporto urban area over the mentioned period of time, in 2004. Actual work, shows a pattern of different types of ESBL and different associations of beta-lactamases, by opposition to the clonal characteristic of ESBL-producing *E. coli* isolates in 2000.
Conclusion: The presence of isolates showing different ESBL and different beta-lactamases association patterns, as expected, in sea coastal water in different dates, seems to indicate distinct epidemiological relationships associated to a persistent, incoming of ESBL producers to the natural environment, providing a track for environmental dissemination of resistant bacteria and genes, that may create a source of transferable traits for emerging pathogens, via natural reservoirs of resistance, relevant in terms of public health and environmental protection.

P1918

Antibiotics in the aquatic and terrestrial environment – Is there a problem?

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Introduction: In most cases, antibiotics are excreted in unmetabolized form and are then discharged into hospital effluent or municipal sewage water. Unused drugs and remainders thereof are sometimes disposed of down drains. The drugs and their metabolites that are not eliminated in the sewage treatment plant (STP) pass through it and enter the aquatic environment, where if they are not eliminated or (bio)degraded they may eventually enter drinking water. If they are eliminated by sorption onto STP sewage sludge which is later used as a fertiliser they may later reach soil. Antibiotics may also end up in soil when they are used as growth promoters in animal husbandry. The antibiotics may disturb microbial activity in sewage treatment processes and soils and affect biodegradation of contaminants and natural organic matter. Furthermore, resistant bacteria may be selected in the aeration tank of sewage treatment plants and in soils, and eventually reach humans via drinking water or the food chain.
Methods: In this study antibiotic input into municipal sewage was estimated and its concentration analysed. The biodegradability of some clinically important antibiotics and their effects against environmental bacteria were examined.
Results: The concentration of antibiotics in hospital effluent was calculated as being as high as 300 μg/L for single compounds, and in total approximately 40 μg/L in municipal sewage. As much as 120 μg/L have been analysed for single compounds in hospital effluent. The biodegradability of most compounds investigated was very low. Some compounds such as ciprofloxacin sorbed strongly onto sewage sludge. Concentrations calculated for hospital effluents and the MICs for sensitive bacteria were in the same order of magnitude. The genotoxicity of some compounds was not eliminated.
Conclusion: It can be concluded that the input of antibiotics into the aquatic environment should be reduced. In general, the environmental significance of therapeutic drugs should be included in the curricula of doctors and pharmacists.

P1919

A change in cleaning can reduce environmental bacterial contamination

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Objectives: To determine the levels of total bacterial and methicillin resistant *Staphylococcus aureus* (MRSA) environmental contamination of an Intensive Care Unit (ICU), and to evaluate the effect of measures introduced to reduce the bacterial burden.
Methods: The counts of MRSA and total bacteria were determined before and after cleaning on an ICU on 6 occasions. On each screen 4 samples were taken from each of the bed spaces (under the bed, the workstation, a ledge behind the bed space and the monitor) and MRSA detected using broth enrichment. Total bacterial counts were determined for under the bed and workstation using RODAC plates. Evaluation of the results demonstrated that cleaning was not effective, therefore a change in the cleaning product and positioning of the product within the bed space was implemented. The environment was sampled before and after cleaning as above on a further 11 occasions to determine the effect.
Results: The six evaluations of the cleaning regime which was in place showed higher levels of environmental contamination with MRSA after cleaning on 3 (50%) occasions. When a change in cleaning was implemented higher levels of MRSA after cleaning were only found on 1 (9%) occasion. Before a change in cleaning total bacterial counts were higher under beds after cleaning on 42.6% (20/47) occasions and on the workstations on 53.1% (25/47) occasions. After a change in cleaning protocol these rates fell to 27.7% (23/83) and 31.3% (26/83) for under the bed and workstation respectively. In addition not only was a change in the total bacterial counts after cleaning observed, but the overall counts were lower for both under the bed and workstation.
Conclusions: The cleaning on ICU was shown to be ineffective in removal of bacterial contamination. A change in cleaning protocol resulted in an increased effectiveness of cleaning, thereby lowering the total bacterial burden and decreasing the opportunities for transmission to patients.

P1920

Hygienic quality of some infant formula milk

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Objectives: During the last years research has been focused to improve the quality of infant formula milk by adding substances beneficial to the infant health, such as nucleotides, calcium, and vitamins. Addition of such substances in infant milk seems to promote a beneficial intestinal microflora to newborns by lowering the buffering capacity of the milk. This last condition allows maintenance of a low pH, which inhibits the development of anaerobic putrefactive bacteria and enables the proliferation of a beneficial microflora.
Methods: Two types of infant formula milk were examined in our study (1) Pediasure (Abbot Lab) supplemented milk with calcium, phosphorus, vitamin D and iron and (2) Similac Advance (Abbott Lab) supplemented with nucleotides. Milk powder was homogenized in peptone water, followed by decimal dilutions in Ringer's solution. From each decimal dilution were plated 5 different solid agar media, such as Plate count agar, Chapman agar, Mac Conkey agar, Columbia agar, Blood Columbia agar. Furthermore, the new selective medium LS (Lactose-sulfite) broth was used for detection of *C. perfringens*. This medium permits the specific estimation of *C. perfringens* at 101. An aliquot of the Ringer's solution was heated for 10 mn at 800°C before seeding to a plate for recovering of the germinated spore-forms. A total of 50 milks were been studied from each milk powder.
Results: The main advantage of LS medium is the extremely low level of C.perfringens detection (101). It is important to mention here that the usual agar cultures media detect from 10^2 CFU. In the Pediasure powder, *Bacillus* sp were found in one milk (0.5 %) and spores of *C. perfringens* in 2 (1%), but always in extremely low levels, because the usual detection level of these bacteria associated with foodborne disease is reported to 10^5 microbial cells at least. Concerning the Similac Advance powder milk enriched with nucleotides, just in one case (0.5%) spores *C. perfringens* were found at 101. All isolated strains, were examined for their antimicrobial activity. Both germs showed resistance to vancomycin, ceftiofur, ampicillin, penicillin, neomycin, tetracycline, cephalexin, amoxicillin. Furthermore, *C. perfringens*,was resistant to gentamycin and sulphamethoxazol .
Conclusion: Checking of the microbiogical and hygienic quality of infant formula milk must be done in order to have an estimate of the sources involved in the chain contamination and protect the final quality of the product.

Tuberculosis: clinical aspects and surveillance

P1921

Activity of mycobacteriological laboratories in the Slovak Republic in 2003

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Objectives: One part of TB surveillance in the Slovak republic is report about activity of mycobacteriological laboratories.
Methods: All 17 mycobacteriological laboratories in the Slovak republic received questionnaire containing 19 tables. Natl. reference laboratory carried out data processing with using Excel and Epi–Info 2002 programmes
Results: Mycobacterial laboratories in the Slovak republic investigated 125990 biological samples and carried out 125 897 examinations in 2003. Mycobacterial strains were isolated from 2150 examinations (1.71% positive examinations). Sputum was the most frequent biological sample. From 95,301 samples of sputum were isolated 1899 strains of *Mycobacterium tuberculosis*/ 1.99% positive samples/ in 2003. The strains were isolated from 534 patients. The prevalence of *Mycobacterium tuberculosis* in inhabitants of the Slovak republic was 9.92 in 2003. Multiresistant *M. tuberculosis* strains were isolated from 60 patients .

P1922

Distinctiveness of *Mycobacterium abscessus* genotypes from cystic fibrosis patients

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Objectives: An increasing prevalence of non-tuberculous *Mycobacteria* (NTM) colonisation among patients with Cystic Fibrosis (CF) is observed. Mainly due to the misidentification of *M. abscessus* its role for the clinical outcome in CF is not yet clear. Our purpose was to analyze the sputum samples from CF patients from 2001 to 2004 for the presence of *M. abscessus* and to determine their intra- and interpatient genetic heterogeneity.
Methods: Genomic DNA was prepared from all isolates and for identification the samples were processed for sequencing the hsp65 gene and the 16S–23S intergenic gene sequence (ITS); for analysis of their genetic heterogeneity we performed RAPD-PCR and fluorescent amplified fragment length polymorphism (fAFLP) analysis. RAPD-PCR was performed with four different primer constellations and for fAFLP analysis we used two enzyme combinations and four selective amplification primer pairs.
Results: We screened 1700 sputum samples and identified 46 *M. abscessus* isolates from a total of 7 CF patients. We detected two different hsp65 and correspondingly two ITS sequence variants belonging to *M. abscessus* type I and type II; there were no intrapatient sequence variabilities within the individual sequevars. With regard to the RAPD pattern we detected five main *M. abscessus* types among the patients. Two patients harboured two different *M. abscessus* strains over the time period under study. Four of the RAPD clusters showed identical AFLP patterns also, but one could be resolved in subcluster by AFLP.
Conclusion: Our results show that CF patients may be colonized/infected by different *M. abscessus* strains, over a long time period as well as at the same time point. These findings are important for the characterization of the species and for future analysis to understand the pathogenicity and epidemiology of this organism.

P1923

Polyclonal infection by *Mycobacterium kansasii*

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Objectives: The aim of this work is to trace changes in the genotyping patterns of *Mycobacterium kansasii* isolates under the stress of antituberculous chemotherapy.
Methods: 46 isolates from 20 patients (2–4 isolates per patients, isolated at different times during the course of treatment) have been genotyped by three typing methods: PCR-RFLP of the hsp65 gene, RAPD-PCR using two primers, and AFLP using three different selective primers.
Results: According hsp65 PCR-RFLP genotyping all strains belonged to type I *Mycobacterium kansasii*. All multiple isolates from the same patients generated the same patterns by RAPD-PCR and AFLP typing methods except isolates from 2 patients (patients Bas 071 and Sma 002). The patient Bas 071 was a HIV positive, 30 years old male. Four strains were isolated from pulmonary specimens. The first two strains were isolated in January and February 2000 respectively. After 7 months of treatment, the other two strains were isolated in August and November of the same year. The first 2 strains generated the pattern B1 by RAPD analysis and the pattern 111 by AFLP analysis, while the other two strains generated the pattern A1 by RAPD and the pattern 122 by AFLP.The patient Sma 002 was a 55 years old female living in Bilbao. Three strains were isolated from pulmonary samples in October 1999, Jun 2000, and August 2000 respectively. The first isolate generated the pattern B1 by RAPD analysis and the pattern 111 by AFLP analysis. The second and third strains generated the pattern A1 by RAPD and the pattern 122 by AFLP.
Conclusions: It was curious that in both patients, and nearly after 7 months of initiating treatment, the isolated *M. kansasii* shifted from the cluster 111-B1 to the cluster 122-A1. These results were confirmed by 2 different isolates at 7 and 9 months of starting treatment. This change affected both AFLP and RAPD patterns. This observation may be due to exposure of these patients to a polyclonal infection from an environmental source, and due to some differences in the sensibility to antituberculous therapy; one subspecies was selected under the effect of chemotherapy, while the other was eradicated.

P1924

Emerging epidemiological features of tuberculosis in Italy, and public health-related concerns

R. Manfredi, S. Sabbatani, G. Legnani, F. Chiodo *(Bologna, I)*

Introduction: Tuberculosis (T) is borne by increasing morbidity-mortality rates in industrialized and developing countries. Changes in epidemiology patterns, atypical manifestations, and spread of chemotherapy-resistant strains, are of concern. The modification of predisposing factors is altering T epidemiology and presentation: the HIV pandemic, advancing age and concurrent illnesses, increasing immunodeficiency, alcoholism and drug abuse, and especially immigration.
Methods and Results: An analysis of patients (p) hospitalized from 1996 allowed us to identify 90 p with confirmed T. These 90 episodes were assessed to compare the features of Italian vs extra-Western Europe p with T. Significant differences were found among the 62 Italian p, and the 28 p coming from abroad (50% from northern Africa). Compared with foreigners, Italian p had a greater median age (51.7 vs 29.9 years; $p < 0.001$) and more frequent and varied underlying disorders (prior diagnosis-familial history of T, chronic pulmonary, heart, liver, or kidney disease, diabetes, malignancies, and collagen vascular diseases; $p < 0.05$), a higher incidence of HIV/AIDS (31.1% of Italian cases; $p < 0.001$), with a predominant pleuropulmonary localization compared with lymph node and/or disseminated ones among HIV-infected p vs non-HIV-infected p ($p < 0.01$). Immigrants had more frequent generic risk factors (low income, social-economic problems, cigarette smoke, and drug-alcohol use; $p < 0.05$ vs Italian patients). No appreciable difference was found as to lung radiological findings, when excluding HIV-infected p.
Discussion: Two different T patterns may be observed in our area. While immigrants are represented by young, otherwise healthy p with predominant lung localization, Italian p are mostly represented by elderly, with underlying diseases and specific risk factors for T, a more frequent HIV co-infection (and a more common extrapulmonary disease). The epidemiologic-clinical features reflect a distinct profile between immigrants and Italians, but we cannot exclude that the significant spread of T, the continued immigration, and growing predisposing conditions in the general population, may lead to a further increase of diagnoses. Surveillance and preventive strategies of control of T need an update, to avoid the spread of T, due to the merging of risk factors of immigrants and supporting risks of the natives, which is expected to prompt a health care emergency in inner cities, where integration of immigrants meets a vulnerable native population.

P1925

The phenomenon of emerging tuberculosis in Italy. Which role for concurrent HIV infection?

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Introduction: Tuberculosis (T) is borne by increasing morbidity-mortality rates, due to changes in its epidemiology, presentation, and spread of drug-resistant strains. The recent variation of predisposing factors (advancing age, concomitant illnesses, alcoholism and drug abuse, immigration from endemic regions, and HIV disease), play a significant role.
Methods and Results: Among 98 consecutive patients (p) with confirmed T hospitalized since 1996, significant differences were found between the 63 Italian p, and the 35 p coming from outside of Western Europe. When compared with foreigners, Italian p had a higher frequency of HIV/AIDS (31.7% of Italian cases; $p < 0.001$), with a predominant pleuropulmonary involvement vs with lymph node and/or disseminated one among HIV-infected p vs non-HIV-infected ones ($p < 0.01$). Compared with foreigners, Italians showed a higher median age ($p < 0.001$), and more frequent and varied underlying disorders (personal-familial history of T, chronic pulmonary, heart, liver, or kidney disease, diabetes mellitus, malignancies, and collagen vascular disease treated wih steroids; $p < 0.05$), while immigrants had generic risk factors (low income, social-economic problems, cigarette smoke, and drug-alcohol use; $p < 0.05$ vs Italians). Our 8-year experience shows that 2 different patterns of T may be observed. Italian p are mostly represented by the elderly, with underlying diseases and specific risk factors for T, a more frequent HIV co-infection (and a more common involvement of sites other than pulmonary ones), while immigrants are represented by young, otherwise healthy p with predominant lung disease.
Discussion: Physician awareness of T needs improvement, especially when high-risk p are of concern (like HIV-infected p), to ensure a rapid diagnosis and cure, and reduce T spread. The epidemiologic-clinical features of our T p show a significantly different pattern between Italians and immigrant, but we cannot exclude that the spread of T, the continued immigration, and growing predisposing conditions in the general population may lead to a further increase of diagnoses especially towards extreme life ages. Surveillance and preventive strategies of screening, diagnosis, therapy, and control of T need a continuous monitoring, to avoid further T spread, due to the merging of risk factors of immigrants, which is expected to represent a health care threaten in inner cities, where the progressive integration of immigrants may meet exposed and vulnerable natives, including elderly and HIV-infected p.

P1926

AIDS-associated *Mycobacterium kansasii* infection: a thirteen-year follow-up of microbiological and clinical features

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Introduction and objective: A prompt and effective diagnosis and a timely and appropriate treatment of atypical mycobacteriosis (especially *Mycobacterium kansasii* disease), remains a serious challenge for clinicians engaged in the management of the immunocompromised host, including HIV disease.
Methods and Results: Fifteen HIV-infected patients with a microbiologically-confirmed *Mycobacterium kansasii* infection have been observed in a 13-year period, out of over 3200 hospitalizations performed because of HIV-associated disorders. These episodes were carefully assessed from an epidemiological, bacteriological, clinical, and therapeutic point of view. The proportionally reduced crude frequency of atypical mycobacteriosis as HIV-related complication, which virtually disappeared after the introduction of potent antiretroviral combinations (highly active antiretroviral therapy, HAART) in the year 1996, proves evident, since only three cases were registered since 1997. In early nineties, the lack of effective antiretroviral regimens made frequent the association of this opportunism with full-blown AIDS, a mean CD4+ lymphocyte count of nearly 20 cells/μL, and extremely variable chest X-ray features. The recent detection of three further episodes of pulmonary *M. kansasii* infection was due to a late recognition of a far advanced HIV disease (the so-called 'AIDS presenters'), complicated by multiple opportunistic disorders. All isolated *M. kansasii* strains tested resistant to cycloserine and streptomycin, while ethionamide-resistance regarded 11 strains of 13; a favourable susceptibility pattern was shown to rifampicin and ethambutol (all 13 strains testes sensitive), while protionamide, capreomycin, and kanamicin had 2–5 resistant isolates.
Discussion: *Mycobacterium kansasii* respiratory or disseminated infection continues to occur, and pose relevant diagnostic problems, including late or missed identification due to slow culture and frequently HIV-associated concurrent opportunistic disease. Serious therapeutic difficulties, due to the unpredictable in vitro antimicrobial susceptibility profile of these organisms, and the need to start as soon as possible an effective combination therapy which do not interfere with other medications (especially protease inhibitors and other antiretroviral agents), are of particular concern.

P1927

Epidemiology of non-pigmented rapidly growing *Mycobacteria* in a university hospital

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Objectives: To analyze the epidemiology of rapidly growing *Mycobacteria* by using RAPD during a 12 year period.
Methods: Rapidly growing *Mycobacteria* (RGM) obtained during the period 1990–2002 were maintained frozen since their isolation until the experiments were performed. Bacteria were reidentified and a study using RAPD techniques was performed. We made use of the protocol described by Zhang et al. using primers OPA-2 and OPA-18. Bacteria were grouped by species. We also included strains from an epidemic outbreak of postsurgical infections due to RGM that were sent to us as a reference laboratory as a control for the technique. Band profiles were compared by using the Bio-Profil software (BioGene, USA).
Results: During the study period 53 patients had clinical samples where RGM were isolated. The isolates were identified as *M. chelonae* (23), *M. fortuitum* (15), *M. peregrinum* (8), *M. abscessus* (3) and *M. mucogenicum* (4), 36 isolates (16 *M. chelonae*, 11 *M. fortuitum*, 7 *M. peregrinum*, 1 *M. abscessus* and 1 *M. mucogenicum*) were isolated during the period 1995–1997. No clear relationship between patients could be found after reviewing their clinical charts. RAPD showed readable profiles for all but one strains. The primer OPA-2 demonstrated a discriminative power similar to the OPA-18 one. Using OPA-2 one cluster was detected with a 1% homology coefficient (UPGMA), and 2 more different clusters appeared when a 5% homology coefficient was applied, all of them being *M. chelonae* strains. Using OPA-18 no cluster was detected for 1% homology coefficient, but when 5% homology coefficient was applied 4 clusters were detected. No predominant strain was detected in the isolates of the period 1995–1997.
Conclusions: An increase of the isolates of RGM was detected in a 3-year period, despite no changes in the protocol were

performed. No predominant strain or outbreak was detected, so the increase of the number of isolates could be due to other causes than the existence of predominant strains. RAPD is an easy and valuable technique for evaluating the epidemiological relation between strains when the isolates are included in the same gel.

P1928

Use of the expanded set of VNTR-MIRU loci for the differentiation of *Mycobacterium tuberculosis* Beijing family isolates

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Several studies have shown that a high proportion of Russian TB isolates belong to the Beijing family. A genotyping system capable of discriminating Beijing strains would be useful for this population. The recent identification of variable number tandem repeats (VNTRs and MIRUs) in *Mycobacterium tuberculosis* has raised the possibility of a rapid amplification-based techniques with comparable discrimination to RFLP-IS6110.Objective: to evaluate the effectiveness of an expanded set of VNTR-MIRU loci for discrimination of TB strains in Russia, particularly for Beijing family strains and to determine the most useful polymorphic loci for automated and/or manual screening of isolates. Materials and methods:A total of 185 *M. tuberculosis* strains isolated from civilian and prison patients collected in 2001–2002 in Samara oblast were analysed. Crude DNA extracts were obtained by heating cell suspensions with chloroform at 80°C. All 185 TB isolates were tested using the original set of 12 MIRU loci and 3 ETR loci described previously (Supply et.al., 2001). Beijing strains were identified using spoligotyping and detection of IS6110 insertion in dnaA-dnaN region. Beijing isolates were then subjected to VNTR-MIRU analysis using an expanded panel of loci (0424, 0531, 1955, 1982, 2074, 2163a, 3232, 3239, 3336, and 3690). Primers were designed in order that fragments could be analysed using a capillary electrophoresis system (Beckman Coulter SEQ8000).

Results and discussion: 129 isolates were identified as being of the Beijing family (69.7%). 15 loci VNTR-MIRU analysis yielded 10 clusters (sized from 2 to 75 isolates) and 58 unique VNTR-MIRU patterns. The two largest clusters consisted of 31 strains and 75 strains (223325173533423 and 223325153533423 profiles respectively). For all strains MIRU analysis showed a higher degree of discrimination than that seen with spoligotyping (HGDI 0.747 versus 0.572). However, the discriminative power of 15 loci MIRU analysis was shown to be insufficient for differentiation within Beijing family. 129 Beijing strains were subjected to MIRU analysis using an expanded set of loci (see above). This provided a higher degree of discrimination within the Beijing group (HGDI 0.870 versus 0.625). Overall three loci (MIRU 26, VNTR 1982, and 3232, $0.3 < h < 0.6$) were shown to be sufficiently polymorphic for the differentiation of Beijing family strains and useful for either automated or manual screening.

P1929

Long-term efficacy of a six-month treatment regimen (6HR2Z) for HIV-negative miliary tuberculosis

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Objective: To investigate the long-term efficacy of a six-month treatment regimen (6HR2Z) for miliary tuberculosis (MTB) in the HIV-negative population.

Methods: All HIV-negative patients with MTB attended in our Centre entered a six-month treatment protocol (6HR2Z) and were prospectively followed in our Tuberculosis Unit for a period of 68 ± 53 months. The diagnosis of MTB was based on cultures, pathology of specimens, and/or clinic-radiological presentation. Daily doses employed in the 6HR2Z protocol were: isoniazid (H), 5 mg/kg; rifampin (R), 10 mg/kg; and pyrazinamide (Z), 30–35 mg/kg. Demographics as well as a complete set of tests (including complete physical examination, analysis, BCG, Mantoux, chest x-ray and CT scan, and assessments of treatment tolerance and illness evolution) were collected in each case.

Results: We recruited 25 patients with HIV-negative MTB. There were 8 female and 17 male (68%). Mean age, 55.7 ± 17.7 years; range, 18–90 years. Twenty patients had initial chest x-ray alterations: 17 miliary pattern, 2 cavitation and 1 pleural effusion .The final diagnosis was established in 16 (64%) patients by the demonstration of *M. tuberculosis* in cultures, in 4 patients by the demonstration of caseating granulomas on histopathology specimens, and in 5 patients by clinic-radiological presentation (4 miliary pattern in chest x-ray). All patients were initially treated according to the six-month 6HR2Z protocol. In one patient treatment modification was required and prolonged because fever by rifampin. As to the outcome, 16/24 (66.7%) patients were cured, 5 (20.8%) died of tuberculosis during the first days of treatment, and 3 (12.5%) died of unrelated causes 1, 2 and 3 months after starting treatment, respectively. Sixteen of the 25 patients who entered the study (64%) completed the treatment as initially planned (i.e. 6-month therapy); any of them had some late sequelae. Six patients developed adverse reactions to the treatment (abdominal dyscomfort in 2; arthralgias in 2, peripheral neuropathy in 1 and eruptive rash in 1) and 2 toxic hepatitis; all these complications resolved spontaneously so that no treatment modification was required. Mean follow-up in this group of 16 patients was 68 ± 53 months (range, 8–141; person–months, 1088).

Conclusion: Our results suggest that a six-month treatment regimen (6HR2Z) is optimal for miliary tuberculosis in the HIV-negative population.

P1930

Is directly observed therapy necessary in all patients with tuberculosis?

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Objective: To identify frequency and predictors of nonadherence to antituberculosis treatment, treatment failure and relapse rates.

Methods: All patients with tuberculosis (TB) attended in our TB Unit from 1990 to 2004 were prospectively followed by a medical doctor. The diagnosis of TB was based on cultures, pathology of specimens, and/or clinic-radiological presentation. Demographics as well as a complete set of tests including sex, age, employment, marital status, previous TB, homeless, alcoholism, injection drug use, HIV infection, concomitant treatments, regimens of antiTB treatment, side effects, nonadherence, resistances and illness evolution were collected in each case. We use educational strategies directed at the patient and patient́s family, and reminder phones calls to patients who defaulted. The patients were revisited at first, second month, and at the end of treatment; if they were any side effects or treatment default were revisited more frequently. A logistic-regression analysis was performed to identify predictors of nonadherence to antiTB treatment, treatment failure and relapse.

Results: We recruited 1159 patients, 689 (59.4%) males, 470 female. Mean age, 38.9 ± 19.7 years (range 10–95). Single 572, separated-divorced 50, unemployed 135, homeless 8, previous TB 132, alcoholism 191(16.5%), injection drug use 66 (5.7%), HIV 57, concomitant treatments 229. Fixed-dose combination therapy 1.137 (98.1%) [772 with HRZ]; 6-month regimens 912 (90.5%) [877 with Z] and 247 others. Serious hepatic toxicity 104 (9%) patients and other side effects 332 (28.6%). The therapy regimen was unobserved in 1105 patients and 49 default (4.4%). The predictors of treatment default were: injection drug use (OR 40; IC 95%: 15.6–61.7) and alcoholism (OR 3.9; IC 95%: 1.9–7.8). The therapy regimen was directly observed in 54 (4.6%) [23 alcoholism, 5 homeless, 4 injection drug use, 22 others] and all completed treatment. Resistances: H 1.9% and R 0.2%. Treatment failure 15/1.159 (1.3%) and relapses 15/1.159. The treatment default was predictor of treatment failure (OR 482.3; IC 95%: 61.7–3.771) and relapses (OR 13.2; IC 95%: 2.6–67.4). Mean follow-up 27 ± 25 months (range, 1–157); person-months, 31.304.
Conclusion: The treatment programme is very satisfactory in our unit. The treatment failure and relapses are low and are associated with treatment default. DOT can get better treatment adherence in patients with injection drug use and alcoholism.

P1931

Ways to reorganise the laboratory service for TB diagnosis in Russia

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Presently one of the most urgent objectives is to rationalize organization of microbiology studies. This will improve effectiveness and quality of microbiology diagnosis of TB in Russia. Thereupon, many Russian regions have started centralization of microbiology studies and establishment of a common laboratory network involved in microbiology diagnosis of TB. It will include laboratories subject to different departments. The whole network is based on the system of quality assurance. All elements of this system will be implemented by the central bacteriology laboratory of the regional TB service. To coordinate performance of bacteriology laboratories and assure quality of studies in Russia, it is planned to establish a network of reference-laboratories, according to the Russian Health Ministry order. The network will consist of 2 federal reference-laboratories, 7 reference-laboratories of the federal districts and regional reference-laboratories. Draft statute to regulate reference-laboratories performance is prepared. Establishment of reference-laboratories network will allow to standardize microbiology methods of studies, obtain comparable data, carry out laboratory monitoring of level and degree of *M. tuberculosis* drug resistance. In 2003 113993 new TB cases were registered. Out of them only 4104 were detected by microbiology methods, which was 3.6%. The radiological method with bacterioscopy considered 26.1%, culture–48%. Quality control of subject laboratories implemented by reference-laboratories, continuous staff training and supervision of laboratories will enable to significantly improve effectiveness of TB detection and treatment.

P1932

Epidemiology of tuberculosis in Marageh (Iran) between 2001 and 2003

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Objectives: The tuberculosis have been an important problem of human society in last centuries. Human had spent his big financial sources for fight with the problem mentioned above. This problem established by a special bacteria which named *Mycobacterium tuberculosis* The aim of this research was the epidemiologic study of the tuberculosis in maragheh area and also assessment of its treatment programmes .
Methods: The method of study in this research was a descriptive cross-sectional. The recent study was a research which in maragheh centre for gampaigan to tuberculosis done on the 1430 person who they were doubtful to tuberculosis between 2001 and 2003. we collected sputum from doubtful persons and studied by culture and direct examination methods. We used radiologic and clinical signs and symptoms for definite diagnosis sometimes. finally we used SPSS software and descriptive statistic.
Results: We found tuberculosis in 66 person, where 65 per cent of them were female and 35 per cent were male. Also 58 per cent of them were staying in town and 42 per cent in villages of maragheh. The results of culture and direct examination were corresponded to each other.
Conclusion: Based on the findings which mentioned above the frequency of tuberculosis in Maragheh were same between the 2001 and 2003. Also the involvement of the female two times more than male were corresponded with the previous results which done in Tehran and Qhom. Extra pulmonary tuberculosis were seen more than the pulmonary tuberculosis which was an interesting result and need to more research and screening methods, on the extra pulmonary tuberculosis.

P1933

Epidemiological characteristics of non-tuberculosis *Mycobacteria* isolated from adult patients in a teaching hospital in Madrid from January 2000 to August 2004

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Objectives: 1) To determine the *Mycobacteria* isolated from adult patients in a Teaching Hospital in Madrid from January 2000 to August 2004. 2) To study the epidemiological characteristics of the atypical *Mycobacteria* isolated in that population.
Methods: We evaluated retrospectively 20228 samples sent to the Microbiology laboratory for mycobacterial research from January 2000 until to 2004 in adult patients. Samples were processed following standard procedures and cultured in solid medium (Coletsos) and liquid medium (modified Middlebrook 7H9). The growth in solid medium compatible with *Mycobacteria* and those detected as positive in the liquid medium system were stained with the Ziehl–Neelsen technique. Identification was performed by hybridation probes (Accuprobe-Gen Probe) for M. tuberculosis and MAC, and in a national referral centre for the rest of them. Epidemiology information was obtained for further studies.
Results: From the total of the 20228 samples sent to the laboratory, 1015 were rejected as inadmissible (5.02%), 623 were contaminated (3.08%) and 17954 were negative after 2 months (88.76%). 636 samples were positive for *Mycobacteria* (3.14%) being 71.70% *M. tuberculosis* (456 samples), 21.38% slow grow *Mycobacteria* (136 samples of which 86 were MAC, 13 *M. simiae*, 13 *M. xenopi* and others), 6.29% rapid grow *Mycobacteria* (40 samples with 14 *M. fortuitum*, 12 *M. chelonae* and others) and 0.63% not viable atypical *Mycobacteria* (4 samples). Sputum was the most common sample sent to the laboratory (60.55%) and the Neumology Department was the origin in 39.44% of the cases. In 100 cases the patients were male (55.55%) and in 80 cases female (44.45%). We considered significant the isolation of the same *Mycobacteria* in two or more samples and 34 patients

had this condition being the microorganisms isolated: MAC in 18 patients, *M. simiae* in 4, *M. xenopi* in 3, *M. chelonae* and *M. kansasi* in 2 and *M. gordonae*, *M. abscessus*, *M. lentiflavum*, *M. mucogenicum* and *M. senegalense* in 1 patient. In 18 of them the isolation was in more than three samples.
Conclusions: Non tuberculosis *Mycobacteria* are an important burden of work in our laboratory and the clinical significance of these microorganisms remains controversial in an important number of cases.

P1934

Mycobacterial diseases in an intensive care unit

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Objectives: Our aim was to study retrospectively the clinical presentation of mycobacterial diseases (MD) in a ICU over a period of 10 years (1993–2003).
Methods: The diagnosis was carried out by means positive acid-fast bacillus smears, positive Lowenstein cultures of different clinical specimens, and/or histologic evidence of granuloma formation.
Results: Of 6356 admissions, a total of 36 patients were diagnosed MD, 34 *M. tuberculosis* (Mt),1 *M. avium* (Ma) and 1 *M. xenopii* (Mx). The average age was 59.51 (25–82) years, 23 were male and 13 female. The mean APACHE II score, on the first day of ICU was 20.10 (10–30). Twenty-one patients had risk factor (1 HIV,7 immunosuppressant drugs treatment, 4 alcoholism,6 silicosis and 3 previous MD) Twenty patients had pulmonary disease (8 pneumonia and 12 ARDS), 2 had meningitis, 6 had disseminated diseases and 9 patients admitted to the ICU for other reasons were found to have MD. The diagnosis was clinically suspected in 22 patients. The average time to clinical suspicion of tuberculosis after ICU admission was 2.5 (1–15) days. Definitive diagnosis was made 18.21 (10–62) days after ICU admission. Twenty-six patients were treated. In 15 patients the treatment had commenced before confirmation of diagnosis.The average ICU length of stay was 17.4 (1–47) days and 20 patients died in the ICU.
Conclusion: The incidence of MD in our ICU over study period is 5.6 of 1000 admission.Mt is the organism most commonly isolated. The most common form of MD was pulmonary tuberculosis (55.5%). Twenty-six per cent of the patients were treated for another condition. The ICU mortality for patients with MD was 50%

P1935

Erythema induratum, what is the role of *Mycobacterium tuberculosis*?

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Introduction: The name Erythema Induratum (EI), was given by Bazin to a nodular eruption occurred on the leg of young women who had tuberculosis. For the past few decades, many investigators have examined EI lesions histopathologically and microbiologically. Some authors reported that at least 50% of EI lesions were positive for *Mycobacterium tuberculosis* on PCR test.
Case Report: We are reporting 2 cases, 63 and 35 year old Saudi females, both of them were presented with lower limbs painful lesions typical of erythema induratum confirmed by characteristic biopsy findings. Both patients were having positive PPD skin test and high ESR tests, but culture from the biopsy for mycobacterium tuberculosis and PCR were negative. They received anti-tuberculosis treatment for a total of 6 months and they showed a dramatic clinical response with gradual decline in ESR.
Discussion: There are some controversies in the literature regarding erythema induratum and its status as a tuberculous infection. More reported cases supporting the association with *Mycobacterium tuberculosis* based on positive PPD test, marked response to anti TB treatment and low concentration MTB-DNA in some lesions by PCR. In our reported cases we failed to document a positive culture for *Mycobacterium tuberculosis* or a positive PCR but both cases showed a dramatic response to anti-TB treatment. With the increased incidence of EI cases in our hospital we recommend to start an anti-TB therapy for any case of EI and wait for results of investigation 6–8 weeks later as evaluations need to be done.

P1936

Tuberculous pericarditis at a national guard hospital: experience over 10 years

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Background: Pericarditis is a rare manifestation of tuberculous disease. Tuberculosis (TB) is common in Saudi Arabia, particularly in our institution. TB is responsible for 4% of cases of acute pericarditis, 7% of cardiac tamponade, and 6% of constrictive pericarditis.
Methods: A retrospective chart review for cases of TB-pericarditis was studied at our hospital, i.e. King Fahad National guard Hospital. TB-pericarditis case definition includes any case of pericarditis with microbiologic culture of *Mycobacterium tuberculosis*, or histopathologic evidence of pericardial biopsy for tuberculosis, or response to anti-TB therapy.
Results: Number of cases with TB-pericarditis was 10 cases, between January 1992 and December 2001. The mean age for patients was 50 years, and 90% were males. Clinical features of TB-pericarditis patients were fever (70%), dyspnea (70%), chest pain (50%), and pericardial rub (40%), laboratory tests were normal white blood count (100%), and elevated erythrocyte sedimentation rate (90%). All cases had cardiomegaly on chest x-ray. Cases who had pericardial effusion were 100% (50% had large, moderate 10%, and mild 40%) on echocardiography. Five cases had pericardiocentesis and they showed evidence of infection. 50% of the cases did not have a pericardial biopsy done, however, the five other cases had a pericardial biopsy, which showed evidence of tuberculosis in four of them (4/5, 80%). *Mycobacterium tuberculosis* culture was done on 3 patients and it was positive in 2 patients. All patients received anti-TB therapy. 70% received oral steroids. 70% of the patients were subjected to surgical intervention. Among the patients of TB pericarditis, 70% recovered.
Conclusion: TB-pericarditis at our hospital is still rare at an average incidence of one case per year (1.5% cases of TB). Early diagnosis and management is necessary to avoid significant morbidity and mortality.

P1937

Role of bone marrow mycobacterial cultures, histopathology and PCR in the diagnosis of disseminated/miliary tuberculosis

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Objectives: To study bone-marrow aspirate and trephine biopsy in disseminated tuberculosis using LJ and BACTEC TB culture media, histopathology, and PCR for *M. tuberculosis* and to conclude on their clinical relevance.
Methods: Newly diagnosed adult patients with disseminated tuberculosis underwent bone marrow aspiration and trephine biopsy for microscopy, acid-fast bacillus stain, mycobacterial

culture on Lowenstein-Jensen and BACTEC TB20 media, and detection of a polymerase chain reaction product using a MBP64 probe. Other investigations as warranted were carried out.
Results: A total of 46 patients with disseminated tuberculosis, age range 16–65 years (mean 36 + 16) were included and stratified into 2 groups based on microbiological results, I) Confirmed TB when evidence of *M. tuberculosis* could be demonstrated in one or more of the available tissues like sputum, lymph node, aspirates, liver, bone-marrow, and II) Clinical TB when patient had compatible clinical features AND a full clinical response to empirical antitubercular therapy, in absence of a demonstrable positivity. Confirmed TB constituted 43% of the patients. Bone marrow contributed to diagnosis of tuberculosis in 15/46 (32.6%) cases. Eleven had bone-marrow granulomata, and another six had PCR positivity for *M. tuberculosis*. Two cases had both. Bone marrow granulomata were distributed evenly between the confirmed and clinical TB, constituting 25% and 23% of respectively. Eight of the granulomata were amongst military TB category (constituting 50%) whereas only three were from non-miliary disseminated TB (constituting 10%), p value 0.002. Two cases had caseation (both from military TB) whereas one revealed acid-fast bacilli (from non-miliary category) in bone biopsy. None of the bone-marrow aspirate cultures on LJ or BACTEC TB yielded any positivity. PCR on bone marrow aspirate was positive for *M. tuberculosis* in 6/46 (13%), comprising 6.3% and 16.7% respectively of miliary and non-miliary categories, p value 0.32.
Conclusion: Bone marrow granulomata and PCR for M. tuberculosis contributed to diagnosis in a third of cases with disseminated tuberculosis, and were equally likely between the confirmed TB and clinical TB groups. The yield was significantly higher in military tuberculosis. Bone-marrow aspirate smear and culture for *Mycobacteria* were of no value.

P1938

Antibiotic treatment of oedematous *Mycobacterium ulcerans* disease reduces the need for radical surgery and enhances the inflammatory response

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Objectives: Oedematous disease is an acute form of *M. ulcerans* disease (Buruli ulcer) needing aggressive surgery to excise all oedematous tissue. Recently it was shown that when early lesions of MUD were treated with rifampicin and streptomycin for 4 weeks, excised tissue was culture negative. Therefore we have investigated the clinical benefit of antibiotic treatment in patients with oedematous disease.
Methods: 21 patients presenting to 3 hospitals in Ghana with oedematous MUD were treated with rifampicin (10 mg/kg) and streptomycin (15 mg/kg) for 2–8 weeks prior to surgery. Surgery to excise necrotic tissue was undertaken when maximum resolution of oedema had occurred. Diagnosis was by punch biopsy before therapy or on excised tissue by staining for AFB, culture on LJ medium, PCR for IS2404 repeat sequence of MU and histology. Resolution of oedema was judged clinically and recorded in photographs.
Results: Patients were aged from 2 to 44 years (mean 11). Oedema involved the majority of one part of a limb, the trunk or the head in all. Tissue from all patients had positive PCR confirming the diagnosis of MUD. In all patients, oedema resolved during antibiotic therapy leaving a much reduced central area requiring excision. Three patients received antibiotics for 2 weeks, 7 for 4 weeks, 1 for 6 weeks and 10 for 8 weeks before oedema resolved and surgery was undertaken. Before therapy, there were large areas of subcutaneous necrosis containing clumps of extracellular *Mycobacteria* but few inflammatory cells. After therapy, a vigorous inflammatory reaction including granulomas was seen with intracellular *Mycobacteria* in macrophages and neutrophils.
Conclusion: Treatment with rifampicin and streptomycin reduced the area requiring excision surgery in oedematous MUD. A vigorous inflammatory response and phagocytosis of *Mycobacteria* was seen after therapy presumably as a result of cessation of mycolactone production.

P1939

Disseminated *Mycobacterium avium* complex in patients with human inmunodeficiency virus. Report of two cases

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Objectives: Describe two cases of MAC plus VIH with particular clinical features
Methods: Brief review plus clinical records.
Results: Disseminated MAC infection is a common complication of late-stage HIV-1 infection, even in the HAART era, in developed countries. However in Spain we find a low incidence of this opportunistic disease. Case 1: Male, 37 years old, remitted to hospital for treatment of ocular pathology related to CMV. Antecedents of VIH, CD4 below 50/μl, receiving antiretroviral treatment plus MAC prophylaxis (azitromycine). Lab findings: leukocytes 2000/mm^3, haemoglobin 10 gr/dl, platelets 249,000/mm^3. GSV 120 mm/h. CT showed multiple intraabdominal adenopaties with no visceromegalia. Bone marrow positive for *Mycobacteria*. The patient received multiple intravenous treatment and suffered 4 episodes of venous thrombosis related to peripheral and central venous catheterization. No hypercoagulability state was proved. He received prophylaxis with LMW heparin.
Case 2: Male, 44, antecedents of consuming inhaled cocaine. Heterosexual. Consulting for weight loss and tiredness. Occasionally haemorrhagic diarrhoea. Dysphagia for solids. Clinical findings: oropharyngeal mycosis, cervical adenopathies. Hepato and splenomegalia. Outer haemorrhoids. Bradypsichia, unsteady wandering and intentional tremor. Lab findings: Haemoglobin 10.3, leukocytes 2090 (2 CD4/μl), platelets 179,000. GSV 40. Biochemical parameters were normal except LDH 703μ/l and Beta2microglobuline 3.71 μg/ml. Total body CT: cavitary lesions in upper pulmonary lobes. Bone marrow biopsy was normal. Gastroscopy revealed duodenal lyphangiectasis. Mantoux 0mm. Microbiological findings: HIV positive (new diagnosis), more than 750,000 copies/ml. BAL positive for *Mycobacteria*. CMV antigen positive, as well as hepatitis C serology. The duodenal biopsy gave the certain diagnosis of *Mycobacterium avium* intracelulare. Treatment of MAI was established with perspectives of HAART instauration but the patient died dew days after diagnosis.
Conclusions: Case 1 makes us think in MAI as playing a main role in the patophysiology of thrombosis for direct toxicity against endothelium. Case 2 suggested us at the beginning a lymphoma, but it turned into a rare case of intestinal affectation of MAI and disseminated disease as cause of death in a naïve HIV patient. As potential serious complications can derive from MAI, prophylaxis seems to be indicated.

P1940

Chronic bursitis by *Mycobacterium chelonae*

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Objectives: *Mycobacterium chelonae* is one of the most antibiotic-resistant species of the pathogenic rapidly growing *Mycobacteria* (RGM). It has been implied in three basic types of infectious disease: the most common type is the disseminated cutaneous disease in chronically immunosuppressed patients, the second type are localized infections following trauma or surgical procedures in normal hosts and the third one is catheter-related infections. The objective is to present first documented case of *Mycobacterium chelonae* olecranon bursitis in our country.
Case report and methods: A 70-year-old patient who had been treated with oral corticosteroids for chronic arthropathy during 15 years was referred to study a chronic olecranon bursitis in right elbow. A drainage was performed to establish the diagnosis. Specimen was gram stained and seeded in blood agar, chocolate agar and into a liquid system BACTEC 9120 (Becton Dickinson).
Results: Microscopic examination of specimen was negative. Culture in liquid medium showed growth of *Mycobacteria* after 5 days. Culture was confirmed as rapidly growing *Mycobacteria*, nonpigmented. *Mycobacterium chelonae* was identified in basis of biochemical and growing characteristics. Susceptibility testing of the isolate was performed by disk-diffusion method (Kirby–Bauer technique) and revealed resistance to cefoxitin, amikacin, sulfamethoxazole, doxycyclin but susceptibility to clarithromycin. Intermediate sensibility was observed to ciprofloxacin and ofloxacin. On three consecutive occasions *Mycobacterium chelonae* was isolated from fluid aspirated from olecranon bursa. At the moment, surgical debridement combined with appropriated antimicrobial therapy is under evaluation.
Conclusions: *Mycobacterium chelonae* has been involved in localized infections following trauma and sporadic localized wound infections following medical or surgical procedures. However, in our case neither of these situations have been presented, only immunosuppressive therapy seems to be the predisposing factor to develop this infection. We want to draw the attention of microbiologist and clinicians to this pathogen because of the increasing incidence of interaction between humans and *Mycobacteria* in coming years.

P1941

Genito-urinary tuberculosis: review of 34 cases

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Objectives: Genitourinary tuberculosis (GUTB) is the most frequent extrapulmonary affection following pleural and ganglionar forms of the disease. As it usually presents with subtle clinical manifestations, diagnostic delay is frequently observed. The objectives of the study were to describe the clinical and epidemiological findings of GUTB, as well as clinical consequences and sequellae.
Methods: We retrospectively reviewed 34 adult cases of HIV-negative patients with a diagnosis of GUTB based in a positive urine culture for *Mycobacterium tuberculosis* and/or a biopsy with caseating granulomes from the genitourinary tract, between 1996 and 2004.
Results: 34 cases of GUTB were reviewed (22 men), with a mean age of 59.52 years. Underlying diseases were present in 18 patients (52.94%). 2 patients had previously had tuberculosis (1 pleural, 1 orquiepididimitis). 2 patients had tuberculosis in other organs. Two or more genitourinary structures were involved in 16 patients: kidney (23), urethers (14), urinary bladder (9), testes (6), prosthata (3) and anexial affection with peritoneal implantation (1). More than one clinical manifestation was present in 18 patients: urinary irritative syndrome (17), lumbar pain (13), hematuria (10), fever (10), genital pain (6), toxic syndrome (3), chronic scrothal supuration (2) and methrorrages (1). Time between first symptom and treatment was 15.06 months. Urine Ziehl–Neelsen (ZN) was positive in 4 of 33 patients and Löwenstein culture was positive in 26 of 33 patients. 15 of 17 biopsies were diagnostic. ZN of biopsied material was positive in 3 of 10 patients, as was Löwenstein culture in 5 of 6 patients. Nephrectomy was performed in 10 patients, percutaneous nephrostomy in 5, ureteral cateterization in 4, orquiecthomy in 5, cistoplastic augmentation in 3, prostatectomy in 1 and laparoscopic exploration in 1. Mean clinical and microbiologic follow-up was 27.53 (4–59) and 26.33 (3–59) months respectively. 63.33% patients remained asymptomatic, 37.50% developed renal failure (2 requiring dialysis). Microbiologic cultures were negative in all surviving patients. 3 patients died (1 unknown cause, 1 disseminated disease, 1 not-related cause).
Conclusions: Diagnostic delay is still frequent, so high clinical suspect is required for GUTB diagnosis. Antituberculous therapy is curative in most patients, but many of them require nephrectomy or reconstructive surgery, and a significant proportion develop renal failure.

P1942

Optochiasmatic tuberculoma and tuberculous lymphadenitis developed during treatment of multiple intra-cranial tuberculomas with anti-tuberculous agents: a case report with 18 months follow-up by MRI

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Case: A 15-year-old girl was admitted with complaints of fever and vomiting for 7 days and progressive alteration of sensorium for 4 days. Examination of the cerebrospinal fluid (CSF) revealed protein concentration of 85 mg/dL and a cell count of 140 leukocytes/mm^3 with predominant lymphocytes. Smear of CSF revealed acid-fast bacilli. MRI revealed minimal contrasting in the dura, and milimetric tuberculomas were determined in the left cerebellum, left temporo-occipital and right fronto-temporal areas. A diagnosis of intra-cranial tuberculoma was made and she was treated with anti-tuberculous drugs (isoniazid, rifampicin, pyrazinamide and streptomycine), and dexamethasone (started to reduce the dose on day 15 and ceased on day 30). Since the patient's condition deteriorated despite the treatment, her CSF and radiological findings were re-evaluated one month later. Cell count of CSF increased from 140 mm^3 (35% PNL, 65% lymphocytes) to 970 mm^3 (35% PNL, 65% lymphocytes). Prominent contrasting (abscess) in dura, increase in the size of tuberculomas and additional new tuberculomas in right pontocerebellar, craniocervical junction, bulbus anteriorum, cerebellar tonsils and localization towards the optic nerve were seen in MRI. The *M. tuberculosis* determined in the first culture was found to be sensitive to all antituberculous drugs and thus the same treatment continued. The patient was followed up and CSF and MRI findings were evaluated at three monthly intervals. Three and a half months after the treatment, it was determined that tuberculous lympadenitis developed in the right side of the neck (extracranial involvement) turned into conglomerate LAP (smear negative) which was then excised and healed within 10 months. In the follow-up

of the patient, it was observed that dural contrasting, number and size of intracranial and chiasmal tuberculomas were increased despite the treatment, and then full recovery of dural contrasting (3rd month check up), CSF findings (12th month check up), opthalmological findings and tuberculomas in the optic chiasma (15th month check up) and intracranial tuberculomas (18th month check up) were seen. The treatment was then ceased on the 18th month.
Conclusion: The case presented here has shown that tuberculomas in the chiasma opticum and extracranial localization developed during treatment may completely recover with application of efficient treatment and thus not require addition of long term steroid administration to the treatment.

P1943

Postoperative infection of a prosthetic aortic valve by *Mycobacterium chelonae*

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Postoperative endocarditis can be caused sometimes by atypical pathogens. We present a case of an infection of a bio-prosthetic aortic valve/of the aortic root by *Mycobacterium chelonae* in an immuno-competent host.
Case report: A 72-year-old woman with aortic stenosis of degenerative origin underwent in February 2003 an elective replacement of the aortic valve by a bio-prosthesis Shelhighâ and of the enlarged aortic root by a tube Shelhigh N°23. In November 2003 she was re-hospitalised because of cardiac decompensation, inflammatory parameters and nocturnal transpiration without weight loss. Echocardiography revealed a displacement of the aortic valve without vegetations. Blood cultures, even with prolonged incubation periods, for microorganisms remained sterile. Angiography before surgery showed 'sacculations' of the aortic root; suspecting an infection. In December 2003 surgery was performed. The surgeons remarked several cavities of cold abscesses in the aortic root. The annulus was destructed by these abscesses protruding also into the pulmonary artery. Infection could not be excised in total. An intervention according to Bentall by insertion of a new Shelhighâ bio-prosthesis was performed and the coronaries reimplanted. An atrio-ventricular block III° necessitated a permanent pacemaker. Acridine strain revealed the presence of *Mycobacteria* spp. which did not grow in culture. PCR sequencing technique identified *M. chelonae*. A large empiric intravenous antibiotic therapy including amicacin, rifampicin, ethambutol, co-trimoxazol, clarithromycin and ciprofloxacin was begun. The i.v. regimen was changed to a peroral regimen three weeks after by omitting amikacin. After confirmation of M. chelonae the antibiotic sixtatherapy was reduced to a peroral regimen of co-trimoxazol, ciprofloxacin and clarithromycin, scheduled for at least 6 months. Clinically the treatment was fully successful already after 5 weeks. The origin of this unusual pathogen remained unclear, the possibility of contamination during the operation or contamination during fabrication could be a possibility. In March 2004 the patient suddenly died of ventricular fibrillation. Autopsy identified a dehiscence of the implantation of the right coronary artery as the cause. There were no signs of activity of the former infection. The abscesses had disappeared.
Conclusion: Successful empiric antibiotic therapy with surgery even for large size prosthetic aortic valve and aortic root is possible.

P1944

Central nervous system tuberculosis: a 4-year review

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Objective: To compare clinical presentation, laboratory, imaging data and outcome among definite, probable and possible/uncertain tuberculous meningitis (TBM).
Methods: We retrospectively studied 106 cases (60 M, 46 F, mean age 34.7) of CNS tuberculosis (TB), most of them with TBM, diagnosed between 2001 and 2004 at an infectious diseases centre in Bucharest. The inclusion criteria were diagnosis of CNS TB and/or culture of *M. tuberculosis* (MT) from CSF. For CSF changes consistent with TBM, we evaluated the diagnosis according to the following criteria: (1) culture of MT from CSF-definite TBM, (2) a) evidence of TB elsewhere or b) chest x-ray consistent with miliary/pulmonary TB and/or hydrocephalus on CT brain scan + improvement on treatment-probable TBM (3) cellular CSF and/or raised CSF protein and/or reduced CSF glucose and a favorable response to specific therapy-possible/uncertain TBM.
Results: We compared definite (39 cases-36.8%), probable (28–26.4%) and possible/uncertain (39 cases) TBM. We noted clinical findings (mean duration of symptoms before presentation 14.3/9.8/11.4 days, respectively, fever 36/21/35, abnormal mental state 29/17/15, localizing neurological signs 19/11/11, history of contact with/prior TB 16/88/10), laboratory data (CSF cells number, mean CSF protein level 228/255/381 mg/dl, low sodium level in 17/9/7 patients). In 3/67 cases of definite/probable TBM, the patients with paraparesis had spinal TB (arachnoiditis-myeloradiculitis in 1 case and tuberculoma, single or multiple, in 2 cases). In 2 patients with spinal TB we remarked an extremely high protein level: 5980 and 4260 mg/dl. The results of MRI (spinal TB) and cerebral CTs of 27/21/14 patients with TBM are shown. We studied the correlation between the stage of the disease at presentation (stage 1–45 (42.5%) patients, stage 2–41 (38.7%), stage 3–20 (18.9%)) and the outcome: 19 (17.9%) patients died, 73 (68.9%) survived, 14 (13.2%) were lost. We found a statistically significant difference between stage 3 at presentation and death for definite TBM compared with the two other categories (33.3%/17.9%/2.6%). In 4 cases MT was isolated from CSF, but the patients did not receive specific therapy before isolation, with an unfavorable outcome.
Conclusion: CNS TB is a serious threat in Romania (39 definite cases in one institution), the diagnosis is difficult, TBM might be over diagnosed (39 patients were defined as having clinical TBM) or under diagnosed (4 cases).

P1945

The evaluation of 38 patients with tuberculous meningitis

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Objectives: In this study, 38 tuberculous meningitis (TbcM) patients were evaluated for symptoms, clinical signs, laboratory findings, therapy and prognosis, retrospectively.
Methods: In this study, 38 patients with TbcM were evaluated in GATA Haydarpasa Training Hospital Department of Infectious Diseases between 1992 and 2004. Im the study patients had undergone the following investigations: chest radiographs, Mantoux test, sputum, urine, blood and cerebrospinal fluid smear and culture for acid fast bacilli, Compureterized Tomography scan and serial Magnetic Resonance Imaging (MRI) of the brain. The definitive diagnosis was performed with positive CSF

smear, culture for acid fast bacilli, PCR and MRI findings. Probable diagnosis was performed with chest radiographs, manthoux test and other CSF examinations.
Results: In our study, 38 consecutive patients were evaluated. The ages of the patients ranged from 20 to 63 years (mean 23.3 ± 9.6 years). Thirty-five (92.1%) patients were male and three (7.9%) were female. The mean duration of neurological symptoms prior to admission was 35.4 ± 21.9 (7–100) days. The most frequent presenting symptoms were headache (97.4%), fever (65.8%) and fatigue (52.6%). The most frequent presenting signs were alterations in consciousness in nine cases, speaking disorders in four cases and convulsion in four cases. The definitive diagnosis was performed in two cases (5.3%) patients with identification of bacilli from cultures, in two patients (5.3%) with polymerase chain reaction and in 16 (42.1%) cases with MRI findings. Antituberculosis therapy was used along 12–18 months in all cases. Short-time corticosteroid therapy was given to 13 cases. Sequels developed in six (15.8%) cases. The sequels were paralysis of third cranial nerves in two cases, sixth cranial nerves in two cases, and fifth cranial nerves paralysis in one case. Mortality developed in five (13.2%) patients.
Conclusion: Because untreated TbcM is almost always fatal, it must be evaluated in differantial diagnosis of central nervous system infections.

P1946

Non-culturability of *Mycobacterium smegmatis* in stationary phase

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Objectives: The latent tuberculosis infection is believed to keep a possibility to reactivate in the human host due to presence of population of dormant cells. *Mycobacterium smegmatis*, the fast growing relation of *Mycobacterium tuberculosis*, is known to form 'non-culturable' (NC) cells in stationary phase in response to growth under suboptimal conditions, and such cells could be reactivated (Shleeva et al, 2004). We have suggested that NC cells in vitro may be considered as dormant and serve a model the persistent state of MTB in the human host.
Methods: *M. smegmatis* strain mc2 155 was cultured into modified Hartman's-de-Bont medium. Respiratory rate of the cells was detected polarographically. The level of DPI-reducing activity was measured spectrophotometrically. Inhibitory compound was isolated from supernatant by absorption chromatography followed by TLC and analyze by NMR, IR and MS.
Results: We found that the addition of bovine serum albumin into the growth media facilitates the transition of *M. smegmatis* cells to NC state during the cultivation in the stationary phase. After 70 hours growing in the modified Hartman's-de-Bont medium with the presence of 0.5 % BSA cfu of the culture is zero. Such NC cells could be resuscitated by co-cultivation with *Micrococcus luteus* cells. The level of NC cells respiration was found very low (about 1–2% of the active cells respiration level). Similarly, the rate of DPI-reductase activity of NC cells was close to zero simultaneously. NC cells are characterized by elevated floating density probably due to different changed protein/lipid ration in NC and active cells. The formation of NC cells is accompanied by accumulation of inhibitor in the supernatant. This inhibitor represents the mixture of fatty acids, and the major component of this mixture is oleic acid. It is not detected in the supernatant of active culture. The addition of oleic acid to active cells on concentrations similar to that found upon transition stopped cell growth and decreases respiration rate and DPI-reductase activity.
Conclusions: 1.1.The presence of 0.5 % BSA in medium significantly facilitates transition of viable cells of *M. smegmatis* to NC cells. 2.2.NC cells are characterized by very low rate of metabolic activity. 3.3.Cells during transition to NC state produce an inhibitor, which represents the mixture of free fatty acids.

P1947

Atypical cerebrospinal fluid and clinical presentation of tuberculosis meningitis: a case report followed by MRI

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Objectives: It was presented a tuberculous meningitis case that exhibited polymorphonuclear (PMN) dominant pleocytosis in cerebrospinal fluid (CSF) throughout her clinical course, suppressed with non-specific antibiotic therapy over the two months, and followed by serial MRI for detecting the disappearing time of meningeal lesions.
Case: A 15-year-old girl admitted in our clinic with a low-grade intermittent fever, sore throat, headache, nausea, vomiting, and loss of consciousness for the last 4 days. The biochemical and microbiological features revealed a CSF glucose of 7 mg/dL (blood glucose 102 mg/dL), CSF protein 370 mg/dL, 1450 white blood cells (WBC) per μl (86% polymorphonuclear) and organisms were absent on Gram and acid-fast stain. A CSF polymerase chain reaction (PCR) for *M. tuberculosis* was negative. According to the clinical and CSF findings, bacterial meningitis diagnosis was made and empirical ceftriaxone + ampicillin were started. On second day of this therapy, her clinical condition was improved. After 5th day of cessation of this treatment, she was become lethargic and meningeal irritation findings were become positive. A second lumbar puncture (26th day of admission) was performed and WBC was 130/mm^3 (73% polymorphonuclear), no microorganisms were found at smears, cultures and PCR. Then, vancomycin and chloramphenicol was started. On day 3rd, fever and clinical findings were again improved. At 40th day of her admission, *Mycobacterium tuberculosis* grew on Lowenstein Jensen medium that cultured from the first performed CSF. She was continued on the anti-TB medications (600 mg rifampicin, 300 mg isoniazid, 2 g morphazinamide, 1 g streptomycin and 10 mg pyridoxine daily), and dexamethasone was commenced. Antituberculous therapy discontinued at 12th month. In serial MRI follow ups, pathological changes were continue still at 20th months of clinical course even her clinical condition was totally improved.
Conclusion: This was a rare case report demonstrates that polymorphonuclear pleocytosis can be found over the months of the tuberculosis meningitis. When evaluate the clinical findings with PML pleocytosis, a misdiagnosis can be made as bacterial meningitis. Being interesting in this case is clinical improvement with non-specific treatment. After 8 month of the antituberculous treatment, going on the meningeal enhancement is the other important finding that can contribute to the current literature.

P1948

High rates of tuberculosis infection in recent immigrants from high prevalence regions in Greece

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Introduction: Persons emigrated from areas of the world with high rates of TB have incidence rates approaching those of their

countries of origin for the first several years after arrival in Greece. For this reason they are considered as a high-risk group for developing active TB disease.

Aim: To identify the incidence of TB infection in immigrants recently arrived from high prevalence countries in the island of Zakynthos.

Method-Materials: All immigrants in Greece are evaluated for TB in order to receive a "green card" (residence permit). In Zakynthos there is an excellent opportunity to study TB infection as all subjects are evaluated in a single office in our hospital. Tuberculin skin testing was performed by the Mantoux method, using 5 IU PPD-S Merieux.

Results: 793 immigrants, 20–45 years old, (648 males and 145 females) were registered during the last 2 years (2001–03). They were mainly from Albania (668), ex-USSR (118) and other Balkan countries (27). From the patients without history or scar of BCG vaccination, 172 males (26.5%) and 56 females (38,6%) responded with a skin reaction 15–19 mm, while 64 males (9.8%) and 20 females (13.8%) with history of BCG vaccination responded with ≥20 mm.

Conclusion: High raters of TB infection among recent immigrants from high prevalence countries, require tuberculin testing for administrative purposes to be replaced by targeted tuberculin testing, to identify person with Latent Tuberculosis Infection who would benefit by treatment.