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

P430 Community-acquired pneumonia caused by 'atypical' pathogens: clinical discrimination with a predictive model and scoring system

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Objective: To develop a bedside predictive model and a scoring system to identify patients with atypical community-acquired pneumonia (CAP), in order to select candidates for tailored antibiotic therapy with macrolides.
Methods: Data from a prospective study of CAP conducted at a Spanish hospital during two consecutive periods, October 99–September 00, and October 00–September 01, were analysed. An extensive non-invasive microbiological investigation was performed in all patients, including detection of *Legionella pneumophila* and *Streptococcus pneumoniae* urinary antigen, and acute and convalescent serologic testing. During the first 12-month study period, serum biochemical markers of bacterial infection [C-reactive protein, procalcitonin and lipopolysaccharide-binding protein (LBP)] were also performed. Clinical, radiological and laboratory data of patients with atypical CAP were compared with data from patients with non-atypical CAP by univariate and multivariable analysis. With the best-discriminating variables from the multivariable analysis, a scoring system model was devised. Each variable was assigned 1 point. ROC curves were used to describe sensitivity and specificity of the model. Two separate models were run.
Results: A total of 493 patients were included. On multivariable analysis, patients with atypical pneumonia were more likely to have air-conditioning exposure (OR 2.64), normal WBC count (OR 2.58), birds at home (OR 2.18), age under 65 years (OR 2.07), non-associated comorbid conditions (OR 2.04), absence of pleuritic chest pain (OR 1.95), and elevated GOT levels (OR 1.87). When the variables age was <65 years, normal WBC, birds at home, elevated transaminase levels, and a negative pneumococcal urinary antigen assay were included in the scoring system, a score of $\geq$4 captured patients with atypical pneumonia with a sensitivity of 33% and a specificity of 95%. A second scoring system was constructed with the 240 patients from the first 12-month study period. When the variables age <65 years, normal WBC count, birds at home, LBP levels <14 mcg/mL and negative urinary antigen assay were included, a score of $\geq$4 captured patients with atypical pneumonia with a sensitivity of 49% and a specificity of 95%.
Conclusion: Selected patient factors and additional diagnostic testing can aid to discriminate atypical pneumonia with high specificity. This information could be useful to ensure effective and safe use of macrolides as empirical monotherapy in CAP.

P431 Effects of a pneumococcal vaccination programme in adult invasive *Streptococcus pneumoniae* disease: a 3-year experience

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Objectives: *Streptococcus pneumoniae* is a leading cause of serious infections. The prevention of pneumococcal disease has become an important public health issue as a result of the rapid increase in the prevalence of pneumococcal resistance to antibiotics. Our aim was to evaluate the effect of a pneumococcal vaccination programme in elderly people (started in autumn, 1999) in the incidence of invasive disease, and the changes observed in serotypes prevalence and antibiotic resistance during the study period.
Methods: Our institution covers a mainly rural area of approximately 160 000 adult population in the northwestern area of Spain. We retrospectively reviewed all invasive *S. pneumoniae* (blood, CSF) isolates recovered at our laboratory between 1998 and 2003, 3 years before and after the programme started, named periods A and B respectively. Identification and susceptibility testing were performed following standard recommendations. Pneumococci were classified as susceptible, intermediate and resistant (S, I, R) according to 2001 definitions of NCCLS. Serotyping was performed at a national reference laboratory.
Results: A total of 165 invasive pneumococci were recovered during the whole period (period A, 1998–2000: 99 isolates; period B, 2001–2003: 66). The rate of invasive disease dropped from an average of 20.6 cases per 100 000 persons in period A to an average of 13.8 in period B (33% lower). A similar reduction was observed in those 65 years and older (49.2 per 100 000 vs. 37.8 per 100 000, 23.2% reduction). Penicillin susceptibility (S, I, R) in the two periods was: A: 73.4, 18.4, 8.2%, and B: 67.2, 31.2, 1.6% (P 0.05). Cefotaxime susceptibility was: A: 83.7, 13.3, 3%, and B: 85.9, 12.5, 1.6%. Erythromycin susceptibility was: A: 84.7, 1, 14.3%, and B: 68.7, 1.6, 29.7% (P 0.05). The most frequent serotypes were (per cent): period A: 4 (16.9), 3 (15.7), 8 (12), 14 (10.8); and B: 3 (16.7), 14 (16.7), 19 (11.7), 6A (8.3).
Conclusions: A significant reduction (33%) in the incidence of pneumococcal invasive disease was observed after the vaccination programme started. The slight increase in penicillin non-susceptible strains (26.6% vs. 32.8%) was because of intermediate susceptible ones, with a decrease in those highly resistant (8.2% vs. 1.6%). Erythromycin resistance duplicated in the study period (14.3% vs. 29.7%).

P432 Effect of initial treatment on bacteraemic *Streptococcus pneumoniae* pneumonia (BSPP) mortality

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Objectives: Community-acquired BSPP is associated with a high mortality. Recent studies have suggested that monotherapy may be sub-optimal for this infection. The aim of this study was to evaluate the effect of initial antimicrobial therapy on the outcome of BSPP.
Methods: Retrospective study of adult cases (>18 years) of community-acquired BSPP hospitalised at our institution from 1990 to 2003. Severity of pneumonia was assessed using PORT-score. The impact of penicillin susceptibility and initial empirical treatment (combined therapy vs. monotherapy) on the 30-day mortality were assessed by univariate and multivariate analyses.
Results: A total of 343 cases were evaluated. There were 220 males (64%); the mean age was 56.9 years (range, 18–94). Most patients had chronic predisposing conditions and 32% of them had HIV infection. Decreased susceptibility to penicillin (MIC > 0.06 mg/L), cefotaxime (MIC > 0.5 mg/L), and erythromycin (MIC > 1 mg/L) was observed in 34% (108/320), 10% (14/135), and 21% (61/292) of strains. Patients were empirically treated with a beta-lactam (50%), a beta-lactam plus macrolide (26%), or other regimens of monotherapy (10%) or combination therapy (14%). Overall mortality rate was 19%. Mortality was significantly associated ($P < 0.001$) with PORT groups: class I = 1/30 (3%), class II = 5/59 (8%), class III = 5/61 (8%), class IV = 25/123 (20%), class V = 28/70 (40%). The mortality rate was higher in patients with penicillin-resistant strains than in patients with susceptible strains (25% vs. 14%; $P = 0.03$), but was similar in patients receiving monotherapy or combination therapy (16% vs. 22%; $P = 0.13$). Multivariate analysis showed

that the presence of septic shock and advanced age (>65 years) were the most important factors associated with mortality.
Conclusions: PORT score accurately identifies the mortality risk of community-acquired BSPP. Decreased susceptibility to penicillin seems to be associated with a worse prognosis. Our study has not confirmed the beneficial impact of empirical combination therapy for BSPP.

P433 Comparative, randomised, open, multicentre trial assessing the efficacy and safety of intravenous/oral azithromycin compared with intravenous/oral clarithromycin for the treatment of community-acquired pneumonia due to *Legionella pneumophila*

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Objective: To evaluate the efficacy and safety of intravenous (i.v.) /oral (p.o.) Azithromycin (AZM) compared with i.v./p.o. clarithromycin (CLA) for the treatment of community-acquired pneumonia (CAP) caused by *Legionella pneumophila*.
Study design: Randomised, open-label, multicentre phase III clinical trial.
Inclusion criteria: Subjects over 18 years with clinical and radiological findings consistent with CAP with no requirement for hospitalisation in ICU and positive determination of *Legionella pneumophila* urinary antigen.
Treatment: (A) Patients (pts) not requiring hospitalisation and therefore, will receive only oral treatment: AZM 500 mg/day during 3 days or CLA 500 mg every 12 h during 10–14 days. (B) The hospitalised subjects will receive: (1) AZM: 500 mg once a day, by i.v. route, during at least 2 days and for a maximum of 5 days. After the second day the investigator will assess whether the therapy can be switched to oral route. The treatment will continue to complete a total of 7 days. (2) CLA: 500 mg twice a day, i.v. route for at least 2 days and for a maximum of 5 days. After the second day the investigator will assess when to switch to the oral route at doses of 500 mg every 12 h. Total treatment will be 10–14 days.
Results: During the period of study 50 patients were recruited, 25 in each group (40 male, 10 female). The mean age of the study participants was 57 years (27–83 years), mean bodyweight was 76 kg (50–130 kg) and mean height was 168 cm (141–182 cm). No difference was found in demographics between groups. Twenty-four pts (96%) received initially i.v. treatment in AZM group vs. 22 pts (88%) in CLA group. Mean duration in i.v. treatment was 3.7 days in both groups. In the intend-to-treat (ITT) population, clinical success rates both at end of treatment (EOT) and end of study (EOS) were 95.8% for AZM and 100% for CLA. Bacteriological eradication rates at EOS and EOT were 100% in both groups for patients cured or improved. The incidence of treatment-related adverse events (AEs) was similar in both groups and most events were of mild to moderate severity. Incidence of phlebitis was seven episodes (28%) for AZM vs. 13 episodes (52%) for CLA.
Conclusions: AZM i.v./p.o. for the treatment of CAP caused by *Legionella pneumophila* is as efficacious as CLA i.v./p.o. Both treatments were well tolerated but incidence of phlebitis was higher in the group receiving CLA ($P = 0.08$).

P434 *Pseudomonas aeruginosa* as a risk factor in acute exacerbations of COPD

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Objectives: *Pseudomonas aeruginosa* is a frequent cause of severe bacterial exacerbation in advanced stages of COPD. Its role in a complicated course of the disease and for an unfavourable outcome were evaluated.
Methods: Ambulatory and hospitalised subjects presenting with an acute exacerbation of COPD were prospectively evaluated for clinical and radiological parameters, for functional impairment, and for life expectancy. From all patients valid sputum samples were obtained at presentation.
Results: A total of 193 patients with a history of COPD and an acute exacerbation were included. In 114 subjects, potentially pathogenic microbes were isolated, *Pseudomonas aeruginosa* in 12 cases (10.5%). In two patients, bronchiectasis was demonstrated by high-resolution CT scan. Seven individuals infected with *Pseudomonas aeruginosa* died within 1–21 months after initial presentation. Between subjects who did or did not die, there were no significant differences with respect to age, frequency of underlying diseases, smoking status, or treatment with systemic steroids. All individuals who succumbed had stage III disease (ATS classification: FEV1<35% predicted). On average, these had a worse lung function (VC 49.1 ± 9% vs. 78.8 ± 18.9% predicted, FEV1 26 ± 6.5% vs.43.5 ± 13.4% predicted, absolute FEV1 0.8 ± 0.2L vs. 1.0 ± 0.3L). Emphysema (5/0), a medical history with treatment in an ITU (5/1), and with preceding mechanical ventilation (3/1) were more frequent in subjects with a fatal outcome.
Conclusion: Presence of *Pseudomonas aeruginosa* in individuals presenting with an acute exacerbation of COPD is associated with a high fatality rate short-term outcome. Particularly severe underlying airflow obstruction and a medical history of treatment in an ITS or mechanical ventilation is associated with an unfavourable outcome.

P435 Community outbreak of *Legionella* in Hospitalet (Spain). Usefulness of the epidemiological and molecular data to identify the source

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Legionella is considered responsible for 2–13% of cases of community-acquired pneumonia requiring hospitalisation. Colonisation of cooling towers and evaporative condensers by *Legionella*, with the subsequent production of polluting aerosols, has been associated with community outbreaks of Legionnaires' disease. To date, epidemiological data and microbiological/and molecular studies are both necessary in the investigation of an outbreak.
Objective: To describe a community outbreak of pneumonia caused by *Legionella* in Hospitalet. Catalonia (Spain) and the usefulness of the DNA chromosomic analysis to find the source of the outbreak.
Methods: An observational prospective study was performed. Data from affected patients was requested and water from suspected environmental sources was cultured for *Legionella*. Isolates of *Legionella* from patients and water samples were subtyped by Pulsed Field Gel Electrophoresis. From each positive environmental plate five colonies were picked up and re-seeded on BCYE, in order to know the clonal distribution of *Legionella*.
Results: From August 16–December 16 (2002), 13 definitive cases of pneumonia caused by *Legionella* were reported . The median age of the patients was 72 years: 92% were males. Of the cases, 11 were hospitalised. The case fatality rate was of 7.7%. The attack rate of the outbreak was 0.029%. The majority of cases were located within a radius of 200 m around the implicated cooling tower (HC) with a risk ratio of 17.4 (CI 95% 3.8–80.6). Of the 17 samples taken from cooling towers and evaporative condensers, nine were positive for *L. pneumophila*. A single DNA subtype was observed among the three clinical strains (subtype A). Two different subtypes were found in the HC cooling tower, one of them (subtype A) matching exactly with the clinical subtypes. Several subtypes were found in the other cooling towers none of them matching the clinical subtypes. Moreover each cooling tower had its own subtypes of *Legionella*. After closing the HC cooling tower no new cases of pneumonia caused by *Legionella* appeared.
Conclusions: The results of epidemiological and microbiological data suggested the HC cooling tower as a source of a community outbreak of LD. The diversity of DNA subtypes even in such a small area facilitate the identification of the outbreak source.

P436 Significance of early *Mycoplasma pneumoniae* serodiagnosis in hospitalised patients with community-acquired pneumonia

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Objectives: The use of serological methods in early diagnosis of pneumonia is controversial due to delayed production of antibodies. Therefore the temporal course of specific serological response was investigated in hospitalised patients with *Mycoplasma pneumoniae* CAP.
Methods: Hospitalised patients with suspected CAP were prospectively recruited for the study. The aetiology of *Mycoplasma pneumoniae* was serologically tested by particle-immuno-assay (PIA). A positive serological diagnosis was made if the acute phase serum titer was more than 1:160 or paired samples taken 2–4 weeks apart showed a fourfold rise in serum titer. The PIA does not differentiate between IgM and IgG. Furthermore respiratory specimens were investigated by PCR. In patients with predefined clinical parameters *Mycoplasma pneumoniae* was diagnosed in cases of positive PIA and / or positive PCR.
Results: *Mycoplasma pneumoniae* was identified as a causative agent in 50 patients (25 female, 25 male). 41 patients (82%) had a positive serology and 45 patients (90%) had a positive PCR result. In 28 patients (56%) significant PIA titers were detected on hospital admission. On day 3 of hospitalisation cumulative significant titers were measurable in 34 cases (68%) and on day 4 in 42 cases (96%). A correlation of these laboratory results was proofed with clinical findings. 48 patients (96%) were complaining about cough, 43 (86%) about fever, 28 (56%) about dyspnoea and expectoration and 26 (52%) had auscultatory findings.
Conclusion: The clinical course of mycoplasma pneumonia in patients requiring hospitalisation justifies the early use of serological methods (PIA) in acute diagnosis.

P437 Prevalence and degree of *Legionella* colonisation in cooling towers

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Cooling towers have been frequently implicated in community *Legionella* outbreaks. Since July 2003, Spanish regulation obligate test water from cooling tower systems for *Legionella* and perform a chlorine shock if colonisation are above 1000 CFU/L. This last measure cause important logistic and economic consequences troubles. Other guidelines increase the cut-off to above 10 000 CFU/L *Legionella* counts, considering little concern levels between 1000 and 10 000 CFU/L Prevalence of *Legionella* colonisation in cooling towers, degree of colonisation, and its relationship with the outbreak are underknown. Because *Legionella* levels fluctuate over time in the same cooling tower. and the real risk of this colonisation is not known, the established cut off to initiate shock measures could be overestimated.
Aim: To know the prevalence and degree of *Legionella* colonisation in cooling towers in Catalonia (Spain).
Methods: Five hundred and fifty-four cooling tower samples were randomly screened during a 3-year period (2001–2003). Water specimens were collected and transported following the national guidelines. Specimens corresponding to outbreaks were ruled out. Samples were processed according to the standard procedure ISO17131:98 for quantitative *Legionella* analysis.
Results: One hundred and two samples (18.41%) had detectable levels of *Legionella* equal to or above 10 CFU/L. The average level of a positive sample was 76.391 CFU/L, (range: 9–3 000 000 CFU/L). According to the Spanish regulation of hazard categories: a total of 30 samples were between 100 CFU/L and 1000 CFU/L, 35 samples were between 1000 CFU/L and 10 000 CFU/L and 17 samples were above 10 000 CFU/L. *Legionella pneumophila* sg. 1 was identified in 69 (67.64%) of the positive samples, *L. pneumophila* sg.2-14 in 21 (20.58%) and *Legionella* no-pneumophila in 17 (16.7%).
Conclusions: *Legionella* is a frequent inhabitant of cooling tower systems, being *L. pneumophil*, the specie most prevalent. As a result of inoculums greater than 1000 CFU/L found in most of the colonised cooling towers, a less restrictive cut off should be probably considered.

P438 Epidemiology of community-acquired pneumonia revisited: a large population-based prospective study in the Mediterranean coast of Spain

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Objective: Over the last few years there have been significant advances in microbial diagnosis and therapy of community-acquired pneumonia (CAP). The impact of these changes on the epidemiology of CAP is unknown. The aim of this study is to provide a comprehensive overview on current epidemiological features of CAP.
Methods: A 2-year population-based prospective study conducted from October 1999 through September 2001 in consecutive adults with CAP at a teaching hospital in the Mediterranean cost of Spain. An extensive non-invasive microbiological investigation was performed, including detection of *Legionella pneumophila* and *Streptococcus pneumoniae* urinary antigens, and acute and convalescent serologic testing to detect antibodies against 'atypical' and viral pathogens.
Results: A total of 493 patients (62.5% men, mean age 56 years) were included. The annual incidence rate of CAP was of 1.03 cases per 1000 inhabitants. In 265 (53.7%) patients there was one or more underlying diseases. 75.1% were included in Fine's categories I-III. 361 (73.2%) were admitted to hospital, 6 (1.2%) of them to the ICU. A total of 276 microorganisms (69 bacteria, 109 atypical pathogens, and 29 virus) in 250 (50.7%) patients were identified. In 243 (49.3%) cases the microbial aetiology remained unknown. In 20% of the cases, the microbial diagnosis was made by detection of urinary antigens. The most frequent organisms were *S. pneumoniae* 38%), *M. pneumoniae* (18%), *L. pneumophila* (10.4%), *C. pneumoniae* (9.6%), influenza virus (8.8%) and Gram-negative bacilli, including *Pseudomonas* species (14.8). In 30 (12%) cases, infection was considered mixed. The most frequent combination was *S. pneumoniae* with *M. pneumoniae* or *L. pneumophila* (three cases each). Microbial diagnosis of CAP varied according to age, site-of-care and co-morbidity. A total of 27 patients died with an overall mortality rate of 4.9%. During the second year of the study there was a decrease in the proportion of patients admitted to hospital (79.2% vs. 67.2%, $P = 0.03$) and in the mortality rate (7.1% vs. 2.8%, $P = 0.03$).
Conclusions: Incidence of CAP in this study at the Mediterranean coast of Spain was lower than previously reported. New technologies allowed a rapid etiological diagnosis of CAP in many cases and disclosed a significant proportion of mixed infections. Mortality rate of CAP may be decreasing. The results of this study may aid in the management of antibiotic treatment in patients with CAP.

P439 Factors influencing treatment outcome and length of stay in patients hospitalised for community-acquired pneumonia

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Objectives: To identify factors affecting treatment outcome and length of hospital stay in patients hospitalised with CAP.

Methods: Data were obtained retrospectively by chart review from 2183 patients with discharge or death diagnosis of CAP during a 12-month period in Germany (D), France (F), Japan (J) and the USA. Exploratory analyses were performed to evaluate predefined prognostic factors affecting treatment success (logistic regression; clinical success = patients discharged [+/- antibiotics]; microbiological success = all CAP-relevant organisms were or were presumed to be eradicated) and length of hospital stay (linear regression and analysis of covariance).
Results: Mean age ranged from 62.9 years (D) to 69.9 years (F). Mean Fine scores were lowest in J (79.2) and highest in F (102.3). In total, 80.7% of patients achieved successful clinical and microbiological outcomes. The highest success rate for combined clinical and microbiological outcomes was seen in J (92.8%), while the USA had the lowest combined success rate (68.1%). Median hospitalisation time varied from 6 days (USA) to 14 days (J). Antibiotic treatment, Fine score, age and social status were identified as prognostic factors at the $P = 0.05$-level in final models. Appropriate antibiotic treatment during hospitalisation increased treatment success in J (odds ratio [OR] 0.2), microbiological success in D and J (OR 0.5, 0.3, respectively) and reduced hospitalisation time in D and J (Cox relative risk [RR] 1.3, 1.5). In all countries, monotherapy with antibiotics other than penicillins and derivatives reduced hospitalisation time (RR 1.3–1.7) and a high-risk Fine score (class V) reduced treatment success rates compared with low risk scores (classes I–III) (OR 2.4–8.7). Longer hospitalisation was related to high-risk Fine score in F, J, and USA (RR 0.4–0.6) and to greater age in D, F, and J (RR 0.8–0.9 per 10 years). In F, 'living in nursing homes' correlated with lower treatment success rates than 'living with families' (OR 4.5).
Conclusions: The importance of risk factors varied between countries and resulted in different success rates and lengths of stay. Appropriate antibiotic treatment for CAP covering the spectrum of common causative pathogens — even monotherapy with antibiotics other than penicillins and derivatives — and a low risk assessment at the time of hospitalisation were identified as the most important prognostic factor for successful treatment and a short hospitalisation time.

P440 Bacteriological outcomes in hospitalised patients with community-acquired pneumonia treated with i.v. azithromycin plus ceftriaxone vs. i.v. ceftriaxone plus clarithromycin or erythromycin

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Objective: Several treatment recommendations, such as the updated IDSA Guidelines, include a beta-lactam/macrolide combination for hospitalised CAP pts. We compared clinical efficacy and safety of sequential therapy with i.v. ceftriaxone (CEF; 1–2 g q.d.) and azithromycin (AZM; 500 mg q.d.) followed by oral AZM (500 mg q.d.) vs. i.v. CEF (1–2 g q.d.) and either clarithromycin (C; 500 mg q12h) or erythromycin (E; 1 g q8h) followed by oral C (500 mg q12h) or E (1 g q8h) in pts with moderate to severe CAP.
Methods: This international multicentre study was a randomised and open label clinical trial. Total length of therapy: CEF/AZM was 7–10 days and CEF/C or E was 7–14 days. Clinical and bacteriological outcomes were assessed at end-of-treatment (EOT; days 12–16) and at end-of-study (EOS; days 28–35).
Results: A total of 135 and 143 pts were treated in the CEF/AZM and the CEF/C or E groups, respectively. 52.6 and 51.1% of pts in the CEF/AZM and CEF/C or E groups, respectively, were classed within the Fine risk categories IV and V. The most frequently isolated baseline pathogens were *Streptococcus pneumoniae, Haemophilus influenzae*, and *Staphylococcus aureus*; their results are presented below. The number of pts with other pathogenic organisms was too small, based on this study alone, to allow any definitive conclusions to be drawn about the efficacy of CEF/AZM or the comparator treatment. The MITT clinical success rates at EOT were 84.3% for AZM and 82.7% for CEF/C or E; EOS success rates were 81.7% for AZM vs. 75.0% for CEF/C or E.
Conclusion: In a relatively sick patient population, AZM/CEF followed by oral AZM showed comparable bacteriological efficacy to CEF/C or E followed by oral C or E in the treatment of hospitalised patients with CAP.

	EOT (d 12–16)		EOS (d 28–35)	
Outcome	CEF/AZM % (N)	CEF/C or E % (N)	CEF/AZM % (N)	CEF/C or E % (N)
Bacteriologic Eradication				
MITT population	73.2 (30/41)	67.4 (31/46)	68.3 (28/41)	60.9 (28/46)
E valuable	80.0 (24/30)	81.3 (26/32)	72.7 (16/22)	75.0 (24/32)
Clinical success by baseline pathogen* in bacteriological MITT population				
S. pneumoniae	81.0 (17/24)	70.0 (21/32)	75.0 (15/24)	66.7 (20/32)
H. inflenzae	92.3 (12/13)	50.0 (4/8)	92.3 (12/13)	37.5 (3/8)
S. aureus	83.3 (5/6)	100 (2/2)	83.3 (5/6)	100 (2/2)
Clinical success in pts with positive blood cultures				
MITT	66.7 (8/12)	58.8 (10/17)†	66.7 (8/12)	52.9 (9/17)†

*19 pts had ≥2 pathogens at baseline (6 and 4 pts were cured in AZM and C/E groups respectively).
†Not significantly different compared with CEF/AZM.

P441 First post-marketing data on treatment of community-acquired pneumonia with moxifloxacin i.v.

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Objectives: This post-marketing surveillance study investigated the efficacy and tolerability of moxifloxacin i.v. therapy in community acquired pneumonia under general hospital treatment conditions.
Methods: This open, prospective, non-controlled, non-randomised multicentre study was conducted in German hospitals with patients diagnosed with community acquired pneumonia (CAP) and treated with moxifloxacin (MXF) i.v. or with MXF sequential therapy (i.v. and oral). The exclusion criteria were the contraindications mentioned in the summary of product characteristics. Documentation included demography, anamnesis, antibiotic pretreatment, concomitant diseases and medications, daily recording of MXF therapy and symptom status, overall assessment of therapy with moxifloxacin i.v. and reporting of all adverse events observed within the treatment period.
Results: A total of 1749 patients treated with 400 mg MXF once daily were documented until April 2003, 56.4% males and 43.5% females, mean age 66.2 (SD 15.5) years. Duration of MXF therapy was up to 4 days in 12.8% of patients, 5–7 days in 38.3%, 8–10 days in 36.6% and >10 days in 12.4% of patients. In case of sequential therapy (77.9% of patients), MXF i.v. was given for up to 2 days in 14.3% of patients, 3 days in 25.4%, 4 days in 29.7% and more than 4 days in 30.6% of patients. 209 Patients (11.9%) were excluded from the analysis of symptom improvement and overall efficacy. These cases were considered as having rather hospital aquired pneumonia than CAP. Improvement of clinical symptoms in the 1538 CAP patients is shown in Figure 1. Improvement was reported in 58.2% of CAP patients at day 3, 84.9% at day 5 and 89.8% at day 7. Mean duration until improvement was 3.4 days and 7.2 days until cure. Overall efficacy of MXF i.v. therapy was rated by the physicians as very good or good in 83.5% of CAP cases. Tolerability was rated in 94.3% of all patients as very good or good. For 92 patients (5.3%) adverse events were recorded, but only in 32 patients (1.8%) events were considered to be related to the MXF therapy.
Conclusions: The new moxifloxacin i.v. formulation is a safe and effective treatment of CAP in the general hospital setting. Because of the rapid symptom relief, Moxifloxacin allows an early change from the i.v. to oral application for patients with CAP treated in hospital.

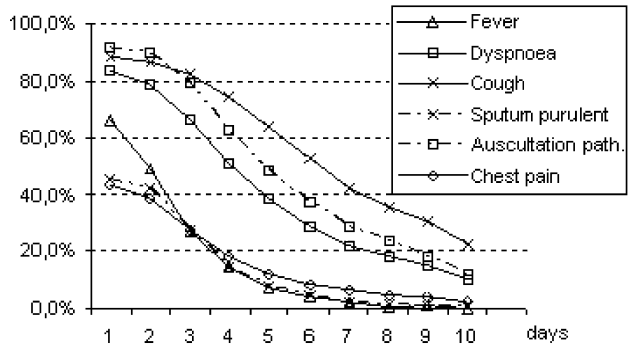

Figure 1. Decrease of clinical symptoms over 10 days from start of MXF therapy

P442 Atypical pathogens in pathogenesis of bronchial asthma in children and efficacy of azithromycin as a part of complex therapy

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Aim: The aim of this study was to evaluate the prevalence of atypical pathogens such as *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP) and *Chlamydia trachomatis* (CT) in children with bronchial asthma and assess the efficacy of azithromycin (Sumamed, Pliva, Croatia) in acute asthma exacerbation as a part of complex therapy.
Methods: Children with acute asthma exacerbation were enrolled in the study. In all children PCR (sera and sputum), serological tests (IgM and IgG) for MP, CP and CT, detection of MP antigen (AG) in nasal aspirate by using immunofluorescence method (IFA) were performed.
Results: A total of 76 children, aged 6 months–14 years, with acute asthma exacerbation were enrolled in the study. 29 of them have mild asthma, 28 – intermediate and 19 – severe asthma. Previous history of the disease was 1 month–10 years. Acute respiratory infections were the trigger of asthma exacerbation in majority (88.2%) of children. The prevalence of MP, CP and CT infections among children found to be very high (54–89%). As a result of high prevalence of MP, CP and CT infections in children, with asthma exacerbation, 5-day azithromycin was added to the complex antiasthmatic treatment. Clinical success of the treatment was achieved in majority of children (91.3%), regardless the severity of asthma exacerbation. We found rapid regression of fever and symptoms of intoxication after 2–3 days of onset of treatment. During the follow-up period (6 months) we found that 79.1% of children did not suffer from acute infections and asthma exacerbations.
Conclusions: The prevalence of MP, CP and CT infections among children included in the study found to be very high. The results suggest that such patients may have benefit with short courses of azithromycin. Nevertheless, further studies are needed in order to assess the role of azithromycin in chronic persistence of atypical pathogens in respiratory tract in children with asthma.

P443 Management of community-acquired pneumonia in a hospital in Norway: penicillin still works!

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Objective: To assess the management and severity and outcome of disease in patients hospitalised with community-acquired pneumonia (CAP) in one of the Oslo city hospitals in Norway.
Methods: Consecutive patients from 18 years of age with CAP were enrolled. CAP was classified in five risk classes according to The Fine severity score (FSS). Antibiotic treatment, outcome and complications were evaluated. Comparison between admission rates and FSS and between antibiotic therapy given and Norwegian Guidelines was performed.
Results: A total of 119 patients were included from 1 Jan 2003 to 31 July 2003. The annual incidence of hospitalised CAP in our region is estimated to 2.3/1000 inhabitants. 5.2% of patients hospitalised in medical department had CAP. Median age was 82 years (range 24–102). Male–female ratio was 1:1. FSS distribution was: 1 = 4.2%; 2 = 6.7%; 3 = 8.4%; 4 = 47.9% and 5 = 32.8%;. Based on IDSA recommendations regarding FSS, 10.9% (classes 1 + 2) could have been treated ambulatory, 8.4% (3) would require a brief observation period and 80.7% (4 + 5) hospitalisation. The median length of stay was for all patients 8 days (1–56), related to FSS risk classes: 1 = 3 days, 2 = 5.5 days, 3 = 9 days, 4 = 9 days, 5 = 10 days. Mortality was: 1–3 = 0%, 4 = 12.3%, 5 = 33.3%, totally 20/119 patients. We found no complications in FSS class 1 and 2. Totally 22 patients were admitted to the intensive care unit (3:20,0%, 4:21,0%, 5:20,5%). Norwegian Guidelines suggest penicillin (pen) or macrolides (mac) in monotherapy as empiric therapy in risk-free CAP, and in combination with aminoglycosides (aglyc) in severe CAP. In our hospital 159 antibiotics were prescribed in 119 CAP (1.34 antibiotics/CAP). 44.5% (53/119) received pen as a monotherapy in the whole treatment period, in 19.7% (13/66) pen was changed to other antibiotic. Mac was given as the primary therapy in 2.5% and in 2.5% as the secondary treatment. In 5/7 patients pen and aglyc combination was converted to pen alone
Conclusions: The relatively high median age in our patient population may explain the large number in high FSS risk classes. The FSS risk classification seemed to correlate with risk of complication and death. Nearly 50% vs. 20% of patients were treated with penicillin resp. aminopenicillin in monotherapy without change in therapy during stay. The good 'pen effect' can be explained with the favourable situation regarding bacterial resistance in Norway.

P444 A case-control study on acute respiratory infections of patients in general practices in the Netherlands, October 2000 – September 2003

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Objectives: Acute respiratory infections (ARI) are very common in the general population: In the Netherlands, yearly 3.2 million patients visit general practitioners with symptoms of upper respiratory disease. To reduce diagnostic deficit, we studied the incidence and aetiology of ARI. Additionally, we studied ARI risk factors, healthcare demand and burden of disease.
Methods: From October 2000 to October 2003, the incidence of influenza-like illness (ILI) and other ARI in general practices and the role of a broad range of pathogens in the Netherlands were studied. General practitioners from a Dutch sentinel network registered all patients consulting them for ILI and other ARI. Weekly, general practitioners randomly sampled one of these patients (case) and one patient of the same age group, visiting the general practitioner for non-respiratory symptoms (control). Samples were analysed for respiratory viruses and bacteria. Participating patients completed a questionnaire on risk factors and burden of disease.
Results: Forty practices registered all patients consulting them for ILI or other ARI. Twenty practices participated in the case-control study. Per year on average, 1600 consultations for ILI and 5000 for ARI were registered, leading to an incidence of 130 and 410 per 10 000 persons respectively. In total 647 cases and 559 controls were sampled. In more than 65% of the ILI cases and 50% of the ARI cases, viruses were detected, compared with 20% in controls. Bacteria were detected in more than 30% of both cases and controls.
Conclusion: Despite three unusual calm influenza seasons, influenza was the most common pathogen in ILI-patients. Rhinovirus was most often detected in ARI patients. Incidence for both ILI

and ARI was highest for 0–4-year olds. More data on the incidence of ILI and ARI and associated pathogens will be presented.

P445 Hospitalised community acquired pneumonia: characteristics, aetiology and outcome

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Objective: To analyse characteristics of patients with community acquired pneumonia (CAP) at the Department of Internal Medicine in the Central Infectious Disease Hospital during a 3-year period (2001–2003).
Methods: All the patients with CAP were included. Recorded data were sex, age, PORT score, presence of underlying diseases, main clinical features and symptoms, radiological appearances and outcome. Blood samples for culture from all patients, sputum when it was possible and serological tests were performed for *Chlamydia pneumoniae, Mycoplasma pneumoniae* and *Legionella* spp. In some cases direct antigen detection (urine or sputum) and serology tests for viruses and other pathogens (influenza, RSV, coxiella etc.) were carried out.
Results: One hundred and fifty-nine consecutive patients were included with a mean age of 54.2 years (range 15–96), 98 male and 61 female. The distribution of patients according to the PORT score was: class I, 20%; II, 29%; III, 25%; IV, 20%, V, 6%. In 45% one, in 36% two or more diseases considered as risk factors were present. The most prevalent of them were chronic pulmonary diseases, alcohol abuse and chronic uraemia. Admission to ICU was necessary in 10 cases (mean age 42,6, PORT score class III, 1; IV, 8; V, 1). Pathogens were identified in 62% of patients. The most frequent were *Legionella* spp. (34%), *S. pneumoniae* (15%) *M. pneumoniae* (15%), *C. pneumoniae* and *psittaci* (15%). All of the invasive *S. pneumoniae* strains were fully penicillin-sensitive. Overall mortality was 9.8%, directly attributable to pneumonia in 7%. All of the fatal outcomes with detected pathogens were caused by *Legionella pneumophila* infection.
Conclusions: The leading causes of hospitalisation of patients with mild pneumonia were fever and/or extrapulmonary clinical symptoms. More thorough ambulatory examinations of patient (X-ray, blood count etc.) would have decreased the number of hospital admissions. The causative pathogens were revealed in 62%, most of them were 'atypical'. The unusual distribution of pathogens probably results from the special situation of our hospital. Nevertheless, more frequent examination of sputum and BAL would result in the increase of 'typical' pathogens. The PORT score was useful to identify patients with poor outcome.

P446 Comparison of additional antibacterial usage in patients with community-acquired pneumonia receiving telithromycin or clarithromycin: results from two double-blind, randomised clinical trials

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Objectives: This analysis was undertaken to compare use of additional respiratory-related antibacterials in patients with community-acquired pneumonia (CAP) treated with the ketolide telithromycin (TEL) or the macrolide clarithromycin (CLA).
Methods: Data from two similar, comparative, double-blind, randomised trials were pooled for this analysis. Adult outpatients with mild to moderate CAP were randomised to receive either TEL 800 mg once daily for 5, 7 or 10 days or CLA 500 mg twice daily for 10 days. In both studies, clinical outcome was assessed in the per-protocol (PP) population at the post-therapy/test of cure (TOC; days 17–24) visit. Patients were followed to days 31–36 (late post-therapy visit) to assess the use of additional respiratory-related antibacterials in the intent to treat population (TEL, $n = 612$; CLA, $n = 411$).
Results: Clinical cure rates in the PP population were equivalent in the two treatment groups [88.8% (428/482) for TEL and 90.1% (272/302) for CLA; 1.3, 95% CI (−6.0, 3.4)]. A total of 155 patients required additional respiratory-related antibacterial therapy: 14.2% (87/612) of patients treated with TEL and 16.5% (68/411) of CLA-treated patients [−2.3, 95% CI (−7.0, 2.4)]. Shorter courses of TEL were not associated with increased requirements for additional antibacterials [14.0% (27/193) for 5 days TEL vs. 14.3% (60/419) for 7/10-day TEL; −0.3, 95% CI (−6.6, 6.0)]. Cephalosporins, macrolides and quinolones were the most common additional antibacterials used, accounting for more than half of supplementary respiratory-related antibiotics in both treatment groups. Duration of additional antibacterial therapy was 174 days/100 patients with 5-day TEL, 145 days/100 patients with 7/10-day TEL and 181 days/100 patients in CLA-treated patients. For those patients who received additional treatment with intravenous (i.v.) antibacterials, the duration of additional treatment was 41 days/100 patients with 5-day TEL, 28 days/100 patients with 7/10-day TEL and 54 days/100 patients with 10-day CLA.
Conclusions: TEL 800 mg once daily for 5, 7 or 10 days is as effective as CLA 500 mg twice daily for 10 days for the treatment of CAP. The tendency towards a reduced frequency and shorter duration of use of additional antibiotics, particularly i.v. therapy, suggests a potential for cost savings when TEL is used to treat patients with CAP.

P447 Seroprevalence of *Chlamydophilia pneumoniae* infection in patients with chronic stable asthma

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Objectives: Asthma is an inflammatory disease in which the airways become blocked or narrowed, constriction in the bronchi and bronchioles and a feel of tightness in the chest. *Chlamydophilia pneumoniae* like genetic and environmental factors contribute to the development of asthma. This study was designed to investigate the presence of *C. pneumoniae*-specific IgG, IgA and IgM antibodies in sera samples of 50 adults with a clinical history of chronic stable asthma and 50 healthy individuals as control group.
Methods: The correlation between *C. pneumoniae*-specific antibodies in chronic stable asthma cases and eosinophilia cationic protein (ECP) levels were also evaluated. 50 stable chronic asthma cases (12 male, 38 female) between the ages 14 and 70 were evaluated for FEV (forced expiratory volume), ECP, allergic state and smoking habits. *C. pneumoniae*-specific IgG, IgA and IgM antibodies were also investigated for this group.
Results: Healthy control group was matched with patient group for age, gender, locality and smoking habits *C. pneumoniae* seropositivity (a past *C. pneumoniae* infection) was found in 68% (34) and 58% (29) of stable asthma cases and control groups, respectively ($P > 0.05$). *C. pneumoniae*-specific IgA was found higher in 34% (17) and 18% (9) of stable asthma and control groups respectively ($P > 0.05$). The indication of chronic *C. pneumoniae* infection (IgG > 1/512 and IgA > 1/40) was found in 28% (14) and 10% (5) of stable asthma cases and control group, respectively ($P < 0.05$). A statistically significant difference was not found between chronic stable chronic asthma and control groups for chronic *C. pneumoniae* seropositivity and smoking. Also a statistically significant difference was not found between ECP positive and negative patients for chronic *C. pneumoniae* infection seropositivity ($P > 0.05$).
Conclusions: As conclusion, this study supports that there can be a relationship between chronic *C. pneumoniae* infections and stable asthma cases.

P448 Severe community-acquired pneumonia: impact of empirical antimicrobial treatment on outcome

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Objectives: To assess the impact of empirical antimicrobial therapy on the outcome of severe community-acquired pneumonia (SCAP).
Methods: Prospective observational study of patients with SCAP admitted to the ICU of a Hospital of Chest Diseases since January 1998. Immunocompromised patients and patients with a known causative microorganism at the time of admission were excluded. After initial laboratory testing empirical antimicrobial treatment was started by the attending physician.
Results: Sixty-seven patients were studied (45 male, mean age 56 ± 18 years, mean APACHE II score 15 ± 6), fifty-one of whom needed mechanical ventilation (76%). Empirical antimicrobial regimens included combination of two to four antibiotics. A combination of a beta-lactam with a macrolide or a quinolone was administered to 54 patients (80%). Twenty-seven (40%) received an aminoglycoside, 16 (24%) received antistaphylococcal agents and 14 (21%) antibiotics against anaerobic microorganisms. Strict adherence to management guidelines of ATS and/or BTS was noted in 39 cases (58%). In most cases (72%), non-compliance consisted of using more antimicrobial agents than those recommended. Overall mortality was 43%. Patients treated with a beta-lactam+macrolide or quinolone combination had significantly lower mortality compared with those treated with other regimens (37% vs. 77%, $P = 0.01$). Concomitant use of other agents had no impact on outcome. adherence to management guidelines was not shown to influence mortality.
Conclusions: Non-adherence to management guidelines was frequently encountered among ICU patients with SCAP. Overuse of aminoglycosides and/or antistaphylococcal agents was the main cause of divergence from guidelines. However, this was not shown to affect mortality. Use of a combination of beta-lactam+macrolide or quinolone was associated with lowest mortality rate.

P449 Efficacy of linezolid for the treatment of pneumonia caused by penicillin (PRSP) or multi-drug resistant (MDRSP) *Streptococcus pneumoniae* (SP)

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Objective: SP is becoming resistant to many antimicrobials commonly used to treat pneumonia, a common and potentially fatal infection. Linezolid is a new antimicrobial with bactericidal activity against SP. The objective of this analysis was to assess the efficacy of linezolid for the treatment of pneumonia caused by SP, including PRSP and MDRSP.
Methods: A meta-analysis was performed pooling data from seven studies evaluating linezolid for the treatment of community- or hospital-acquired pneumonia. Patients were required to have symptoms compatible with pneumonia and a chest X-ray showing an acute infiltrate. Adults received 600 mg linezolid i.v./p.o. q12 h, and children received 10 mg/kg q12 h, for a mean of 11 days. For this analysis, patients with SP isolated from sputum and/or blood were considered; these patients were included in the modified intent-to-treat (MITT) population. Patients who met stricter criteria constituted the microbiologically evaluable (ME) population. PRSP were defined as isolates with an MIC > 2 mcg/mL; MDRSP were defined as isolates resistant to at least two of the following classes: penicillin, second-generation cephalosporins, macrolides, tetracyclines, or TMP/SMX.
Results: Of 536 SP isolates, including those from patients receiving control regimens, the MIC90 for linezolid was 1 mcg/mL, with a range of <0.125 mcg/mL to 4 mcg/mL. There were 275 SP patients in the linezolid-treated MITT population, and 212 in the ME population; clinical cure rates in these two groups were 85 and 90%, respectively, among patients whose outcomes could be assessed. There were 34 MITT and 25 ME patients with PRSP, with clinical cure rates of 74 and 84%, respectively. In bacteraemic ME patients with SP the clinical cure rate was 61/66 (92%) and in those with PRSP it was 7/8 (88%). Amongst the ME patients with PRSP and whose isolates were resistant to at least one other class of antimicrobial (MDRSP), clinical cure rates and microbiological eradication rates ranged from 75 to 100%.
Conclusion: Linezolid is an effective agent for the treatment of pneumonia caused by SP, including strains resistant to other antimicrobials.

P450 Pathogenic role, epidemiology, and susceptibility to antimicrobial agents of *Neisseria meningitidis* isolated from lower respiratory tract secretions of adult patients

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Objectives: Meningococcal pneumonia has been a recognised clinical syndrome for over 20 years. Because of the nasopharyngeal carriage of *N. meningitidis* (NM), the ability to establish the diagnosis based on the culture of respiratory samples (RS) alone is hazardous. To assess the incidence of respiratory infections caused by NM, the serogroups, and the antimicrobial susceptibility of the isolates, we reviewed the 20 cases of NM isolated from RS of adult patients which occurred at our institution from 1999 to 2003.
Methods: Ours is a large teaching institution (1800 beds) serving a population of 640 000 inhabitants. All significant RS of patients suspected of having pneumonia were quantitatively cultured onto blood agar, chocolate agar, and MacConkey agar. Isolates were identified by standard procedures. Susceptibility testing was performed by the broth microdilution method with 5% lysed horse blood. Beta-lactamase production was detected using the nitrocephin test. Serogroups were determined at the National Reference Laboratory for Meningococci in Spain.
Results: Over the study period, 15 003 RS were processed. Among those, 40.7% were positive. NM represented 0.4% of all positive samples. The evolution of cases was: 1999, two cases (0.03/1000 admissions); 2000, 0 cases (0/1000); 2001, seven cases (0.11/1000); 2002, two cases (0.03/1000), 2003, nine cases (0.14/1000). The origin of isolates was bronchial aspirate (13 cases), bronchoscopic specimens (4), and sputum (3). Serogroups were B (17); C, Y and 29E, one case each. 80% of the isolates were non-susceptible to penicillin (MICs 0.12–0.25 mg/L). None of the isolates produced beta-lactamase and all were fully susceptible to cefotaxime, rifampin and ciprofloxacin. Fifteen patients were hospitalised in ICUs. Nine patients were diagnosed of pneumonia (four monomicrobial and five polymicrobial) and 11 were colonised. None developed meningococcal bacteraemia.
Conclusions: The pathogenic role of NM in lower respiratory tract infections of adults is probably underestimated because its isolation is difficult. In our area the most frequent serogroup was B, and we found a high incidence of penicillin-resistant (non-susceptible) isolates.

P451 Efficacy of a 7-day course of oral telithromycin 800 mg once daily in community-acquired pneumonia caused by resistant *Streptococcus pneumoniae*

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Objectives: The ketolide telithromycin has a targeted spectrum of antibacterial activity for the treatment of community-acquired

respiratory tract infections, that provides coverage of all relevant pathogens, including atypical/intracellular organisms and *Streptococcus pneumoniae* resistant to penicillin (PRSP) and/or erythromycin (ERSP). Previous studies have shown that oral telithromycin 800 mg once daily for 7–10 days is an effective treatment for patients with community-acquired pneumonia (CAP). The efficacy and tolerability of 7-day telithromycin in patients with CAP, including those with CAP caused by PRSP and/or ERSP, were investigated further in this multicentre multinational study.
Methods: A total of 858 patients (aged ≥13 years) with CAP received telithromycin 800 mg once daily for 7 days in an open label, non-comparative study that aimed to recruit a high proportion of patients with pneumococcal aetiology. Clinical and bacteriological outcomes were assessed in the clinical and bacteriological per-protocol populations (PPc, $n = 723$; PPb, $n = 274$) 10–17 days post-therapy [test of cure (TOC) visit].
Results: Clinical cure and bacteriological eradication were achieved in 15/16 (93.8%) patients infected with resistant pneumococci: 14/15 (93.3%) patients with ERSP and 7/7 with PRSP. Overall, satisfactory bacteriological outcome was achieved in 87.6% (240/274) of patients at TOC, with 94.6% (122/129) of *S. pneumoniae*, 87.8% (108/123) of *Haemophilus influenzae*, 80% (16/20) of Moraxella catarrhalis and 80.8% (21/26) of *Staphylococcus aureus* strains being eradicated/presumed eradicated. In total, 89.3% (646/723) of patients were assessed as clinically cured at the TOC visit. Telithromycin treatment was well tolerated. Overall, 15.7% (135/858) of patients experienced treatment-emergent adverse events (TEAEs) classified as possibly related to study medication, the majority of which (occurring in 112 patients) were mild in intensity. The most common possibly treatment-related TEAEs were diarrhoea [4.7% (40/858)] and nausea [3.3% (28/858)].
Conclusion: A 7-day regimen of oral telithromycin 800 mg once daily is an effective and well-tolerated first-line treatment option for CAP, including infections caused by resistant pneumococci.

P452 Efficacy of telithromycin in patients with community-acquired respiratory tract infections caused by *Streptococcus pneumoniae*, including resistant strains

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Objectives: The ketolide telithromycin has a targeted spectrum of antibacterial activity for the treatment of community-acquired respiratory tract infections (RTIs), providing coverage of all relevant RTI pathogens, including *Streptococcus pneumoniae* resistant to penicillin [PEN (PRSP)] and/or erythromycin [ERY (ERSP)]. This analysis of data pooled from 14 Phase III/IV studies evaluated the clinical and bacteriological efficacy of telithromycin 800 mg once daily in the treatment of pneumococcal community-acquired RTIs.
Methods: Isolates of *S. pneumoniae* were collected from patients with community-acquired pneumonia, acute exacerbations of chronic bronchitis or acute bacterial sinusitis in 14 Phase III/IV clinical studies. Patients received telithromycin 800 mg once daily for 5, 7 or 7–10 days. Isolates were tested for susceptibility to PEN, ERY and telithromycin by MIC testing at a central laboratory. Clinical and bacteriological outcomes were assessed at post-therapy (Day 17–24) in the bacteriological per-protocol (PPb) population.
Results: *S. pneumoniae* was identified as a causative pathogen in 555 telithromycin-treated patients in the bacteriological modified intent to treat (bmITT) population; 493/495 (99.6%) evaluable isolates were susceptible to telithromycin at an MIC of ≤1 mg/L. PPb clinical cure and satisfactory bacteriological outcome rates for all *S. pneumoniae* infections were 92.7% (404/436) and 94.3% (411/436), respectively. Clinical cure and satisfactory bacteriological outcome rates were both 82.4% (28/34) for PRSP (PEN MIC ≥2 mg/L), both 84.6% (44/52) for ERSP (ERY MIC ≥ 1 mg/L) and both 83.3% (30/36) for PEN-intermediate (PEN MIC 0.12–1 mg/L)/PRSP/ERSP infections. Of 82 patients with pneumococcal bacteraemia, 74 (90.2%) were clinically cured, including 5/7 and 8/10 with PRSP or ERSP, respectively. Telithromycin was well tolerated with the majority of treatment-emergent adverse events (TEAEs) of mild to moderate intensity. TEAEs considered by investigators to be possibly related to treatment were reported by 1071/4045 (26.5%) patients, the most frequent being diarrhoea (7.6%), nausea (5.3%) and headache (1.5%).
Conclusion: Telithromycin 800 mg once daily for 5–10 days is an effective and well-tolerated treatment for community-acquired RTIs caused by *S. pneumoniae*, including isolates resistant to PEN and/or ERY.

P453 Epidemiology of hospitalised patients with AECB (acute exacerbation of chronic bronchitis) and Gram-negative bacilli in the sputum

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Objectives: To describe the epidemiological, clinical and microbiological characteristics of patients admitted to hospital with an acute exacerbation of chronic obstructive pulmonar disease (COPD) and Gram-negative bacilli in the sputum.
Methods: During a 6-month period, we studied prospectively all patients admitted to hospital with an acute exacerbation of COPD and Gram-negative bacilli in the sputum. Diagnosis of COPD and acute exacerbation were defined following GOLD and Anthonisen criteria, respectively. Age, gender, smoking history, pulmonary function (FEV1), presence of bronchiectasis, previous use of antibiotics, previous hospital admission, Emergency Room visits and microbiological data at entry were recorded.
Results: From July to November 2003, 111 patients were admitted to hospital for AECB. Thirty patients (27%) had Enterobacteriaceae or *P. aeruginosa* in the sputum. There were 28 men, mean age 75.2 years (10), 22 were ex-smokers and four were current smokers and 46% of the patients had been exposed to antimicrobials in the previous 3 months. Nineteen had been admitted to hospital and another 10 had been in the Emergency Room in the past year, respectively. Of the 22 patients who received antimicrobial therapy on admission, treatment was modified in six (20.7%) according to the sputum microbiological findings. The mean length of stay of 12.3 days (6.95). Mean FEV1 during stable phase of the illness was 805 mL (47.6) (40.89% of predicted). *P. aeruginosa* was isolated in 15 of the 30 patients (50%), multiple Enterobacteriaceae in 12 patients, *S. marcescens* in one patient and *K. pneumoniae* in two patients. Bronchiectasis were present (thoracic scanner) in 75% of patients.
Conclusions: The prevalence of bronchial colonisation/infection with Gram-negative bacilli in COPD patients with AECB and mean FEV1 values of approximately 40%, are higher than those reported previously in retrospective studies. More than 2/3 of the patients had been admitted to hospital during the past year and half had been treated with antimicrobials in the recent past. A striking finding was the prevalence of underlying bronchiectasis, present in 75% of this population.

P454 Procalcitonin and neopterin correlates with aetiology and severity in adults with pneumonia

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Clinical outcome of pneumonia depends of a multifaceted treatment approach. Not only diagnostic methods but also early indicators of the degree of inflammatory response can aid in therapeutic decisions.The aim of this study was to evaluate the

usefulness of procalcitonin (PCT) and neopterin in distinguishing among aetiologies and severity of pneumonia.
Methods: The study population were patients with clinical signs of lower respiratory tract infection and pathological findings on the chest radiograph. Samples were collected at admission for microbiological procedures in order to establish the aetiological diagnosis. Were eligible for the study entry only those diagnosed of pneumococcal or *Legionella* pneumonia. Patients were grouped into five risk classes according to the Pneumonia Severity Index (PSI). Patients were also grouped by the presence of unilobar or multilobar radiographic pulmonary infiltrates. PCT was measured by immunoluminometric assay (Lumitest PCT, Brahms Diagnostica) and neopterin was measured by enzyme immunoassay (Neopterin ELISA, IBL).
Results: Eighty-seven patients were included in the study. Seventy-six patients were diagnosed of pneumococcal pneumonia. *Streptococcus pneumoniae* was isolated in blood culture in 15 patients. The rest were diagnosed by detection of pneumococcal capsular polysaccharide in urine samples by counter immunoelectrophoresis. Twenty-one patients were diagnosed by urinary antigen detection of *Legionella pneumophila* serogroup 1 by enzyme immunoassay (Bartels EIA Legionella Urinary Antigen). Patients with pneumococcal pneumonia presented elevated both procalcitonin (mean 11.11 ng/mL) and neopterin (mean 87.43 ng/mL) levels, being higher in bacteraemic than in non-bacteraemic pneumonia ($P = 0.059$ for PCT and $P = 0.015$ for neopterin). Patients with *Legionella* pneumonia presented elevated neopterin levels (mean 86.89 ng/mL) and slightly elevated PCT levels (mean 1.04 ng/mL). When comparing the levels of both markers according to PSI class, patients in high risk class (IV and V, 33 patients) had significantly more elevated PCT levels ($P = 0.021$) than patients in low-risk class (I to III, 64 patients). Neopterin presented a lowest correlation ($P = 0.091$). Both PCT and neopterin yielded a significant correlation to the radiographic extent of the pneumonia ($P = 0.002$ and $P = 0.020$, respectively).
Conclusion: PCT and neopterin levels show a significant correlation to the aetiology and the severity of the pneumonia.

P455 Rapid centrifugal method for *Legionella* antigen concentration in urine samples

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The aim of the present study was to evaluate the utility of the antigen concentration method by centrifugal ultrafiltration (Amicon Ultra-4. Millipore Corporation, Bedford, MA, USA) for detecting Legionella pneumohila antigen in urine samples by immunochromatographic (ICT) method, comparing it to the passive selective ultrafiltration (Urifil-10. Millipore Corporation).
Materials and methods: Group 1: we studied 35 urine samples from patients with pneumonia caused by *L. pneumophila*. Legionnaires' disease was diagnosed by detection of soluble antigen from *Legionella pneumophila* serogroup 1 in urine samples by Binax EIA. The second group consisted of 35 urine samples from patients with pneumonia of other aetiologies (15 *Streptococcus pneumoniae*, 15 *Mycoplasma pneumoniae*, and five *Chlamydia pneumoniae*). The third group consisted of 15 urine samples from patients with no clinical or radiological signs of pneumonia, the pacients had urinary tract infections (10 *Escherichia coli*, one *Pseudomonas aeruginosa*, two *Proteus mirabilis*, one *Klebsiella pneumoniae*).
Results: The results of the ICT test using urine samples concentrated by passive and centrifugal ultrafiltration were identical in the 35 samples from group 1 patients (100% agreement). Urine samples, concentrated by both methods, from patients with pneumonia of other aetiologies or no clinical symptoms or signs of pneumonia but with urinary tract infection (groups 2 and 3) were all negative by ICT. The overall agreement between both the concentration methods, considering the three patient groups, was 100%.
Conclusions: (1) The antigen detection of *Legionella* in urine samples concentrated by passive ultrafiltration and centrifugal ultrafiltration shows a concordance of 100%. (2) The centrifugal concentration is an easy and rapid system (15–30 min). Therefore, in less than 1 h we could obtain the result of *Legionella* antigen detection. (3) The concentration of urine samples by centrifugation did not represent a decrease in the sensitivity and specificity of the antigen detection.

P456 Usefulness of PORT-score to decide hospitalisation of patients with community-acquired pneumonia: analysis of 509 consecutive patients classified in categories I and II

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Objectives: Pneumonia Patient Outcomes Research Team (PORT) has developed a prediction rule to identify patients with community-acquired pneumonia (CAP) who are at risk for death and other adverse outcomes. The authors recommend outpatient management for Fine categories I and II.
Methods: Observational study of patients with CAP and class I and II according to PORT-score at a tertiary general hospital. Prospective data collection has been done according to a protocol of CAP (October 1996–November 2003). We analysed epidemiological, clinical, radiographic and laboratory data and outcome of hospitalised patients compared with outpatients. Decision about admission or discharge was made on clinical and not algorithms judgement.
Results: Five hundred and nine (23.8%) of 2139 patients with CAP were classified in Fine categories I and II; 376 (73.6%) were hospitalised (364 in general wards and 12 (3.2%) in an intensive care unit (ICU)), representing 19.4% of all CAP admissions. Mean age (44 vs. 39; $P = 0.002$), CRP level (17.3 vs. 13.2; $P = 0.004$) and PORT-score points (51 vs. 39; $P = 0.0001$) were significantly higher in hospitalised patients compared with outpatients. Mean oxygen pressure on blood was significantly lower in the inpatient group (68 vs. 78; $P = 0.0001$). Chronic alcoholism (7% vs. 2.3%; $P = 0.046$), chronic lung disease (34.3% vs. 15.8%; $P = 0.0001$), HIV infection (10.1% vs. 1.5%; $P = 0.002$), shortness of breath (48.9% vs. 19.7%; $P = 0.0001$), pleural effusion (11.2% vs. 2.3%; $P = 0.002$) and diabetes (5.6% vs. 1.5%; $P = 0.05$) were significantly more frequent in the hospitalised group. None of the patients died.
Conclusions: In our series, 73.6% of CAP patients in Fine categories I and II were admitted to the hospital (3.2% in ICU). Our findings suggest that some conditions (alcoholism, chronic lung disease, HIV infection and diabetes) not considered in the PORT-score are important medical contraindications on physicians' judgement for outpatient care. Additional consideration is also provided by physicians to patients who have low pO2, subjective shortness of breath or pleural effusion. PORT-score is probably good to predict dead and helps physicians make decisions about hospitalisation even though the prognostic score should not supersede clinical judgement in the decision to hospitalise as some patients designated as being at low risk may have important medical contraindications to outpatient care.

P457 Sputum colour as marker for clinical success in patients with acute exacerbation of chronic bronchitis (AECB) treated with levofloxacin

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Objectives: Bacterial exacerbations of chronic bronchitis are an ongoing therapeutic challenge with increasing incidence in elderly patients. Levofloxacin, due to its enhanced activity against the most common pathogens in AECB, is well accepted as a standard treatment for this indication. The objective was to investigate

efficacy and safety in AECB-patients, documented by signs, symptoms and sputum colour.
Methods: In a post authorisational survey during the season 2002/2003 a total of 1.297 outpatients (median age 59.8 years) were treated with Levofloxacin 500 mg tablets o.d. for 7 days. 1.228 patients suffered from AECB. Concomitant diseases were documented in 49% and concomitant medication for 69% of the patients. Two AECB episodes (median) occurred during the previous 12 months with a median time interval of 15 weeks between the previous and the actual episode. The change of sputum colour characterised by a pretrial defined colour table based on the Stockley criteria was documented by the patients on a daily basis during the treatment.
Results: A purulent sputum was found in 95% of the 1.228 patients before treatment, but at the end of treatment in only 15% ($P < 0.001$). For 1.034 patients sputum colour was rated. At day 5 a change from green/dark yellow to clear/white was observed in 45% of the patients and in 76% at day 7, end of treatment. Body temperature decreased in 94%, improvement was observed for cough in 93%, for breathing difficulties in 83%, for auscultation findings in 88%, and for general physical condition in 86% of the patients (each $P < 0.001$). At the end of observation the clinical outcome was rated as successful in 98% of the patients (60% cured, 38% improved). A complete release of symptoms was reached after 6.6 days (mean) and normal everyday activities were resumed after 6.9 days (mean). Adverse drug reactions were reported in only two patients (0.15%).
Conclusions: The clinical outcome of a 7-day treatment with Levofloxacin, assessed by signs, symptoms and the change of sputum colour as an additional marker, demonstrates a high efficacy in the present population of elderly AECB-patients with various concomitant diseases, accompanied by an excellent safety profile, resulting in a subsequent resumption of normal activities at the end of treatment.

P458 Outcome and clinical differences between pneumococcal pneumonia with and without bacteraemia

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Background: Few attempts have been made to compare bacteraemic and non-bacteraemic pneumococcal pneumonia, mainly because the difficulties to gain agreement on which cases represent non-bacteraemic pneumococcal pneumonia. Recently, immunochromatographic assay for detection of *S. pneumoniae* urinary antigen has been successfully evaluated for the diagnosis of pneumococcal pneumonia.

	Bacteremic	Nonbacteremic	*P*
Mean Age	39.8	70.8	<0.001
COPD	18%	40%	0.033
Liver Disease	16%	0%	0.028
CHF	7%	32%	0.004
Current Smoker	40%	16%	0.024
Former Smoker	17%	48%	0.004
Alcohol Abuse	30%	8%	0.021
Drug Abuse	19%	0%	0.014
HIV Infection	23%	0%	<0.001
Days of Intravenous Antibiotic Treatment	6	4.5	0.006
Temperature $\geq$ 38.5°C	41%	20%	0.044
Length of Hospitalisation	7.9	6.8	0.071

COPD: Chronic obstructive pulmonary disease. CHF: congestive heart failure. HIV: human immunodeficiency virus

Objectives: (1) To examine and compare clinical and radiological features, risk factors and outcome associated with bacteraemic and non-bacteraemic groups. (2) To study vaccine indications in patients with pneumoccocal pneumonia according the 1997 ACIP recommendations.
Methods: A retrospective study (1995–2003) analysing the clinical records of patients diagnosed of pneumococcal pneumonia in our institution was performed. *S. pneumoniae* were identified by blood cultures (bacteraemic group) and detection of urinary antigen (non-bacteraemic group).
Results: There were 95 patients (70 bacteraemic and 25 non-bacteraemic). In seven non-bacteraemic cases another aetiology was detected (*Legionella*, one case and *C. pneumoniae*, six cases). Table 1 shows the main differences between the two groups. Overall mortality was 9.5% (without group differences). 81% cases fulfilled the ACIP vaccine recommendations, although different strengths of evidence were observed (grade A, 57%).
Conclusion: (1) In our study, smoking is the leading risk factor for pneumococcal pneumonia. However, current smokers have an increased risk of bacteraemic forms and former smokers and COPD developed non-bacteraemic forms more frequently. (2) Bacteraemic patients need a more prolonged intravenous antibiotic treatment than non-bacteraemic patients. (3) In accordance with previous studies, the majority of our patients fulfilled the ACIP recommendations. Then, pneumococcal vaccination should be emphasised in persons at highest risk.

P459 Comparative assessment of moxifloxacin and macrolides in acute exacerbation of chronic bronchitis: clinical efficacy and influence on the long-term prognosis

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Objectives: Comparative clinical studies usually show similar clinical effectiveness of different antibiotics in acute exacerbation of chronic bronchitis (AECB). In connection with this it seems useful to estimate the influence of antibiotics on the long-term prognosis for patients with AECB, i.e. the length of symptom-free period after disease exacerbation.
Methods: It was the open randomised comparative study. Patients with AECB who had at least two exacerbations per year were included into the study. One group of patients with AECB were treated by moxifloxacin (MXF) 400 mg per day during 5 days and the other group by macrolide antibiotic (azithromycin, clarithromycin, or spiramycin) during 7 days. After treatment the patients were followed up for 12 months.
Results: A total of 60 patients with AECB were included into the study, 29 of which were treated by MXF, and 31 by macrolide antibiotic. The average age of the patients was correspondingly 58.1 and 57.3 years, length of chronic bronchitis – 10.8 and 11.8 years. Number of AECB in previous year was similar in both groups. Clinical cure rate estimated a week after the end of treatment was 96.6% for MXF and 93.5% for macrolides. The minimal period to the next exacerbation of chronic bronchitis was 107 days in the group of MXF and 14 days in the group of macrolides. During the follow-up period (6 and 12 months) the incidence of AECB (requiring prescription of antibiotics) was 3.6 and 15.4% in the group of patients who were prescribed MXF, and 17.2 and 44.0% in the group of patients who were treated by macrolide antibiotics. Average length of symptom-free period after MXF treatment was 49 days longer than in the macrolide group.
Conclusion: MXF was superior to macrolides in long-term prognosis in AECB and prolongs the period till the following exacerbation.

Bacteraemia and endocarditis

P460 Human leptospirosis in a regional leptospira laboratory, Piemonte (Italy)

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Objectives: Human leptospirosis is underdiagnosed in Italy, and often unrecognised because of the difficulty of clinical and laboratory diagnosis. The aim of the study is to report clinical and epidemiological features of human leptospirosis in a North-West area of Italy with some professional risk factors (agriculture, rice cultivation, animals farm, abattoirs).
Methods: Samples from 292 patients with suspected *Leptospira* infections were examined. The serodiagnosis was performed by microscopic agglutination test, using cultures of 20 reference *Leptospira interrogans* strains (kindly provided by National Centre for Leptospirosis, Rome, Italy). Cultures were performed on EMJH medium (with and without 5-fluorouracil) and incubated at 30°C for 2 months.
Results: Diagnosis of leptospirosis was confirmed in 42 (14.4%) patients. The clinical symptoms were: fever (100%), jaundice (76.2%), meningitis (9.5%), renal failure (73.8%), enlarged liver (73.8%) and conjunctival suffusion (14.3%). Cultures were positives in two patients, who later died. *Leptospira* strains were isolated from blood of a 88-year-old patient and from both blood and urine of a 35-year-old patient. The strains were identified as *L. interrogans* serogroup icterohaemorrhagiae, serovar icterohaemorrhagiae by National Centre for Leptospirosis. The largest number of infections was ascribed to occupational activities (43%). Water contaminated with animal urine emerged as probable source of infection (77%). The typical leptospiral seasonal trend, with a peak during the summer months, was observed.
Conclusion: The results confirmed leptospirosis as an important cause of infection in our region. However, strict laboratory diagnosis protocol must be followed in order to recognise this rare but very severe re-emerging infection. Clinicians should be aware of leptospirosis, especially in area with high risk factors.

P461 *Streptococcus pneumoniae* bloodstream infections (SPBI): a clinical study of 460 episodes in the era of penicillin resistance

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Objectives: SPBI with decreased susceptibility to penicillin are increasing in prevalence in most European countries. The aim of this study was to evaluate the clinical spectrum of disease in adults and the influence of penicillin susceptibility on the outcome of SPBI.
Methods: Retrospective study of adult cases (>18 years) of SPBI diagnosed at our institution from 1990 to 2003. The impact of penicillin susceptibility and initial empirical treatment on the 30-day mortality were assessed by univariate analyses.
Results: A total of 460 episodes of SPBI were evaluated. There were 290 males (63%) and the mean age was 57.6 years (18–94). Most patients had chronic predisposing conditions and 28% of them had HIV infection. Decreased susceptibility to penicillin (MIC > 0.06 mg/L), cefotaxime (MIC > 0.5 mg/L), and erythromycin (MIC > 1 mg/L) was observed in 36% (154/433), 9% (17/196), and 21% (83/400) of strains. The most common form of SPBI was pneumonia (81%), followed by bacteraemia without focus (7%), meningitis (6%), peritonitis (1%), and others (5%); 46 episodes (10%) were nosocomially-acquired. Patients were empirically treated with a beta-lactam (50%), a beta-lactam plus macrolide (20%), or other regimens of monotherapy (9%) or combination therapy (21%). Overall mortality rate was 22% and was similar among patients with pneumonia (21%), bacteraemia (27%), meningitis (23%), or peritonitis (20%). Mortality rate was higher ($P < 0.05$) in patients with penicillin-resistant strains (27%) than in cases with susceptible strains (18%), and in nosocomial infections (54%) than in community-acquired infections (18%). The mortality rate was similar in patients receiving monotherapy or combination therapy (21% vs. 23%).
Conclusions: Community-acquired pneumonia remain as the most common cause of SPBI, but an increasing proportion of the cases (10%) are hospital-acquired infections. Decreased susceptibility to penicillin and hospital-acquired infections are associated with a worse prognosis in most clinical forms of SPBI.

P462 *Streptococcus bovis* bacteraemia: clinical characteristics of 41 episodes

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Objectives: To assess the clinical and the therapeutic aspects and the outcome of *S. bovis* bacteraemia occurring in our 858-bed academic hospital during the last 11 years.
Methods: We reviewed the clinical files of all pts with *S. bovis* bacteraemia (^{3}1 blood culture) from January 1990 to December 2001.
Results: *S. bovis* bacteraemia (mean positive blood cultures 2.7 per pt) was detected in 41 pts (26 male, 15 female, mean age 64.5 years, range 27–90). All except four episodes were community-acquired infections. Thirty-one of the 41 patients (75%) had at least one underlying disease: neoplasia (12), cirrhosis (12), diabetes (seven), haemodialysis (two) and corticosteroids (eight). Eleven had valvular disease (nine with prosthesis). A primary focus of infection was found in only eight pts (19%) (six angiocholitis and two gynaecological infections). Transthoracic and/or transoesophageal echography, performed in 30 of the 36 patients who survived more than 7 days, confirmed endocarditis (BE) in 22 (73%). All nine pts with valvular prosthesis had BE (>12 months after surgery). Metastatic foci of infection were found in 10 of the 22 pts with BE (spondylodiscitis (4), cerebral (4), splenic (3) and renal (3) emboli). Valvular replacement was performed in two pts. In 11 pts (27%), no primary focus of infection or BE was found (primary bacteraemia). Colonoscopy (performed in 25 of the 36 pts who survived more than 7 days) showed one or more lesions in 23 of them (92%): polyp (16), carcinoma (3), diverticulitis (5) and ulcero-haemorrhagic colitis (1). Thirteen pts died during hospital stay (31%), nine because of *S. bovis* infection. Pts with BE more frequently suffered from neoplasia or cardiac diseases, digestive symptoms, secondary focus of infection and lesions of the colon and presented with longer duration of symptoms before diagnosis ($P < 0.05$) as compared with patients without BE.
Conclusion: Bacteraemia caused by *S. bovis* was mainly associated with endocarditis or primary bacteraemia. Colonoscopy should be performed in all pts considering the high rate of colic lesions. Morbidity and mortality associated with *S. bovis* was high in this group of elderly pts with underlying diseases.

P463 Infective endocarditis caused by *Leptospira grippotyphosa*

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Case: A 42-year-old man with no significant previous medical history was hospitalised for fever lasting 9 days, chills, headache, arthralgia, vomiting, diarrhoea, and dehydration. On admission, a marked cardiac murmur and hepatomegaly were the only pathological findings. Few days later the patient developed conjunctival

petechiae and splinter haemorrhages. Three blood cultures (Bactec aerobic and anaerobic) collected before antibiotic treatment remained sterile. Transoesophageal echocardiography showed fibromatous thickening of both cusps of the mitral valve, mitral prolapse and regurgitation; two fluttering 10-mm vegetations grew from the cusps. The patient also developed severe renal failure, aseptic meningitis and hepatic abnormalities. Serum agglutinins against *L. grippotyphosa* rose from 1:200 to 1:3200 in the sixth week of the disease. The source of the infection could be an inadequately protected water well at the patient's rural cottage. The patient was successfully treated with intravenous amoxicillin/clavulanate and ampicillin. No relapse has occured in following 2 years.

Discussion: The case nearly meets the Duke criteria of 'definite endocarditis'; we feel endocarditis was present. We believe the simultaneous occurrence of two infrequent diseases (leptospirosis and culture-negative endocarditis) is unprobable.

Conclusion: We suggest the patient underwent leptospiral endocarditis. To our best knowledge this is the second report on leptospiral endocarditis in the world literature.

P464 Optimal dosage of azithromycin for the treatment of mild scrub typhus

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Objectives: Scrub typhus is one of the most important endemic infections in Asia-Pacific region and the isolation of doxycycline-resistant Orientia tsutsugamushi prompted a search for effective regimens alternative for doxycycline therapy. Although azithromycin proved to be effective against doxycycline-resistant and sensitive strains, its optimal dosage is not confirmed yet. We conducted a prospective, nonblinded, randomised trial to search optimal dosage of azithromycin for the treatment of mild scrub typhus.

Methods: Febrile patients with skin rash or eschar satisfying eligibility criteria were randomly assigned to a single 500-mg dose of azithromycin group, a single 1000-mg dose of azithromycin group, or 1 week of daily oral 200-mg doxycycline group. Treatment outcome was assessed by time to defervescence (the time interval for body temperature to fall below 37.3°C after the first dose of antimicrobial agents is administered).

Results: From September to November 2003, 84 patients were assessed for eligibility. Seventy-three patients were randomly allocated to each of three groups and 66 patients were completed per protocol. Fifty-five cases (500-mg azithromycin group, 21; 1000-mg azithromycin group, 17; doxycycline group, 17) were serologically confirmed and analysed to compare the efficacy. The mean time to defervescence was shortest for single 1000-mg dose of azithromycin group (31.6 ± 23.3 h), shorter for single 500-mg dose of azithromycin group (39.5 ± 26.2 h) and longest for doxycyline group (42.9 ± 34.4 h), but statistically not significant ($P > 0.05$). There were no treatment failure or relapses after completion of therapy.

Conclusion: A single 500-mg dose of azithromycin is as effective as a single 1000-mg dose of azithromycin or conventional doxycycline therapy for the treatment of mild scrub typhus in South Korea.

P465 Clinical characteristics of infective endocarditis: descriptive review of 262 episodes and risk factors for death

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Objectives: To determine the clinical characteristics, site of involvement, microbiological findings, and outcome of infective endocarditis (IE), and to identify factors associated with an increased risk of mortality.

Methods: All cases of IE observed between January, 1980, and December, 2001, were reviewed, and cases which satisfied the Duke criteria for definite diagnosis were evaluated. Data were collected with regard to demographic, clinical and laboratory characteristics, blood culture results, HIV serostatus, and outcome. HIV test was not available for patients admitted before 1985.

Results: There were 262 episodes in 246 patients (165 males). The age of patients was 37.9 ± 14.6 years (range 16–83). 145 (58.9%) patients were intravenous drug users (IDUs), 51 had a history of valvular heart disease, and 22 had a prosthetic heart valve. 80 patients were HIV-infected, and 76 of them were IDUs. The most common organism was *S. aureus*, isolated in 43.1% of cases, followed by streptococci in 23.3%. In 14.1% of cases blood cultures were negative.The left side of the heart alone was involved in 52.7% of cases, the right side of the heart alone in 38.9%, and both sides in 4.2%. Tricuspid valve involvement was present in 37.4% of cases, compared with mitral and aortic valve involvement in 27.5% and 20.6%, respectively. More than one valve was involved in 8.8% of cases. Staphylococci more frequently affected IDUs (77.6%), patients younger than 30 years (44.6%), and the right side of the heart (53%). In contrast, streptococci more frequently affected patients with valvular heart diseases (39.3%), older than 50 (29.5%), and the left side of the heart (80.3%) ($P < 0.001$).Overall mortality rate was 16.2%. In multivariate analysis negative blood cultures, left sided and multivalvular IE were associated with an increased risk of death. The mortality rate among HIV-positive patients was 25.3% compared with 11.9% in HIV-negative patients with an attributable mortality of 13.4%.

Conclusions: Clinical characteristics of IE in our institution probably reflect the predominance of IDUs. The causative organisms were significantly associated with the predisposing condition, age of patients, and site of heart involvement. Negative blood cultures, and left side or multivalvular heart involvement were strong risk factors for mortality. Work funded by Ricerca Corrente IRCCS.

P466 Risk and prognosis of community-acquired bacteraemia caused by *E. coli* and other members of Enterobacteriaceae in patients with diabetes mellitus

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Objective: Diabetic patients are considered at high risk for severe infections originating foremost from the urinary tract. We conducted a population-based study to examine whether patients with diabetes have an increased risk or a poorer prognosis for community-acquired Enterobacteriaceae bacteraemia when compared with non-diabetic patients.

Methods: We included all patients above 15 years of age in the North Jutland County Bacteraemia Registry, Denmark, with a first hospitalisation for community-acquired monomicrobial bacteraemia because of a member of the family Enterobacteriaceae from 1992 through 2001. Ten gender- and age-matched population controls per case were selected, using a unique personal identifier. Diabetes was determined by record-linkage with the County Prescription Database (for prescriptions for antidiabetic drugs) and the Hospital Discharge Registry. We did conditional logistic regression analyses to estimate the risk for bacteraemia among diabetic and non-diabetic persons, with adjustment for a range of comorbid diseases using Charlson's comorbidity index. Additionally, we compared case fatality rates for diabetic and non-diabetic patients with bacteraemia, using Cox proportional-hazards regression analyses to adjust for possible confounding factors influencing case fatality.

Results: A total of 1317 incident cases with a first hospitalisation for Enterobacteriaceae bacteraemia were identified. 225 cases (17.1%) had diabetes recorded before the date of hospitalisation with bacteraemia, compared with 779 (5.9%) of controls. The adjusted OR for bacteraemia in persons with diabetes was 2.9 (95% CI: 2.4–3.4). The highest relative risk was noted in adults under 65 years (adjusted OR = 5.9, 95% CI: 3.9–9.0) and in

persons without any other recorded comorbidity (adjusted OR = 4.9, 95% CI: 3.7–6.6).The case-fatality in diabetic patients compared with non-diabetic patients was 17.3% vs. 13.4% after 30 days, and 23.6% vs. 19.5% after 90 days. After adjustment for gender, age, comorbidity, and focus of infection, the 30-day mortality rate ratio for diabetic patients was 1.4 (95% CI: 1.0–2.0) compared with the non-diabetic patients.
Conclusions: Diabetes seems to be a strong risk factor for community-acquired bacteraemia caused by *E. coli* and other Enterobacteriaceae. Furthermore, patients with diabetes appear to have a higher case-fatality in this severe infection than non-diabetic patients.

P467 Predictors of bacteraemia in hospitalised patients with infectious cellulitis

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Objectives: Among patients with cellulitis, the development of bacteraemia is associated with a high risk of adverse outcomes. We sought to identify predictors of bacteraemia at the time of presentation to the hospital emergency department in patients with cellulitis.
Methods: Review of medical charts of all adult patients hospitalised for community-acquired cellulitis (Jan 95–Dec 02) who had blood cultures performed on admission. Cellulitis complicating diabetic foot ulcers, orbital cellulitis, HIV-infected patients, and drug addicts were excluded. For the purpose of the study, patients with bacteraemia were compared with patients without bacteraemia.
Results: There were 329 cases of cellulitis (184 female); mean age of 61.5 years. The infection was microbiologically documented in 115 cases (53 bacteraemia, 65 needle aspiration, and 20 surgical sample). The organisms isolated most frequently were *Staphylococcus aureus* 34, *Streptococcus pyogenes* 25, groups B, C, and G streptococci 19, *Pseudomonas aeruginosa* 14, and five *Escherichia coli*. Overall, 53 (16%) patients had bacteraemia and 276 patients had negative blood cultures. The development of in-hospital clinical complications (32% vs. 12%; $P < 0.001$) and overall mortality (<30 days) (19% vs. 2%; $P < 0.001$) were more frequent in patients with bacteraemia than in patients without bacteraemia. Factors significantly associated with bacteraemia by univariate analysis were: male sex (55% vs. 42%), age >60 years (77% vs. 56%), presence of multiple comorbidities (25% vs. 14%), duration of symptoms <2 days (54% vs. 33%), hypoalbuminaemia (<30 g/L) (46% vs. 28%), renal insufficiency (creatinine > 150 mmol/L) (25% vs. 6%), and shock (15% vs. 2%). Multivariate analysis identified age >60 years (OR 1.88), duration of symptoms <2 days (OR 2.19), and shock (7.58) as independent predictors of bacteraemia.
Conclusion: The predictors of bacteraemia delineated in this study may help clinicians to identify a subset of patients with cellulitis at a high risk of complications and mortality.

P468 Cytokine profile and nitric oxide levels in sera of patients with acute brucellosis

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Objectives: This case control study, aims to investigate serum cytokines profile (TNF-alpha, IL-1 beta, IL-2R, IL-6, and IL-8) and nitric oxide (NO) in untreated human brucellosis and assay correlation of such parameters with each other.
Methods: The study covered 67 subjects. It comprised a total of 37 patients with brucellosis. Control subjects were 30 healthy individuals with no history of Brucella infection. Brucellosis was identified according to the positive blood culture and raised Brucella antibodies in serological tests in addition to compatible clinical symptoms. Cytokine profile analysis was performed according to the Immulite chemiluminescent enzyme immunometric assay. Nitrites/nitrates are representatives for NO. Their serum level was measured by Griess method.
Results: Patients with brucellosis had significantly elevated serum levels of nitrites/nitrates, IL-2R, IL-6 and IL-8 compared with healthy controls whereas levels of TNF-alpha and IL-1 beta were not changed. We could not find statistically significant correlation between any of the studied cytokine levels and nitrate/nitrite concentration except for that between IL-2R and direct nitrite ($r = 0.471$). There was also positive correlation between direct nitrite and total nitrite ($r = 0.671$).
Conclusion: Certain cytokines are only elevated in acute brucellosis and such extent of elevation depends on severity of illness. Moderate elevation in serum NO was observed that is comparable with previous experimental studies. This explains absence or very rare occurrence of septic shock in brucellosis but it may prolong intracellular survival of Brucella and subsequent development of chronic infection. Nonetheless some cytokines and NO altogether may take part in disease process of Brucella infection and affect its outcome.

P469 The various clinical manifestations of brucellosis: a review of 43 cases

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Objective: Brucellosis is a zoonotic disease that involves many systems such as musculoskeletal, gastrointestinal, genitourinary, cardiovascular, central and peripheral nervous systems. In this study we aimed to investigate the clinical courses, laboratory features and prognosis of cases with brucellosis.
Results: We evaluated 43 patients (25 M, 18 F, of mean age 41.4 years). Acute, subacute and chronic forms of infection's rates were 53.5, 30.2 and 16.3% respectively. The main symptoms were malaise (83.7%), fever (62.8%), sweating (53.5%), arthralgia (48.8%), anorexia (46.5%), myalgia (27.9%), headache (25.6%), weight loss (23.3%) and cough (14%). The most frequent signs were hepatomegaly (27.9%), splenomegaly (16.3%), lymphadenomegaly (11.6%), spondylitis (27.9%) and peripheral arthritis (7%). Laboratory findings were as follows: Anaemia (39.5%), leucopenia (7%), lymphocytosis (16.3%), pancitopenia (4.7%), increase of ESR (34.9%), CRP (30.2%), and liver enzymes (25.6%). Standart tube agglutination test was positive in 90.7% of the patients. Total culture positivity was 39.5% (32.55% of blood, 4.7% of CSF, 2.3% of abscess specimens). Focal diseases were observed in 48.9% of the patients (spondylitis 27.9%, neurobrucellosis 11.6%, paravertebral abscess 7%, endocarditis 2.3%). Based on the clinical response different treatment regimens were given and uncomplicated patients were treated for 6–12 weeks with rifampin–doxycycline or streptomycin–doxycycline.Complicated cases such as neurobrucellosis, endocarditis and paravertebral abscess were treated with appropriate combinations for 6–16 months. During follow up, one patient had pulmonary complication because of brucellosis; four patients underwent surgery because of paravertebral abscess (three cases) and endocarditis (one case). Treatment failure was seen in two patients and the therapy was changed. There was no relapsing case in our follow-up period.
Conclusion: Brucellosis is an infectious disease that can cause various clinical manifestations and complications. In all the cases with fever, arthralgia, and malaise brucellosis should be considered especially in endemic areas.

P470 Brucellar spondylitis (clinical manifestations and outcome of treatment in 32 cases)

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Objectives: Brucellar spondylitis is common in endemic regions of brucellosis.The purpose of this study was to assess the clinical manifestations and outcome of treatment in brucellar spondylitis.
Methods: From September 1998 to June 2003, 32 cases of brucellar spondylitis who attended the Department of Infectious Diseases,

Babol Medical University were studied. All cases were treated with cotrimoxasol plus rifampin or doxycycline plus rifampin for 4 months and all cases were followed for 1 year after completion of therapy. Clinical manifestations and outcome of treatment were recorded.
Results: Thirty-two cases (24 male, eight female, mean age, 46 ± 17 years, ranged 18–77 years) were evaluated. Disease was acute and subacute in 28 (87.5%) cases. Severe back pain, sweating and fever were the most clinical symptoms and were seen in 100, 62.5 and 47% cases respectively. Involvement of lumbar, dorsal and cervical regions were seen in 26, 2 and 4 cases, respectively. Seventeen and 15 cases were treated with cotrimoxasol plus rifampin or doxycycline plus rifampin for 4 months, respectively. Only one case in doxycycline plus rifampin treated group had relapse ($P > 0.05$).
Conclusion: Severe back pain, sweating and fever were the most clinical symptoms. Four months of therapy is sufficient in the treatment of brucellar spondylitis.

P471 Successful treatment of *Brucella melitensis* endocarditis with antibiotic combination

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In July 2003 a 22-year-old woman presented at the hospital with fever, shivering and weight loss. The agglutination test for *Brucella* was positive at a titer of 1/320. A transoesophageal examination showed vegetations and *Brucella* endocarditis was suspected. A blood culture growth of *Brucella melitensis* biotype 1 confirmed our dignosis. The treatment was started with 600 mg/day rifampin, 2×100 mg/day doxycycline and TMP-SMX 160/800 mg twice daily. Two months later TMP-SMX therapy ceased, rifampin and doxycycline therapy continued a further 6 months. At the clinical follow up, there were no signs of heart failure or peripheric embolism. On the seventh day of therapy the fever decreased. After treatment a repeat transoesophageal echocardiography showed that the vegetations at the aorta valve disappeared. At 1-year follow up, the patient was healthy. If there is no history of congestive cardiac failure and prosthetic valve involved and only moderate extravascular cardiac involvement medical treatment may be a valid alternative to surgical therapy as advised in some literature where the patient's ilness is not prolonged.

P472 Clinical features and prognosis of B melitensis vertebral osteomyelitis. A descriptive study of 148 cases

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Objectives: To describe current clinical, radiological, diagnostic and therapeutic characteristics and prognosis of Brucelar Vertebral Osteomyelitis (BVO).
Methods: We carried out a prospective, descriptive study of 446 patients with Vertebral Osteomyelitis (VO) from January 1983 to October 2003 in two tertiary hospitals. Diagnosis of VO was established by the presence of spinal pain unrelieved by rest or fever and spinal pain on physical examination, together with imaging compatible with VO. Diagnosis of BVO was established according to one of the following criteria: (1) *Brucella* spp isolation in vertebral, paravertebral, or epidural tissue or a psoas sample. (2) *Brucella* spp isolation in blood cultures, high titers of *Brucella* antibodies or seroconversion in the presence of a compatible clinical and radiological picture of VO. All patients were treated with doxycycline 100 mg b.i.d. for 3 months plus streptomycin 1 g i.m. q.d. for 3 weeks or doxycycline 100 mg plus rifampin 900 mg q.d. both for 3 months.
Results: One hundred and forty-eight patients of the total sample (32.2%) had BVO. 109 (73.6%) were male and 39 (26.4%) female. The mean age was 51.4 + 13.6 years. Cervical level was involved in 14 cases (9.5%), dorsal or dorso-lumbar in 32 (21.6%), lumbar or lumbo-sacral in 98 (66.2%) and multiple levels were affected in four cases (2.76%). The mean duration of symptoms prior to diagnosis was 3.2 months. Eighty per cent of patients had fever, 91.9% inflammatory spinal pain and 41.3% had some neurological deficit. Blood cultures were positive in 42% of cases and B melitensis was isolated in all of them. The mean number of affected vertebral bodies was 2.1 (range 1–5). Paravertebral masses were detected in 62 cases (42.9%), epidural masses in 44 (29.7%) and psoas abscesses in 14 (9.5%). Fifty patients (33.8%) required surgical treatment. The rate of therapeutic failure, relapse and attributable mortality were 14.9, 2 and 1.2% respectively. The mean hospital stay was 36.6 days and 29 patients (19.6%) had severe functional sequelae. A high diagnostic delay, cervico-dorsal level and more than two vertebral bodies affected were associated with a worst prognosis.
Conclusions: BVO represent a high percentage of VO in endemic areas. In this type of VO, non-invasive diagnostic yield is very high. Although BVO mortality is very low, BVO frequently requires surgical treatment, long hospital stay and results in severe functional sequelae.

P473 Psychotic disorder related with *Brucella* infection: three case reports

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Objective: Three patients who were brought to hospital because of psychotic symptoms and diagnosed as brucella infection and treated with antibiotics were presented.
Cases: Two of the cases were hospitalised in psychiatry clinic and one of them was hospitalised in infectious disease clinic. The average age of three cases is 33. All of the patients had a history of cheese eating. One case had a history of treatment and as acute psychotic attack 4 years ago. All of them had shared symptoms of fever, headache, sweating, weight loss, back pain, vertigo, irritability and restlessness. In psychiatric examination patients were evaluated as irritable, sleep disturbance, impaired orientation, attention and memory, difficulty in cooperation, acceleration and deceleration in thought flow and auditory and visual hallucinations. In one case there were symptoms of neuralgic disorders like nystagmus, ataxia and strabismus. All cases have positive *Brucella* agglutination test in 1/640 titer. Two cases had positive BOS culture for *Brucella* and all of them positive blood culture for *Brucella*. After the antibiotic treatment patients' clinic and laboratory states came to normal. Psychiatric symptoms disappeared.
Conclusion: Acute psychotic presentation and increasing psychotic symptoms can be seen in neurobrucellosis. In all of the cases who have psychotic symptoms and also fever and infection; neurobrucellosis should be considered in differential diagnosis.

P474 Pulmonary involvement in brucellosis

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Objectives: Brucellosis is a multisystem infection that may present with a broad spectrum of clinical presentations. Pulmonary involvement is extremely rare in the course of brucellosis with an estimated rate of <1–5% of cases. A variety of pulmonary manifestations have been documented in the literature, including bronchitis, bronchopneumonia, lung abcess, empyema, pleural effusion, granulomas, solitary nodules, hilar and paratracheal lymphadenopaty. The aim of this study was to determine the incidence and forms of pulmonary involvement in the course of brucellosis.
Methods: A prospective study was carried out in 110 patients with brucellosis who were admitted to the Infectious Diseases and

Clinical Microbiology Department, Ankara Training and Research Hospital, between October 2001–December 2003. All the patients were questionnaired about their pulmonary symptoms including cough, expectoration, chest pain, dyspnoea and haemoptysis. All the patients underwent a thorough physical examination, chest radiography and when pulmonary pathological findings were present, underwent additional diagnostic evaluations including computerised tomography of the thorax and pulmonary function tests.

Results: Of these 110 patients, 11 (10%) patients (six female, five male) presented with pulmonary involvement. Eight (72%) patients had pulmonary symptoms including dyspnoea, dry cough and productive cough with expectoration. Six patients had chest radiography findings but two had not. Three patients had no pulmonary symptoms but had findings in chest radiography. Chest radiography findings were compatible with computerised tomography findings of the thorax. Radiological findings were as follows: Parenchymal nodules in eight patients, lobar pneumonia in one, paratracheal lymphadenopathy in one, parenchymal nodules, lobar pneumonia and minimal pleural effusion in one patient. Of 11 patients with pulmonary involvement, four (36%) patients had coexisting chronic obstructive pulmonary disease. All the patients were treated with a combination of rifampin and doxycycline or streptomycin and doxycycline. Clinical and radiological findings of pulmonary involvement were recovered in all patients except four patients who had coexisting chronic obstructive pulmonary disease.

Conclusion: Pulmonary involvement is a rare event in the course of brucellosis but the rate could be higher than estimated. In endemic regions, brucellosis should be considered as a causative agent in patients with pulmonary symptoms.

P475 Same involvement of brucellosis in two brothers: epididymoorchitis

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Objectives: Brucellosis is a very polymorphic disease and could affect any organ. Epididymoorchitis occurs in up to 2–20% of patients with brucellosis. In this report, two brothers with epididymoorchitis were presented.

Case 1: A 43-year-old male sheep breeder was admitted with a-6-week history of fever, sweating, headache, pain in his shoulders and neck. Six weeks ago he had applied to an urologist with a history of painful swelling and redness of the left testis and fever. In ultrasonographic examination increasing of testicular diameter, thickening of scrotal wall and testicular hypoechogeneity had been detected. After 10 days of antibiotic therapy, his testicular symptoms had improved. On physical examination restricted mobility of the neck and hepatomegaly were evident but testis was normal. The complete blood cell count and biochemical findings were normal. Standard tube agglutination testing (SAT) was positive at titer of 1/640. *Brucella melitensis* was isolated in both of blood and bone marrow culture. Magnetic resonance imagining revealed cervical spondylitis. Cerebrospinal fluid examination revealed findings of meningitis and SAT was positive at titer of 1/16. Osteoarticular and neurological involvements of brucellosis were diagnosed and rifampicin plus doxycycline (24 weeks) plus seftriaxon (4 weeks) treatment was administered. The clinical and laboratory findings improved with this treatment.

Case 2: A 45-year-old male sheep breeder was admitted with a 3-month history of pain in his right shoulder and wrist. Before these symptoms, painful swelling and redness of the right testis and fever had developed and he had applied to an urologist. His symptoms had improved with antibiotic therapy. On physical examination there was only hepatomegaly. WBC was 10.500/mm^3, other blood cell count and biochemical findings were normal. Serum SAT was positive at titer of 1/640. Blood and bone marrow culture were negative. Rifampicin plus doxycycline (6 weeks) treatment was administered. His clinical findings improved with this treatment.

Conclusions: In both brothers, presenting symptoms of the brucellosis were epididymoorchitis. So that brucellosis must be considered in the differential diagnosis in endemic areas. Although orchitis is a rare complication of brucellosis, it was observed as same involvement in both brothers. These cases suggest the possibility of genetic basis for the occurrence of brucellosis and/or epididymoorchitis.

P476 Human brucellosis: a retrospective evaluation of 75 cases

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Objectives: Brucellosis is an important zoonosis worldwide, mainly in the Mediterranean countries, including Turkey. The annual national incidence of the disease in Turkey is 0.59 per 100 000 persons. The aim of this study was to evaluate the epidemiological, clinical and laboratory findings, therapeutic features and outcomes of patients with brucellosis retrospectively.

Methods: This study was carried out at Cumhuriyet University Hospital, Department of Clinical Bacteriology and Infectious Diseases, Sivas, Turkey, between January 1998 and November 2003. Seventy-five brucellosis patients were included in the study.

Results: Thirty-nine (52%) were female patients and 36 (48%) were male. The mean age was 41 $\pm$ 1.8 (range 16–70 years). Clinical form of the disease was acute in 51 cases, 18 sub-acute, five chronic, and one asymptomatic. The most possible source of brucellosis was the consuming of unpasteurised dairy products, especially raw milk and fresh cheese (68%). Malaise (53%), fever (53%), back pain (45%) and anorexia (43%) were the most common presenting symptoms, and fever and hepatomegaly were the most common initial clinical findings among the patients. Elevated serum C-reactive protein levels was determined in 27 of 33 patients and elevated erythrocyte sedimentation rate in 56 of 75 patients. Cultures (blood 36, cerebrospinal fluid 1, joint fluid 1) were positive in 38 (51%) patients and all strains identified as *Brucella melitensis*. Various treatment regimens were used, mainly doxycycline plus rifampicin. There was no therapeutic failure. Relapse occurred in five of the 75 patients (6.7%).

Conclusion: Brucellosis is still endemic zoonosis in the Central Anatolian region of Turkey. So, we think that the effective heating of dairy products and other potentially contaminated foods is main prevention method of the disease and others.

Diagnositic methods - I

P477 Comparison of proficiency testing among governmental and private sector hospital microbiology laboratories in Tehran

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Objective: The aim of this study was to evaluate proficiency testing of microbiology laboratories in governmental and private sector hospitals.
Methods: Two species of bacteria were sent to microbiology laboratories (71 governmental and 35 private sector) included *Staphylococcus epidermidis* (ATCC 12228) and *Bukhoderia cepacia* (ATCC 2541). We asked all laboratories for identification of each bacteria and susceptibility testing of *S. epidermidis*. Scoring of results performed according of WHO criteria (maximum score of point for identification 3 and for susceptibility testing 5).
Results: Of 111 microbiology laboratories, we received answer from 87 (75%) laboratories. The mean score of points for identification of *S. epidermidis* in governmental hospital microbiology laboratories was 2.8% (SD $\pm$ 0.64). The main score of point in private hospital microbiology laboratories was also 2.8 (SD $\pm$ 0.7376). Statistically there was not a significant difference between two groups of laboratories for identification of *S. epidermidis* (P-value = 0.712). Of 55 governmental hospital microbiology laboratories, 22 laboratories identified *B. cepacia* and obtained 3 score of points. Eleven laboratories obtained zero score of points and other laboratories obtained 0.05–2.5 score of points. The mean score was 1.67 (SD $\pm$ 1.2). Of 30 private sector hospital microbiology laboratories five laboratories produced correct answer for identification of *B. cepacia* and obtained 3 score of points, 18 laboratories zero score of points and other laboratories obtained 0.5–2.5 score of points. The mean score was 0.81 (SD $\pm$ 1.119; P-value = 0.001). The mean score of points for susceptibility testing of *S. epidermidis* in governmental-related hospitals microbiology laboratories was 4.8 (SD $\pm$ 0.38) and in private sector was 4.66 (SD $\pm$ 074; P-value = 0.571).
Conclusion: It is concluded that private sector hospital microbiology laboratories in our county in comparison with governmental hospital microbiology laboratories have poor proficiency for identification of microorganisms such as *B. cepacia*. It may be due to lack of some culture media reagents and unskilled personnel in this sector.

P478 Implementation of particle analysers in the detection and description of biofilm formation

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The growth in a biofilm form enables bacteria to survive in the environment and in human organism. Biofilm bacteria are highly resistant to host immunity and to antibiotic therapy. The ability of bacteria to form biofilm is considered to be an important factor of the pathogenicity and represents a serious medical problem. The proof of the ability and the evaluation of the dynamics of biofilm formation can be helpful for optimal therapy of the infection. The phenotypical methods of the biofilm detection are related with subjective evaluation of the obtained results. Therefore, we tried to find a new method for the detection and description of biofilm formation. The *Staphylococcus epidermidis* strains isolated from blood cultures of patients with bacteraemia were used in this study. Isolates were grouped on the basis of the presence of intact ica-operon, determined by PCR reaction, into two groups, ica-operon positive and ica-operon negative. The phenotypical slime-positivity or negativity was performed by two methods by Christensen tube method in brain heart infusion and by the typical growth on agar with Congo red. Bacteria with uniform (monodisperse) microparticles were cultivated under constant stirring for different time periods. The growth of bacterial biofilm layer on the microparticles was monitored as an increase of microparticle size. Various microparticles of diameters ranging from 10 to 50 μm were used. The size of biofilm-covered microparticles was measured using different independent measuring principles. Time-of-transition (TOT), low angle laser light scattering (LALLS), gravitational field flow fractionation (GFFF) and dynamic shape analysis. The total biofilm increase given by the number of particles can reach several tens of cm^2. Therefore, the changes in size distribution resulting from the biofilm growth are statistically significant. This approach also allows determination of the influence of various cultivation parameters (e.g. pH and chemical composition of the medium, properties of the particles, physical conditions and bacteria species) on the growth and detachment of biofilms. The measurement of microparticle increase as a result of biofilm formation has been shown to be an effective tool for investigation of biofilms.

P479 Laboratory variables in selected neuroinflammatory diseases

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Objectives: The intrathecal synthesis of specific IgG antibodies against the viruses of measles, rubella and varicella zoster, called MRZ reaction (M-measles, R-rubella, Z-varicella zoster) is typical for chronic inflammatory autoimmune diseases of the nervous system. MRZ reaction is present in 89% patients with multiple sclerosis (MS), while in patients with neuroborreliosis (NB) it occurs very seldom (<0.1%). The aim of our study was to detect MRZ reaction and intrathecal synthesis of specific antiborrelia IgG antibodies and to evaluate the relevance of specific antibody indices from first diagnostic lumbar puncture for the differential diagnosis between MS and NB.
Methods: We investigated a cohort of 50 patients: 21 patients were diagnosed as multiple sclerosis, 19 patients were diagnosed as neuroborreliosis and 10 patients with OND (other neurologic diseases) served as negative controls. Serum and CSF samples were analysed at each patient. The diagnostic kit of Human Company, Germany (measles, rubella, varicella-zoster virus human ELISA IgG antibody test) and the diagnostic kit of Test-Line Company, Clinical Diagnostics, Czech Republic (EIA *Borrelia garinii* IgG) were used for the detection of specific IgG antibodies. The intrathecal synthesis was evaluated as specific antibody index (AI according to Reiber's method). Values of AI > 1.4 indicated the intrathecal synthesis.
Results: Intrathecal synthesis of IgG antibodies against measles, rubella and/or varicella zoster viruses (AI > 1.4) was detected in 95% patients with MS. Antibody index against measles was positive in 85.7% patients, against rubella in 52.4% patients and against varicella zoster virus in 38% patients. All these patients had negative intrathecal antibody synthesis against *Borrelia burgdorferi* (Bb), i.e. negative Bb-IgG-AI. In patients with NB 89.5% had positive Bb-IgG-AI. One patient (5%) only had positive anti-measles AI. OND patients had negative MRZ reaction and Bb-IgG-AI. The statistical significance of MRZ reaction vs. Bb-IgG-AI positivity was confirmed by Spearman rank coefficient and Wilcoxon's test.
Conclusion: Positive MRZ reaction is the most valuable diagnostic parameter in diagnostically equivocal cases of MS and NB with neurological first symptoms. Our data emphasise the importance of MRZ reaction and Bb-IgG-AI in differential diagnostics of MS

and NB in early stages when other methods (MRI, oligoclonal IgG bands) do not provide definite diagnosis.

P480 Bactericidal effect of endox against various pathogens

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Objectives: Endox is an instrument used for the sterilisation of the root canal and/or periapical infections after the endodontic treatment. The instrument applies an high frequency alternate current (HFAC) and, for each impulse, generates an electromagnetic field for a short period time (140 ms) in the site of infection. To better understand the mechanism by which sterilisation occurs, the instrument was used to test the bactericidal effect on different bacterial suspensions exposed to the above electromagnetic field.
Methods: Bacterial strains included *Enterococcus faecalis, Staphylococcus aureus*, *Actinomyces* spp., *Escherichia coli, Pseudomonas aeruginosa* and among fungi it was tested with *Candida albicans.* A sample of 20 μL of a saline bacterial suspension was exposed to the electromagnetic field for three times. Survivors were then counted by standard procedure.
Results: Bactericidal effect of 99.99% was found in *E. faecalis, S. aureus, E. coli* and *P. aeruginosa*, instead in *Actinomyces* spp. and *C. albicans* the reduction of bacterial concentration was lower (99.9%).
Conclusions: Sterilisation effect obtained with Endox was very remarkable (99.99%) on bacteria and, even in less extent, on fungi (99.9%). These different results may be explained by the different constitution of cell wall. The Actinomyces, in comparison with other bacteria has shown a less marked reduction of bacterial concentration, this fact can be attributed to its characteristic to produce 'sulphur grains', crystalline and proteinic formations that defend the membrane cell from the electromagnetic field. The extension of the area and the mode of action of the electromagnetic field are under investigation.

P481 Evaluation of *Helicobacter pylori* stool antigen test for the detection of *H. pylori* infection and comparison with other methods

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Objective: *Helicobacter pylori* is the main cause of gastritis, peptic ulcer and gastric cancer in adults. The measurement of *H. pylori* antigens in human stools has been proposed as a valuable, noninvasive diagnostic tool. The aim of this study was to evaluate the usefulness of *H. pylori* stool antigen (HpSA) in diagnosis of *H. pylori* infection in adult patient.
Methods: Seventy-eight patients aged 21–68 years who were admitted to Department of Internal Medicine, Division of Gastroenterology with symptom of dyspepsia for whom the indication of upper gastrointestinal endoscopy was present were included in this study. Stool samples were taken before antibiotic treatment. The patients were evaluated with histology, rapid urease test and HpSA (Linear Chemicals). Urea breath test was carried out only in 38 patients. Patients were defined as *H. pylori* positive, if histology, urease test or urea breath test confirmed.
Results: Fifty-nine patients were positive for *H. pylori*, whereas 19 patients were negative. HpSA was positive in 53 of 59. The sensitivity and specificity of methods were as follows: histology 100 and 94%, urease 61 and 83%, urea breath test 100 and 100%. The sensitivity of HpSA was 89% and specificity of HpSA was 95%.
Conclusion: Faecal tests are noninvasive, easy to perform for the diagnosis of *H. pylori* infection. This test is cheap and effective for the diagnosis of *H. pylori* infection in Turkish population.

P482 Comparison of the Now® ICT malaria P.f./P.v. and the OptiMAL® IT rapid diagnostic tests for malaria in a nonendemic area

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Objectives: Malaria patients require a rapid and accurate diagnosis. Microscopic examination of thin or thick blood smear remains widely used; however, such tests are time-consuming and require experience – especially in case of low parasitaemia often encountered in nonendemic areas. Since last decade, malaria rapid diagnosis tests based on the detection, in blood, of soluble parasitic antigens have been marketed. The aim of our study was to compare the performance of Now® ICT malaria P.f./P.v. (Binax, Portland, ME, USA) and OptiMAL® IT (Diamed, Cressier, Switzerland), two new versions of rapid diagnostic tests for malaria.
Methods: A total of 556 consecutive patients were prospectively enrolled in Lyon, France. The 'gold standard' was a combination of results obtained by thin blood smear film microscopic examination and QBC. PCR and anti-malarial drug assays were performed only on discrepant samples.
Results: One hundred and nine (19.6%) patients had proven malarial attacks. Sensitivity was 96.3% for Now® ICT malaria P.f./P.v. and 79.8% for OptiMAL® IT ($P = 0.0001$), and specificity was, respectively, 98.8 and 98.4%. Likelihood ratios for positive tests were, respectively, 86.1 and 50.9 for Now® ICT malaria P.f./P.v. and OptiMAL® IT. Of 80 *P. falciparum* cases, Now® ICT malaria P.f./P.v. missed two infections. The test detected all 13 *P. vivax* infections. Five false positive results were observed in patients with a recent history of fever, self-treated for malaria. OptiMAL® IT misdiagnosed 10 *P. falciparum* infections with parasitaemia up to 0.1%. Two *P. vivax* infections were not detected. We observed seven false positive cases, with no evidence of previous malaria attacks in six cases. During patient follow-up, Now® ICT malaria P.f./P.v. can persist positive for at least 7 days. OptiMAL® IT turned negative within an average of 3 days being more likely to reflect parasite vitality.
Conclusions: Rapid diagnostic tests for malaria could be helpful as an adjunct test, but could not replace microscopic examination of blood films, which remains the gold standard, including moreover quantitative techniques.

P483 Identification of yeasts and yeast-like microorganisms with a colorimetric card newly developed for the VITEK® 2 system

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Objectives: A new colorimetric card (YST; this new card is not yet available for commercial use) was developed for rapid automated identification of yeasts and yeast-like microorganisms using the VITEK® 2 system. A database created from testing well-characterised strains was used to optimise the computer-assisted algorithm to achieve a high level of performance accuracy.
Methods: The YST card, containing 46 tests that measure enzymatic, acidification and alkalinisation activities, was tested with 1304 isolates representing 13 genera comprised of 54 different species. Strains included 246 recent clinical isolates tested at the University of Texas at San Antonio Health Science Center with the remainder of isolates being stock cultures tested at bioMerieux. Cards were filled with organism suspensions made in 0.45% aqueous NaCl to a turbidity equivalent to a McFarland#2 standard. Inoculated cards were incubated in the VITEK® 2 for approximately 18 h and a computer-assisted algorithm was optimised and used to generate test and identification results.
Results: Of the 1304 isolates tested, 1249 (95.8%) gave a correct identification with 138 (10.6%) low discrimination results [requiring supplemental testing and/or microscopic observation to

discriminate between multiple (up to three) choices]. Twenty-six (2.0%) of the isolates gave an incorrect identification and 29 (2.2%) were unidentified. Results of the subset of 246 recent clinical isolates showed 97.2% correct (including 8.9% low discrimination), 2.0% incorrect and 0.8% unidentified. This slightly higher performance was due to a higher frequency of isolates of the more common species.
Conclusion: The new YST colorimetric card used with the VITEK® 2 system provides an accurate, rapid, and automated method for the identification of yeasts and yeast-like microorganisms.

P484 Compatibility of CPS ID 3, a new chromogenic medium, with the VITEK 2 system

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Objectives: CPS ID 3 is a new chromogenic medium for the diagnosis of urinary tract infections. This new formula improves colour intensity for each enzymatic activity and also increases the growth of Gram-positive bacteria and yeasts. Compatibility studies of CPS ID 3 with the bioMerieux VITEK 2 system were performed. Results were compared with those obtained with colonies isolated on CPS ID 2, the previous formula, and a nonchromogenic media, both being recommended as isolation medium before VITEK 2 testing.
Methods: A set of 180 microorganism strains representative of those commonly encountered in urine and various mechanisms of resistance were streaked on CPS ID 3, CPS ID 2 and a reference medium. Isolated colonies were used to inoculate the appropriate identification and susceptibility card selected among ID-GNB, ID-GPC, ID-YST, AST-N022, AST-N017, AST-P523 and AST-P524.
Results: For ID-GNB, ID-GPC and ID-YST cards the correct identification rates on CPS ID 3 were 96.7, 83.3 and 98.3%, respectively, and statistically there was no difference with those obtained on CPS ID 2. As regards susceptibility test results, the average MIC agreements on AST-N022, AST-N017, AST-P523 and AST-P524 were 99.3, 99.1, 99 and 98.6%, respectively. There was no trend to induce a higher susceptibility or resistance for specific species or drugs. Moreover, there was no statistically significant difference between the MIC agreements obtained on CPS ID 2 and CPS ID 3.
Conclusion: Identification and susceptibility testing on VITEK 2 of common urinary pathogens isolated on CPS ID 3 correlates well with those performed on CPS ID 2. CPS ID 3 is fully compatible with the VITEK 2 system. Consequently, with CPS ID 3, bacteriologists will save considerable amounts of time and reagents, while having a complete solution for the diagnosis of urinary infections, even with the most complex specimens.

P485 New improved VITEK 2 card for identification of Gram-negative bacilli

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Objectives: A new VITEK 2 Gram-negative card (GN; this new card is not yet available for commercial use) has been developed recently to increase identification accuracy of key clinical isolates and expand the number of nonfermentative bacteria claimed by the current ID-GNB card on the VITEK 2 system. The GN card contains 47 biochemical tests enabling the identification of 88 species or groups of fermentative bacteria and 47 species or groups of nonfermentative bacteria.
Methods: A total of 2806 well-characterised isolates from the bioMerieux stock collection were used to build the database. Cards were filled with organism suspensions made in 0.45% aqueous NaCl to a turbidity equivalent to a McFarland #0.5 standard. Inoculated cards were incubated in the VITEK 2 instrument and a computer-assisted algorithm was optimised and used to generate kinetic identification results.
Results: Of the 2806 isolates tested, 2737 (97.6%) gave a correct identification with 123 (4.4%) low discrimination results (requiring simple additional testing). Fifty-seven (2.0%) of the isolates gave an incorrect identification and 12 (0.4%) were unidentified. All the results were obtained within 2–10 h. Overall correct identification is 98.4% for Enterobacteriaceae and other fermentative bacteria. For nonfermentative bacteria, 95.7% of the isolates gave correct identification.
Conclusions: The new GN card used with the VITEK 2 system provides an accurate and rapid method for the identification of a wide range of Gram-negative bacteria. This updated card provides several improvements compared with the current ID-GNB card that include:
- high level (93.2% vs. 77.5%) of one choice identification without any additional testing required and 97.6% vs. 96.9% overall correct when additional testing is included;
- more species claimed for nonfermentative bacteria 47 (vs. 24);
- elimination of mixed taxon 'various nonfermenting Gram-negative bacilli' comprising 26 taxa on the current card.

P486 Identification of Gram-positive bacteria with a new card developed for the VITEK 2 system

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Objectives: A new card (GP; this new card is not yet available for commercial use) was developed for rapid automated identification of Gram-positive bacteria using the VITEK 2 system. An expanded database was generated by testing well-characterised strains from clinical and industrial origin to achieve a high level of performance.
Methods: The GP card, containing 43 biochemical tests that measure enzymatic, acidification and alkalinisation activities, was tested with 1916 routinely and less frequently encountered stock isolates distributed into 122 species. Organisms were grown on three different isolation media and cards were filled with bacterial suspensions made in 0.45% aqueous NaCl to a turbidity equivalent to a McFarland #0.5 standard. Inoculated cards were incubated in the VITEK 2 instrument and the optimal algorithm was established to generate kinetic identification results.
Results: Of the 1916 isolates tested, 1882 (98.2%) gave a correct identification with 99 (5.2%) low discrimination results. Twenty-eight (1.5%) of the isolates gave an incorrect identification and six (0.3%) were unidentified. All the results were obtained within 2–8 h.
Conclusion: The new GP card used with the VITEK 2 system provides a rapid and accurate method for automated kinetic identification of a wide range of aerobic Gram-positive species. Compared with the existing ID-GPC card, the main improvements include:
- more species claimed: 122 vs. 57;
- higher level of single-choice identifications for the clinical species of coagulase negative staphylococci: 95.8% vs. 85%;
- less inaccurate results for nonreactive organisms.

P487 The IDEIA Norwalk-like virus enzyme immunoassay – a rapid method for screening outbreaks of nonbacterial gastroenteritis

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Norwalk-like viruses (NLVs), initially known as small round structured viruses (SRSVs) and now classified as noroviruses (NoVs), are a group of genetically diverse, single stranded RNA viruses belonging to the family Caliciviridae that are recognised as a major cause of nonbacterial gastroenteritis. This study was performed with a view to implementing the DakoCytomation IDEIA NLV EIA within a routine diagnostic laboratory in order to provide a more rapid response to outbreaks of gastroenteritis within the region.

Objectives: To evaluate the DakoCytomation IDEIA NLV EIA as an alternative to screening by electron microscopy (EM) and the Lordsdale SRSV EIA.
Methods: A faecal suspension of each clinical sample was prepared according to the manufacturer's instructions. A 100 μL of faecal suspension was added to coated microwells and incubated with NLV-specific genogroup 1 or 2 conjugate. After incubation, the microwells were washed and the presence of specifically bound conjugate was determined by the addition of a chromogen and enzyme substrate. Clinical specimens with an absorbance value greater than the cut-off values were regarded as positive. All samples were sent to Bristol for confirmation by EM, Lordsdale SRSV EIA and reverse transcription polymerase chain reaction (RT-PCR). Discrepant samples were sent to a second laboratory and resubmitted to Bristol for further analysis by RT-PCR.
Results: A total of 93 faecal samples collected from patients involved in 28 outbreaks of gastroenteritis were tested. The DakoCytomation EIA detected 24 positives and 45 negatives, confirmed by EM, Lordsdale SRSV EIA and RT-PCR. Detection of NLV antigen in 13 faecal specimens by the DakoCytomation EIA were not confirmed by Bristol. Seven faecal samples found to be negative by the DakoCytomation EIA were positive by the Lordsdale SRSV EIA. Further analysis of these discrepant samples was sought by RT-PCR at a second laboratory. The results obtained were in agreement with those obtained by the DakoCytomation EIA. Of the original 20 discrepant samples, 17 were resubmitted to Bristol for RT-PCR. The results obtained for 11 samples were consistent with those obtained by the DakoCytomation EIA and RT-PCR at the second laboratory. However, six samples remained discrepant.
Conclusions: The results of the study indicated that the DakoCytomation IDEIA NLV EIA is a rapid, reliable alternative to EM and the Lordsdale SRSV EIA. The assay was subsequently introduced into routine testing.

P488 Blind-protected telescopic catheter: a simple, reliable and cost-effective method for the diagnosis of the ventilator-associated pneumonia

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Objective: Ventilator-associated pneumonia (VAP) is a serious infection in the ICU (elevated mortality, cost of the diagnostic procedures and the antibiotics). In our hospital, broncoscopic procedures for the diagnosis of the VAP are rarely used. This study evaluated the utility and cost-effectiveness of the blind PTC in the diagnosis of the VAP in a 10-bed ICU.
Methods: In a 2-year prospective study, PTC was performed and found positive in 57 patients with a clinical suspicion of VAP (new pulmonary infiltrate, purulent secretions, hypoxaemia, fever, leucocytosis). We compared these protected telescopic catheter (PTC) patients to 98 others (matched for age and APACHE II score) who were treated empirically for a VAP but did not have a PTC (non-PTC patients). We evaluated (i) the time needed for the quantitative culture of the PTC, (ii) the time necessary for the report of the results, (iii) the accuracy and ability of the method to safely guide a de-escalation strategy.
Results: (i) Approximately 30 min were needed for the culture (desinfection and vortexing of the tip, dilutions, susceptibility tests according to the NCCLS); (ii) results were communicated to the ICU physicians in 48–72 h; (iii) mean age (55 $\pm$ 14 years) and APACHE II score (19 $\pm$ 8 years) were comparable between the two groups as well as the duration of antibiotics before the VAP (5.8 $\pm$ 3 days vs. 6.7 $\pm$ 4 days). Two of the 57 PTCs were contaminates (3.5%), 52 of 55 PTC patients were treated for VAP (94.5% clinical relevance, 5.5% false positives). Finally, in 22 of these 52 PTC patients, Imipenem was switched to Piperacilin/Tazobactam (42.3%). Vancomycin was withdrawn from the empirical antibiotic regimen in 20 of the 52 PTC patients (38.5%). No change was attempted in the empirical antibiotic regimen of the 98 non-PTC patients. The number of patients died was comparable in the groups (13 PTC vs. 24 non-PTC patients; $P > 0.5$).
Conclusions: PTC is a simple and reliable method for the diagnosis of the VAP. Guided by the PTC, physicians can safely discontinue large-spectrum antibiotics. Any additional cost of its use can be easily waged by the discontinuation of unnecessary antibiotics.

P489 Diagnostic and prognostic value of serum adenosine deaminase activity in scrub typhus

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Objective: Scrub typhus is a common endemic febrile illness together with haemorrhagic fever with renal syndrome (HFRS) and leptospirosis in Korea. The aim of the present study was to assess the diagnostic and prognostic value of serum adenosine deaminase (ADA) activity in scrub typhus.
Methods: Serum ADA activity was determined by colorimetric method. A total of 108 cases of serologically confirmed scrub typhus were compared with 16 cases of HFRS and 16 cases of leptospirosis. Indirect immunofluorescence assay was performed for scrub typhus and leptospirosis, while HFRS was diagnosed by particle agglutination test.
Results: Serum ADA levels were significantly higher in scrub typhus (92.46 $\pm$ 33.89 U/L) than HFRS (44.19 $\pm$ 12.36 U/L) or leptospirosis (24.92 $\pm$ 7.92 U/L) ($P < 0.0001$). Serum ADA2 isoenzyme levels also were higher in scrub typhus (70.15 $\pm$ 21.65 U/L) than HFRS (33.16 $\pm$ 10.47 U/L) or leptospirosis (18.96 $\pm$ 9.27 U/L) ($P < 0.0001$). Five patients who died of scrub typhus have more high levels of serum ADA than survived patients (151.3 $\pm$ 60.62 U/L vs. 89.6 $\pm$ 29.7 U/L; $P = 0.002$). Severe scrub typhus patients ($n = 32$) complicating with renal failure, respiratory failure, shock, encephalitis and myocarditis have higher levels of serum ADA than nonsevere patients (115.78 $\pm$ 47.04 U/L vs. 82.64 $\pm$ 19.83 U/L; $P < 0.0001$).
Conclusion: Serum ADA value is sufficiently useful in early differentiation of scrub typhus from other febrile illness and may prove to be useful as prognostic marker of scrub typhus.

P490 Early diagnosis of CJD: detection of 14-3-3 proteins in the CSF of genetic form

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Creutzfeldt–Jakob disease (CJD) belongs to a family of fatal neurodegenerative diseases called transmissible spongiform encephalopathies (TSE). CJD is distributed worldwide and it occurs in sporadic, genetic, iatrogenic forms as well as a variant CJD. The definite diagnosis of CJD is performed usually post-mortem by the detection of prion by several specific methods (Western blot, ELISA, immunohistochemistry). Nonspecific, but sensitive test for diagnosis of CJD which can be positive even in early stages of the clinical course of the disease is the detection of protein 14-3-3 in the cerebrospinal fluid (CSF) using Western blot. This test shows different sensitivity in individual forms of CJD, the highest was demonstrated in the most frequent sporadic CJD, less efficient appears to be the method in human genetic TSEs. In Slovakia, 75% of all CJD patients belong to the most frequent genetic form with mutation of prion protein gene at codon 200 (mutation E200K). Frozen CSF samples have been stored in our laboratory since 1978. The aim of our study, performed on 125 CSF samples, was to determine: (i) the sensitivity of the protein 14-3-3 test in a large, homogenous group of described genetic CJD, and (ii) the time interval of storing, during which the sensitivity of CSF for described test is preserved. Obtained results demonstrate that CSF of definite CJD patients stored more than 15 years showed no positivity for protein 14-3-3 at all. CSF samples frozen longer than 10 years showed positivity between 28.6% (sporadic CJD) and 33.4% (genetic CJD). Standardised method of protein 14-3-3

detection in the CSF of tested genetic CJD cases with E200K mutation (CJD E200K) showed as much as 92.3% sensitivity. Detection of protein 14-3-3 was included into WHO diagnostic criteria considering mainly results obtained in sporadic CJD. Presented results demonstrate that in CJD E200K (the most important genetic CJD) a combined genetic testing (positive CJD-specific mutation) and detection of protein 14-3-3 represent a very reliable marker of early CJD diagnosis, not available in other human TSEs.

P491 A simple, isocratic reverse-phase ion-pair high performance liquid chromatography assay for cidofovir in human body fluids

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Objectives: Cidofovir (1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine dihdrate, HPMPC, GS-0504) is an acyclic nucleoside analogue active *in vitro* and *in vivo* against a range of herpesviruses, poxviruses, adenoviruses and some RNA viruses. Cidofovir is a phosphonate and is phosphorylated to its active diphosphate form (analogous to a nucleoside triphosphate) by cellular enzymes. Not requiring activation by a virally coded enzyme, it can protect uninfected cells. Cidofovir is usually a second line drug for patients suffering severe, life-threatening viral infection. By 24 h after dose 90% is excreted unchanged in the urine, although the intracellular half-life of the diphosphate is between 17 and 65 h. Being nephrotoxic, it should be administered with probenecid. It is not clear whether this toxicity depends on peak levels of the drug or on time above a given level. In order to help to answer this question and to conduct pharmacokinetic studies we have developed a HPLC assay for the detection of cidofovir in human body fluids.
Methods: Chromatography was performed on a stationary phase of Hypersil 5 ODS with a mobile phase of 2.8 mM sodium phosphate and 1.2 mM tetrabutyl ammonium hydroxide, pH 7.5. The mobile phase was pumped at 1 mL/min and Cidofovir detected by UV absorbance at 275 nm. Serum and plasma samples were prepared by deproteinising with equal volumes of methanol, urine samples by 10-fold dilution in mobile phase. An injection volume of 10 μL was used.
Results: In this assay the retention time of Cidofovir is approximately 13 min and it is well resolved from the components of biological fluids and from other drugs. No interference was seen with 31 other antimicrobial agents. The apparent concentration of Cidofovir preparations is unaffected by heat (>22 h in water or urine, >6 h in serum) and freezing and thawing (>18 cycles in water or urine, >8 cycles in serum). The assay is linear over the range of 0.03–8 mg/L. The limits of detection and quantification are 0.03 and 0.06 mg/L (aqueous) and 0.06 and 0.12 mg/L (serum). Recovery from serum is approximately 90%.
Conclusion: This assay is suitable for measuring Cidofovir levels in biological fluids.

Antimicrobial susceptibility in Gram-negative bacteria - I

P492 The activity of some quinolones against *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* clinical isolates in the presence of pump inhibitors

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Objectives: The most common cause of multidrug resistant strains is the efflux mechanism. The presence of such efflux systems was described for *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. In *P. Aeruginosa*, the presence of three efflux systems (MexAB-OprM, MexCD-OprJ and MexEF-OprN) responsible for quinolones resistance was described. These MDR pumps belonging to the RND family are inhibited by Phe-Arg-b-naphthylamide. On the contrary, reserpine does not inhibit RND pumps. The presence of similar efflux systems was shown also in *S. maltophilia* (MexAB-OprM and SmeDEF). Probably, *S. maltophilia* has also other efflux pumps.
Methods: In our study, activity of some quinolones (nalidixic acid, ciprofloxacin) against *S. maltophilia* and *P. aeruginosa* clinical isolates in the presence and absence of pump inhibitors was determined. The following pump inhibitors in two concentrations (20 mg/L and 80 gm/L) were used: Phe-Arg-b-naphthylamide, reserpine and omeprazol. We also looked for a new inhibitors among newly synthesised compounds, 6-(adamant-1-yl)pyrimidines.
Results: From the studied inhibitors only Phe-Arg-b-naphthylamide affected the susceptibility of tested strains to quinolones, first of all to nalidixic acid. Generally, the presence of higher concentration of inhibitor pump (80 mg/L) increased most effectively sensitivity to nalidixic acid both *S. maltophilia* and *P. aeruginosa*. In 90% of *S. maltophilia* and 80% of *P. aeruginosa* the MIC decreased from threefold to 20-fold in the presence of Phe-Arg-b-naphthylamide. On the contrary, this inhibitor affected the MIC of ciprofloxacin only for a few strains. Moreover, Phe-Arg-b-naphthylamide alone has shown the activity against three strains of *P. aeruginosa* (MIC 20, 40 and 160 mg/L). The other studied pump inhibitors did not change generally the MIC of both quinolones. Unlike the inhibitory activity of Phe-Arg-b-naphthylamide agents as reserpine and omeprazole increased slightly the MIC of tested quinolones for some strains of *S. maltophilia*.
Conclusions: Our data confirm that opposed to reserpine the second tested agent Phe-Arg-b-naphthylamide inhibited efflux pumps not only on *P. aeruginosa* but also on *S. maltophilia*. Moreover, obtained results indicated that depending on the structure of antibiotics the quinolones are maybe transported with different effectiveness through the efflux pumps. Additionally, it is possible that in case of *S. maltophilia* reserpine act antagonistically to ciprofloxacin.

P493 Effect of serum susceptibility on the bactericidal activity of antimicrobials against *Haemophilus influenzae*

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Objectives: To determine the serum susceptibility of clinical isolates of *H. influenzae* (HI) and investigate the effect of this on antimicrobial (AM) kill.
Methods: A total of 2650 HI from various countries worldwide were screened for serum sensitivity [fr2 log kill over 1 h in the presence of 20% human serum (HS)]. The bactericidal activity of moxifloxacin (MFX), levofloxacin (LFX), telithromycin (TEL), clarithromycin (CLA), azithromycin (AZI) and amoxicillin-clavulanate (AMC) was then evaluated at 0.25x, 1x and 4x MIC over 6 h against four serum-resistant (SR) and five serum-susceptible (SS) isolates in haemophilus test medium in the presence of HS or heat-inactivated HS (I-HS). Kill was compared by plotting log 10

reduction in viable count over time and measuring the area under the curve (*AUC*) of each graph.
Results: The majority of isolates (82.1%) were found to be SR. The sample subset of five SS isolates was all killed by HS alone without AM (data not shown). Average *AUC* data for AM at 4x MIC against the four SR HI with HS and for all nine HI with I-HS are shown below. All AM were bactericidal against the SR HI in the presence of HS, with the fluoroquinolones (FQs) MFX and LFX being the most active. For one SR HI, only MFX was bactericidal. In contrast, CLA and AMC were not bactericidal and TEL showed weak kills with I-HS. MFX and LFX remained the most active AMs even with I-HS.

AUC	MFX	LFX	TEL	CLA	AZI	AMC
HS (SR only)	20.1	19.4	10.1	8.1	15.1	17.6
I-HS (SR& SS)	14.1	15.1	2.0	0	8.9	0

Conclusions: The activity of HS alone cannot be relied upon to eradicate clinical isolates of HI because the majority of HI are SR. FQs were the most intrinsically bactericidal AMs against HI and only MFX was active against all HI. Most other classes of AM were poorly bactericidal or merely bacteriostatic in the absence of active HS. Eradication of HI may be important for the treatment of chronic infections and the use of highly bactericidal agents unaffected by serum susceptibility such as the FQs may be beneficial in this regard.

P494 Assessment of *in vitro* telithromycin activity against recent *Haemophilus influenzae* isolates recovered from adult patients in Spain

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Background: Telithromycin is a ketolide compound with proven, improved activity against *Streptococcus pneumoniae* isolates. However, its activity against *Haemophilus influenzae* isolates has been studied to a lesser extent. This study assessed the *in vitro* activity of telithromycin against recent *H. influenzae* isolates recovered from adult patients in Spain.
Material and Methods: A total of 246 *H. influenzae* isolates obtained from adult patients (=16 years), one isolate per patient, suffering respiratory tract infections during the 2003 respiratory disease season were studied. Isolates were recovered from 10 Spanish hospitals in different geographic areas. Susceptibility testing was performed as recommended by the NCCLS using microdilution panels (Trek Diagnostic, UK).
Results: Telithromycin activity (MIC range, MIC 50 and MIC 90: 0.25–16, 2 and 4 μg/mL, respectively) was similar to that of azithromycin (0.12–16, 1 and 2 μg/mL), and clearly higher than that of clarithromycin (0.5–64, 8 and 16 μg/mL) and erythromycin (0.5 to >32, 8 and 16 μg/mL). Using 4 and 16 μg/mL to define the susceptible and resistant category for telithromycin, 98.4 and 0.8% of isolates, respectively, were included within each category. The corresponding values within the susceptible category for azithromycin and clarithromycin using the NCCLS criteria (M100-S13) were 98.4% (4 μg/mL) and 68.3% (8 μg/mL), respectively. One isolate was resistant to azithromycin (MIC, 16 μg/mL) but susceptible to telithromycin (4 μg/mL) and two isolates were resistant to telithromycin (MIC, 16 μg/mL) but susceptible to azithromycin (4 μg/mL). These three isolates were resistant to clarithromycin (32–64 μg/mL). Overall β-lactamase production was 20%. Less than 3% of isolates were β-lactamase negative and resistant to ampicillin. It is of note that three (1.2%) isolates were resistant to amoxicillin/clavulanate (MIC, 8/4 μg/mL) and one (0.4%) isolate were resistant to levofloxacin (2 μg/mL), all remaining susceptible to telithromycin (MIC range, 1–2 μg/mL).
Conclusions: *In vitro* telithromycin activity against *H. influenzae* isolates was comparable with azithromycin and higher than clarithromycin. Moreover, telithromycin was active against *H. influenzae* isolates with emerging resistance mechanisms to amoxicillin/clavulanate or fluoroquinolones.

P495 Bactericidal activity and synergy of rifampin alone and in combination against pan-resistant *Acinetobacter baumannii*

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Objectives: The purpose of this study was to know the bactericidal activity and synergy of rifampin (RMP) alone and in combination with imipenem (IMP) and sulbactam (SB) against two clinical strains of panresistant *Acinetobacter baumannii*, including resistance to colistin.
Methods: MIC and MBC (mg/L) were performed using microdilution method (NCCLS). Time-kill curves were used to evaluate the bactericidal activity and the synergy of antimicrobial combinations (RMP + IMP, RMP + SB and IMP + SB) against the strains 99 and 113. For the time-kill curves antibiotics concentrations used were equivalent to the respective MIC and the C_{max} of RMP, IMP and SB obtained in C57BL/6 mice (RMP 25 mg/kg, IMP 30 mg/kg, and SB 60 mg/kg) in time points from 10 to 150 min after a single dose; antimicrobial levels were determined by bioassay method. Antibiotics were considered to be bactericidal when there was a reduction of the original inoculum $\geq$3 log CFU/mL. Synergy was defined as $\geq$2 log decrease in CFU/mL when using the drug combination, relative to the most active component alone.
Results: MIC/MBC: RMP (128/>128) for both strains; IMP (128/>256) for the strain 99 and (256/>256) for the strain 113; SB (>256/>256) for both strains. C_{max}: RMP (13.4 mg/L), IMP (16.7 mg/L) and SB (81.5 mg/L). Bactericidal activity: RMP (MIC) was bactericidal for both stains and not with C_{max}. IMP was not bactericidal for any of the strains using MIC or C_{max}. SB (MIC and C_{max}) was bactericidal for the strain 99 and it was not bactericidal against the strain 113. The following combinations were synergistic: RMP + IMP (MIC and C_{max}) and RMP + SB (MIC and C_{max}) for both strains.
Conclusions: The combination of RMP plus IMP or SB is synergistic against selected clinical strains of panresistant *A. baumannii*. These results suggest that these combinations may be useful in the treatment of experimental infections caused by this agent.

P496 Synergistic activities of nontraditional antibiotic combinations against multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains

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Objectives: Multiresistant *Pseudomonas aeruginosa* and *Acinetobacter* strains are increasingly cause of life-threatening infections and leads to limitation of the therapy. This study was designed to determine the synergistic activity of colistin with rifampicin, doxycycline, meropenem and azithromycin against multidrugresistant (MDR) *P. aeruginosa* and *A. baumannii* clinical isolates.
Methods: The synergistic activity in combination of colistin with rifampicin, doxycycline, meropenem and azithromycin was investigated against randomly selected five *A. baumannii* and five *P. aeruginosa* isolates by using chequerboard titration method.
Results: The combination of colistin and rifampicin was fully synergistic against four of *A. baumannii* and two of *P. aeruginosa* strains. Colistin with meropenem and colistin with azithromycin combinations showed synergistic activity against three of *A. baumannii* isolates, while resulted in additive or indifferent effects on *P. aeruginosa* strains. Colistin and doxycycline combination was

generally partially synergistic and additive effects against all of the isolates.
Conclusions: The results of this study demonstrate that against MDR *P. aeruginosa* and *A. baumannii*, synergy may occur between nontraditional antibiotics. As *P. aeruginosa* and *A. baumannii* have become to resistant to commonly used antibiotics, it is necessary to test various drugs alone and in combinations to find treatment choices for the infections that these organisms can cause.

P497 Cell surface hydrophobicity and adherence of *Pseudomonas aeruginosa* to abiotic surfaces: effect of subinhibitory concentrations of piperacillin/tazobactam

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Objectives: *Pseudomonas aeruginosa* is a human opportunistic pathogen that colonises biotic or abiotic surfaces and has been emerging as the primary source of nosocomial infections. Cell surface hydrophobicity (CSH) of bacteria is a very important physicochemical feature, which has a great influence on the ability of bacteria to adhere to the surface of host cells or medical devices. It has been reported that subinhibitory concentrations (sub-MICs) of antibiotics are able to affect the bacterial surface properties and various phenotypic traits. In this study, the effects of sub-MICs of Piperacillin/Tazobactam (P-T) on bacterial surface hydrophobicity as well as the effect on bacterial adhesion were analysed, using *P. aeruginosa* strains.
Methods: *In vitro* antimicrobial activities were evaluated by microdilution method (NCCLS) against three reference strains (ATCC 27853, PAO1 and AK1), three defined PAO1 mutants with deviating surface characteristics (MT1562, PT623 and PAO1algC) and five *P. aeruginosa* clinical isolates (CIs). Selection of CIs was based on minisatellite-primed MSP-PCR fingerprinting of 100 isolates obtained from patients hospitalised at a Portuguese Central Hospital. The hydrophobicity assay was performed by growing the 11 strains in LB in presence and absence of P-T. The changes on CSH were estimated by calculating the percentage of cells adhering to *n*-hexadecane (1). The effects of 1/2 MIC on bacterial adhesion (1 h) were studied using a modified microtitre-plate assay (2).
Results: There was a significant decrease in CSH of all the strains tested that could explain a decrease in adhesion values in CIs and controls (reference and mutant strains). Treatment of the bacterial cells with subinhibitory concentrations (1/2 MIC) of P-T changed significantly the CSH towards the hydrophilic state compared with nontreated cells, and was found to be strain dependent.
Conclusion: As CSH and Adhesion ability are considered pathogenic traits, these data indicate the potential effectiveness of sub-MIC P-T for the treatment of patients with *P. aeruginosa* infections.
Acknowledgements: This study was supported by a research grant from Fundação Calouste Gulbenkian.
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P498 *In vitro* activity of antimicrobial drugs against *Brucella melitensis* strains in an endemic area

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Objectives: In human beings, brucellosis caused by *Brucella melitensis* is the most important clinically apparent disease and remains a major problem in the Mediterranean region including Turkey. Despite clinical and laboratory studies, the optimum antibiotic therapy for brucellosis is still unclear. There are few reports (mainly from endemic regions) in the literature about the sensitivity studies of *Brucella* spp. Furthermore, these studies still have not been standardised and interpretative standards are not available. The *in vitro* activities of antimicrobial drugs against *B. melitensis* strains isolated from blood and body fluids cultures of the patients with brucellosis were investigated in an endemic area.
Methods: A total of 63 *B. melitensis* strains were collected between January 1998 and November 2003 from 63 patients with brucellosis at Cumhuriyet University Hospital, Sivas, Turkey. Only one strain per patient was included. The isolates were tested for susceptibility to various antimicrobial agents by using the Sceptor (Becton Dickinson Diagnostic Instrument Systems, Towson, MD, USA) automatic system. This is a broth microdilution system that uses plastic microtiter plates with doubling dilutions of desiccated antimicrobial agents.
Results: The aminoglycoside-structured antibiotics generally had good activity. Ceftriaxone had active against 59 of 63 (93.6%) strains (MIC <8 μg/mL). Carbapenems (imipenem, meropenem) showed good activity towards all strains of *B. melitensis* with MICs of 4 μg/mL. All strains tested were susceptible to cefepime (MIC <8 μg/mL) and 62 (98.4%) of 63 strains were susceptible to ciprofloxacin (MIC <1 μg/mL). Fifty-nine (93.6%) strains were inhibited by rifampicin at 1 mg/mL. Sixty-one (96.8%) were susceptible to tetracycline and 58 (92.1%) were to trimethoprim-sulfamethoxazole at concentrations of 4 and 0.5/9.5 μg/mL, respectively. Strains were also tested with other antimicrobials.
Conclusion: In order to reduce the incidence of complications of brucellosis and the development of drug resistance by the pathogen, it is necessary that proper treatment be instituted, following antimicrobial susceptibility testing.

P499 Thermophilic *Campylobacter* resistance to five antimicrobial drugs

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Although *Campylobacter enterocolitis* is often self-limited diseases, in prolonged diarrhoea, severe clinical presentation, immuncompromised patients and postinfective sequels, treatment is necessary. However, appearance of *Campylobacter* spp. strains resistant to erythromycin and also increasing resistance to quinolones may be treat to efficient therapy. The aim of the study is to evaluate sensitivity of thermophilic *Campylobacter* strains to drugs used in therapy of enterocolitis as well as to nalidixic acid used in identification. We investigated sensitivity of 76 thermophilic campylobacter strains isolated from stool of patients with diarrhoea. Sensitivity of *Campylobacter* spp. was tested by agar dilution method in microaerophilic atmosphere for 48–72 h on Columbia agar supplemented with 5% defibrinated horse blood and antimicrobial agent: erythromycin (0.06–4 mg/L), gentamicin (0.25–8 mg/L), ciprofloxacin (0.25–16 mg/L), tetracycline (0.25–16 mg/L), chloramphenicol (1–32 mg/L) and nalidixic acid (4–64 mg/L). The NCCLS interpretative standards for Enterobacteriaceae were used as a tentative breakpoints in MIC determination. For erythromycine sensitivity testing as a breakpoint MIC of 4 mg/L was used. As a quality control, strain *C. jejuni* NCCLS 11951 was included. The resistance to erythromycin was low (4% strains) with MIC50 of 0.5 mg/L and MIC90 of 1.0 mg/L. Strains resistant to gentamicin were not detected. For gentamicin MIC50 of 0.5 mg/L and MIC90 of 1.0 mg/L were determined. Resistance to ciprofloxacin was detected in 38.2% of investigated strains with MIC50 of 0.5 mg/L and MIC90 of 8 mg/L. Resistance to nalidixic acid was higher (42.1%) with MIC50 of 8 g/L and MIC90 64 g/L. Strains resistant to tetracycline were detected in 15.8% of investigated strains with MIC50 of 0.5 mg/L and MIC90 16 mg/L. All investigated strains were sensitive to chloramphenicol with MIC50 of 4 mg/L and MIC90 of 8 mg/L. Occurrence of strains resistant to erythromycin and ciprofloxacin may restrict its effectiveness in thermophilic *Campylobacter* infection therapy and requests its permanent investigation. Resistance to nalidixic acid may diminish the value of the test for preliminary identification of thermophilic campylobacters.This work is a part of the project 'The role of *Campylobacter jejuni* in etiology of some autoimmune diseases' (1612) and supported by the Ministry of Science, Technology and Development, Republic of Serbia.

P500 *In vitro* activity of 12 anti-anaerobic agents against clinical *Bacteroides fragilis* group strains isolated over a 7-month period

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Objectives: Surveillance for antimicrobials resistance of clinical *Bacteroides fragilis* group isolates is necessary for help guide empiric therapy of anaerobic infections. We determine the antimicrobial susceptibility pattern of clinical *B. fragilis* group strains isolated in our institution from May to November 2003.
Methods: Susceptibility testing was performed using a microdilution method according to document M11-A5 (NCCLS). Antimicrobials tested were amoxicillin (AM), amoxicillin-clavulanate (AC), piperacillin (PI), piperacillin-tazobactam (PT), cefoxitin (CE), imipenem (IM), chloramphenicol (CH), clindamycin (CL), metronidazole (ME), moxifloxacin (MO), tetracyclin (TE) and vancomycin (VA).
Results: A total of 182 strains from 127 specimens were tested. The percentage of intermediate resistant strains within each species tested were:

Species (number)	AM	AC	PI	PT	CE	IM	CL	ME	MO	TE
B. fragilis (76)	100	7.9	28.9	2.6	6.6	1.3	46.1	1.3	26.3	81.6
B. thetaiotaomicron (38)	100	7.9	28.9	0	52.6	0	73.7	2.6	31.6	84.2
B. uniformis (18)	94.4	0	16.7	0	16.7	0	55.6	0	72.2	66.7
B. vulgatus (15)	100	6.7	40.0	0	13.3	0	46.7	0	46.7	8.0
B. ovatus (14)	100	28.6	50.0	14.3	64.3	0	71.4	0	50.0	14.3
Other (21)	100	23.8	57.1	4.8	52.4	0	71.4	0	33.3	90.5
Overall (182)	99.5	10.4	33.5	2.7	27.5	0.5	57.7	1.1	36.3	83.0

*All strains well resistant to VA, and susceptible to CH.

Conclusion: PT, IM, ME and CH were the most active agents tested, with at least 97% of susceptible strains. Moreover, AC and CE had also a good activity with a susceptibility rate ranging from 72.5 to 90.0%. The remaining antimicrobials had a poor activity. In spite of the growing use of broad-spectrum antimicrobials, most of these agents remain active against clinical strains of *B. fragilis* group isolated in our institution.

P501 The effect of sub-inhibitory concentration (sub-MIC) of amikacin and ciprofloxacin on the loss of capsular antigen K1 by *Escherichia coli* strains

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Objectives: The aim of this investigation was to examine the influence of 1/2 MIC of amikacin (AN) and ciprofloxacin (CIP) on the loss of capsular antigen K1 by *Escherichia coli* strains.
Methods: Four *E. coli* K1 strains (315, 353, 418 and 662) isolated from urine of children with urinary tract infections were used. The bacteriophage K1A for detecting the capsular antigen K1 was used. The MICs of AN and CIP for each strain in Mueller–Hinton broth were determined by using microdilution method. The frequency of occurrence of surface antigen K1 among 100 clones of each *E. coli* K1 strains (without antibiotics and with 1/2 MIC of AN and CIP) was detected by the method described earlier (1).
Results: In controls, all tested *E. coli* K1 strains revealed the occurrence of 93–96% clones with K1 antigen. The exposure of *E. coli* K1 strains to 1/2 MIC of CIP significantly decreased the percentage of clones with K1 antigen. Only 13% clones of *E. coli* 418 strain possessed K1 antigen. In cases of *E. coli* strains 353, 662 and 315, the presence of K1 antigen was found in 28, 37 and 40% per 100 clones, respectively. We observed that after exposure all *E. coli* K1 strains to 1/2 MIC of AN, the percentage of clones with K1 antigen corresponded to the percentage observed in control.
Conclusions: The findings indicate that only sub-MIC of CIP caused decrease in the percentage of clones sensitivity to K1A phage.
Reference
Jankowski *et al.* (1992). Diagn. Lab. 29, 31–34.

P502 Antibiotic susceptibilities and extended-spectrum β-lactamase production of Enterobacteriaceae from urinary tract infections

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Objectives: ESBL producing Enterobacteriaceae have compromised therapy with β-lactam antibiotics, including third generation of cephalosporins. The objective of this study was to determine the occurence of ESBL phenotypes among different isolates of Enterobacteriaceae and their susceptibility to antimicrobial agents.
Materials and Methods: A total of 1000 strain of Enterobacteriaceae were isolated from urine samples during 1-year period. Suspected strains are presumptively defined as ESBL producers according to result of disk diffusion method, using ESBL marker antibiotics-ceftazidim, ceftriakson and cefotaksim. Those isolates were retested with double-disc sinergy test (DDST)-CAZ, CTR, CTX and amoxicillin-clavulanic acid disks implementation. Enhancement of inhibition zone (or so-called ghost zone) indicated presence of ESBL strain. Antimicrobials susceptibility to β-lactam antibiotics, aminoglikosides, quinolones and trimetoprim-sulfametoxazol evaluated by disc-diffusion method, and the ESBL detection was performed by the DDST, according to NCCLS criteria (2002).
Results: The species distribution as follows: *Escherichia coli* (63.9%), *Klebsiella* spp. (30%), *Enterobacter* spp. (11.1%), *Proteus vulgaris* (2.3%), *P. mirabilis* (11%), *Providencia* spp. (0.8%), *Morganella morgani* (0.2%) and *Citrobacter* spp. (2.6%). Total number of isolates (21.4%) was multiresistant for more than three groups of antibiotics: *E. coli* were 44.39%, *Klebsiella* spp. 8.41%, *Enterobacter* spp. 26.63%, *P. vulgaris* 5.14%, *P. mirabilis* 10.28%, *Providencia* spp. 2.33%, *M. morgani* 0.46% and *Citrobacter* spp. 35%. Thirty-three per cent of all isolates were from hospital samples; 4.3% of all isolates were producing ESBL, and included four different species. *Escherichia coli* (4.06%), *Klebsiella* (23.3%), *Enterobacter* (8.1%) and *Proteus mirabilis* (0.2%). The majority of producers were from clinical specimens (63%).
Conclusions: Gram-negative rods were responsible for high percentage of urinary tract infections. *Escherichia coli* was the most common uropathogen. Multiresistant strain represent 21.4% from Enterobacteriaceae implicated in UTI. The resistance to ampicilin was the most frequent and concerned 62.6% of isolates of all indicated species.

P503 Agreement between disc diffusion and E-test methods to assess the carbapenem susceptibility of four Gram-negative nosocomial pathogens

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Objectives: The aim of this study was to assess the carbapenem susceptibility of four nosocomial pathogens and to evaluate the reliability of the susceptibility results determined by E-test and disc diffusion (DD) methods.
Methods: *Escherichia coli* ($n = 73$), *Klebsiella pneumoniae* ($n = 60$), *Pseudomonas aeruginosa* ($n = 70$) and *Acinetobacter* spp. ($n = 70$) isolated from nosocomial infections in 2002–2003 were included in the study. Thirty-five per cent of the strains were isolated from intensive care units. After determining antimicrobial susceptibility against imipenem and meropenem by DD (10 μg; Oxoid, UK) and Etest (AB Biodisk, Solna, Sweden) methods, the results were categorised as susceptible (S), intermediate (I) and resistant (R) according to the NCCLS criteria. For statistical analyses, the

Table 1. Susceptibility of *Acinetobacter* spp and *P. aeruginosa* strains to imipenem and meropenem by comparing results of DF and Etest Methods

		Etest method		
	DD method	R/I	S	McNemar test p-value
Imipenem				
Acientobacter spp	R/I	37	3	0.250
	S	0	30	
P. aeruginosa	R/I	34	4	1.000
	S	3	29	
Meropenem				
Acinetobacter spp	R/I	32	9	0.021
	S	1	28	
P. aeruginosa	R/I	30	7	0.344

intermediate group was included in the resistant category because of the low numbers of bacteria in the former group.
Results: None of *E. coli* or *K. pneumoniae* strains were resistant to carbapenems, whereas, resistance reached up to 59.0% in *Acinetobacter* spp. and *P. aeruginosa* isolates. By either method, the pattern of the susceptibility of the four bacteria was not statistically significantly different for imipenem vs. meropenem. Total agreement of DD and E-test methods for susceptibility to imipenem was 95.7%, and 90.0% in *Acinetobacter* spp. and *P. aeruginosa*, respectively; and susceptibility to meropenem was 90.0% for both bacteria. However, the difference of the results obtained by either method was statistically significant for *Acinetobacter* spp.
Conclusion: Study results suggest a high resistance rate for *Acinetobacter* spp. and *P. aeruginosa* strains against carbapenem antibiotics in our hospital. Further studies are needed to clarify whether E-test should be used to confirm meropenem resistance of *Acinetobacter* spp. determined by DD method.

P504 Gram-negative nosocomial pathogens in Estonian intensive care units

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Objective: While the most important reasons of mortality and morbidity in intensive care units (ICUs) are nosocomial infections caused by Gram-negative pathogens, our objective was to evaluate susceptibility pattern of those pathogens comparatively in Estonian ICUs by similar protocol. To clear up methodological discrepancies, data of E-test and disk diffusion method were compared.
Methods: During April–November 2003, a total 105 *Acinetobacter baumannii*, 92 *Pseudomonas aeruginosa* and 96 *Klebsiella pneumoniae* strains were collected from clinical specimens from ICUs of North Estonian Regional Hospital, East Tallinn Central Hospital and Tartu University Clinics. For susceptibility testing, E-tests and antibiotic disks (meropenem, imipenem, ampicillin/sulbactam, cefepime, amikacin, piperacillin/tazobactam, ciprofloxacin and ceftazidime) were used accordingly to NCCLS guidelines.
Results: Ninty-five per cent of *A. baumannii* strains were sensitive to meropenem, 98% to imipenem, 61% to ampicillin/sulbactam, 56% to cefepime and 72% to amikacin (MIC50/90 values, respectively, 1/3, 0.75/2, 6/32, 8/32 and 6/64); 80% of *P. aeruginosa* strains were meropenem, 70% imipenem, 78% piperacillin/tazobactam, 68% ciprofloxacin, 75% ceftazidime and 98% amikacin sensitive (MIC50/90 values 1/16, 3/>32, 6/>256, 0.25/12, 1.5/64 and 4/12). The susceptibility of *K. pneumoniae* isolates to meropenem and imipenem were 99%, to ciprofloxacin 92% and to amikacin 97% (MIC50/90 values 0.023/0.19, 0.19/0.5, 0.023/1 and 2/3). Generally, MIC50/90 for meropenem was 0.38/4, imipenem 0.75/6, ampicillin/sulbactam 6/32, cefepime 8/32, amikacin 3/8, piperacillin/tazobactam 6/256, ciprofloxacin 0.125/1.5 and ceftazidime 1.5/64. In all three ICUs, the sensitivity among *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* strains was similar, exept higher resistance to cefepime of *A. baumannii* strains in Tartu University Clinics. Discordance between E-test and disk-diffusion was pathogen specific. In *K. pneumoniae*, one major and seven minor errors were found, whereas in *A. baumannii*/*P. aeruginosa* testing, respectively, 2/4 very major, 13/12 major and 69/33 minor errors occurred. Carbapenems test results correlated better than comparisons of other agents.
Conclusions: Most active agents against all pathogens were carbapenems and amikacin, whereas meropenem and ciprofloxacin had lowest MICs than others. For empirical treatment meropenem is preferred due to high activity against all Gram-negative pathogens and the lowest MIC values. In case of *A. baumannii* and *P. aeruginosa*, E-tests are needed for susceptibility testing.

P505 Effects of subminimal inhibitory concentrations of three antimicrobials on the virulence factors and growth of *Proteus mirabilis*

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Objectives: Proteus is the second bacteria only to *Escherichia coli* as a cause of nonhospital acquired urinary tract infections. The enzyme urease causes the pH of urine to rise, allowing unchecked growth of the bacteria. Adherence to uroepithelial cells and the organisms rapid motility are also involved in the pathogenesis of the urinary tract infections. Antimicrobial treatment is often chosen after determination of the minimal inhibitory concentrations (MIC). Although antibiotics are often present in subinhibitory concentrations (sub-MICs) and may still be effective in reducing bacterial virulence.
Methods: Subminimal inhibitory concentrations of ciprofloxacin, gentamicin and ampicillin-sulbactam at 1/2–1/32 X MIC levels on growth, adherence, urease and swarming characteristics of *Proteus mirabilis* (ATCC 14153) were studied.
Results: Minimal inhibitory concentrations values were 0.125 μg/mL for ciprofloxacin, 0.25 μg/mL for ampicillin-sulbactam and 1 μg/mL for gentamicin. These three antimicrobials had no significant effect on swarming and urease production at the sub-MIC levels. Ciprofloxacin and ampicillin-sulbactam had no inhibitory effect on growth at sub-MIC levels whereas gentamicin inhibited the colony counts at 1/2 X MIC level. We also observed that ciprofloxacin, gentamicin, ampicillin-sulbactam inhibited the adherence to uroepithelial cells at 1/2–1/8 X MIC.
Conclusion: *Proteus mirabilis* causes urinary tract infections in the complicated urinary tracts, especially in indwelling catheters, presence of the structural abnormalities or in elder patients. Ciprofloxacin (quinolone), ampicillin-sulbactam (β-lactam) and gentamicin (amino glycoside) have different effect mechanisms, and side effects. In this study, it was interesting to see that there were no differences on the swarming and urease production of these three antibiotics whereas gentamicin still inhibited bacterial growth at 1/2 X MIC levels. Gentamicin was more effective in 1/2–1/4 X MIC levels on adherence of the pathogen that is known to be the most important virulence mechanism. In conclusion, we may suggest that gentamicin is more effective antibiotics among these three agents.

P506 *In vitro* susceptibility of *Pseudomons aeruginosa* isolated in a burn centre in south Iran, to silversulfadiazine and silver nitrate

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Objectives: Development of microorganisms resistant to antiseptics may increase with the widespread use of these agents. Silver salts and compounds ($AgNO_3$, silversulfadiazine) are among these agents. Silversulfadiazine (SSD) is extensively used in our burn centre in south of Iran (Ghotbedin Hospital, Shiraz). This study was carried out to determine and compare the susceptibility of

Pseudomons aeruginosa isolated from burned patients, burn wards and nonburn patients to SSD and $AgNO_3$.
Methods: Three groups of *P. aeurginosa* were isolated including the strains from burned patients (group I), environmental strains from burn centre (group II) and strains isolated from nonburn patients (group III or control). The MICs of SSD and $AgNO_3$ for these strains were determined by agar dilution method in TYE agar without NaCl in dark. The results were compared by fisher exact test and correlation between MICs was determined as well. Susceptibility of these strains to SSD was also evaluated by agar cup plate method.
Results: From 63 strains in group I, 60 strains were resistant to SSD which 40 of them were highly resistant (MIC >10 mM) and five of them were resistant to $AgNO_3$. In group II, eight strains of 15 were resistant to SSD with the same range of MIC as group I but non of them showed resistance to $AgNO_3$. In group III, all the strains were sensitive to SSD and $AgNO_3$. The differences between MIC of SSD in these groups were significant ($P < 0.001$). Correlation between MIC of SSD and $AgNO_3$ was not significant in group I. The results of agar dilution tests were confirmed by agar cup plate method.
Conclusion: *Pseudomanas aeruginosa*, the epidemic cause of infections in our burn centre could develop a high level of resistance to SSD (MIC >10 mM) which is an important threat for burned patients and warns to revise the effectiveness of this drug. Only five strains of 60 SSD resistant were cross-resistant to $AgNO_3$ (MIC 0.75–1 mM).

P507 Antimicrobial resistance among *Streptococcus pneumoniae* and *Haemophilus influenzae* from Africa and the middle-east: 2002/2003 winter season

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Objectives: Antimicrobial resistance among respiratory pathogens exists worldwide, affecting empirical prescribing choices. Quality surveillance data are needed to monitor the prevalence and spread of resistance to commonly prescribed antimicrobials.
Methods: Respiratory tract isolates of *Streptococcus pneumonia*e (Sp) and *Haemophilus influenzae* (Hi) were collected from patients in three African countries, seven middle-eastern countries and Pakistan in the 2002/2003 winter season. MICs for various antimicrobials were determined using E-test, and susceptibility assessed based on NCCLS breakpoints, where applicable. Quality control strains were tested on each day of testing. Not all antimicrobials were tested in all countries, or against all isolates.
Results: A total of 1154 Sp and 1091 Hi isolates were collected. In Africa 58.6% (136/232) and in the middle-east 42.5% (349/822) of Sp were penicillin susceptible and 4.3% (10/232) and 8.3% (68/822), respectively, were penicillin resistant (PRSP). The highest PRSP prevalence was in Tunisia (17.8%). No PRSP were identified in Egypt, Jordan, Kuwait or Pakistan. In Africa 10.8% (25/232), in the middle-east 12.3% (99/808), and in Pakistan 13.0% (13/100) of Sp were azithromycin resistant. The lowest regional prevalence of Sp resistance to a cephalosporin was to cefprozil [Africa: 2.2% (5/232); middle-east: 1.9% (16/822)], and the highest was to cefaclor [Africa: 10.8% (25/232)] and cefdinir [middle-east: 24.3% (113/465)]. Only 0.4% (1/232) of Sp in Africa and 0.5% (4/821) in the middle-east were resistant to amoxicillin/clavulanic acid. In Africa, 50.0% (5/10) of PRSP were co-resistant to azithromycin, as were 22.4% (15/67) of PRSP from the middle-east. Of Hi isolates, 14.9% (33/221) (Africa) and 21.6% (166/768) (middle-east) were β-lactamase positive. All Hi from Africa and Pakistan and 99.9% from the middle-east were amoxicillin/clavulanic acid susceptible.
Conclusions: Despite an overall prevalence of PRSP in Africa and the middle-east <10%, the higher prevalence of PRSP in countries such as Tunisia may complicate empirical prescribing in those areas. Of further concern is the prevalence of macrolide resistance and PRSP macrolide co-resistance. The high prevalence of β-lactamase production in Hi in the middle-east may make β-lactamase-unstable antimicrobials unsuitable in this region. Agents such as amoxicillin/clavulanic acid, to which both Sp and Hi remain susceptible, may be most appropriate for empirical prescribing in these areas.

Antifungal susceptibility studies

P508 Comparative evaluation of AFST-EUCAST method and Sensititre YeastOne Colorimetric Antifungal Panel with NCCLS reference method for susceptibility testing of *Candida* species

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Objectives: This study was carried out to compare the performance of two alternative methods, Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) method and a commercially prepared Sensititre YeastOne Colorimetric Antifungal Panel with NCCLS M27-A2 microdilution method.
Methods: Two quality controls, seven reference strains of ATCC including various *Candida* species and a clinical isolate of fluconazole-resistant *C. glabrata* were included. Susceptibility for amphotericin B, fluconazole, itraconazole and ketoconazole were performed with AFST-EUCAST, Sensititre and NCCLS microdilution methods while the susceptibility of 5-flucytosine was investigated with Sensititre and NCCLS methods. AFST-EUCAST method was performed with RPMI-1640 supplemented with 2% dextrose, inoculum size of $0.5–2.5 \times 10^5$ CFU/mL and flat-bottom plates. Sensititre method was carried out according to the manufacturer's instructions with an inoculum of $1.5–8 \times 10^3$. The endpoints were determined visually for amphotericin B, visually and spectrophotometrically at the wavelength of 450 and 492 nm for azoles after 48 h in the NCCLS method. The results were read spectrophotometrically at the same wavelengths in the AFST-EUCAST method and only visually in the Sensititre method. The results were compared according to the agreement of MIC values within $\pm$2-fold dilutions and susceptibility categories.
Results: When the agreement between the results of NCCLS and Sensititre method after 48 h was considered 8–9 of 10 strains were within $\pm$2-fold dilutions for amphotericin B, fluconazole and itraconazole and 6–7 were for ketoconazole. According to the results of NCCLS method and AFST-EUCAST method, at 24 and 48 h 8–10 of the strains were within $\pm$2-fold dilutions for all of the agents. When the susceptibility categories were considered there was no very major and major errors.
Conclusion: It can be concluded that both AFST-EUCAST and Sensititre methods are potentially good alternatives for antifungal susceptibility testing of *Candida* species for many antifungal agents when tested with reference strains.

P509 Determination of antifungal activity of caspofungin using flow cytometry

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Caspofungin is an echinocandin that blocks the synthesis of β-(1,3)-D-glucan of the fungal cell wall, which is an essential component of the cell wall of numerous fungal species. The inhibition of its synthesis may result in a fungistatic effect, from blockade of the cell wall synthesis, or in a fungicidal effect, from changes in the integrity of the cell wall. Cytometric methods allow the early establishment of a susceptibility profile and give the possibility to evaluate functional and morphologic changes of the fungal cells (1).

Objectives: To determine susceptibility of clinical isolates of yeast to caspofungin by cytometric methods.

Methods: Two strains of *Candida albicans* with low MIC to caspofungin (determined accordingly M27 A protocol by NCCLS) and one *C. guilliermondii* and one strain of *Cryptococcus neoformans* both with high MIC were studied. The strains were grown overnight in Sabouraud broth and then incubated with caspofungin (Merck), at serial concentrations (MIC, MIC/2, 2x MIC and 4x MIC) during 1, 3 and 5 h, in phosphate buffer saline (Sigma). The suspensions were washed and stained with propidium iodide (PI; Sigma) (a marker of cell death by cell membrane lesion), FUN-1 (molecular probes) an indicator of metabolically integrity of yeast cells and SYTO 16 (molecular probes) a green fluorescent nucleic acid stain. The cells were analysed on a flow cytometer (Beckman Coulter XL-MCL): the morphology (scattergram) and the intensity of fluorescence of the stained cells [FL3 (red) for PI; FL2 (green) for FUN-1 and FL2 for SYTO 16] were evaluated.

Results: Obvious changes of the scatter were noticed from after 1 h of incubation, which increased with increasing incubation time. Five hours of incubation with 4x MIC were necessary to stain sensitive yeast strains with PI. Resistant strains did not stain with this probe. Fun-1 staining increased on sensitive strain after 3 h incubation with MIC or higher concentrations; resistant strains only increased intensity of fluorescence with very high concentrations. SYTO 16 did not allow to distinguish sensitive from resistant strains.

Conclusions: The antigungal effect of caspofungin could be evaluated by cytometric methods. The fungicidal effect was detected with PI after 5 h incubation with the antifungal while fungistatic effect was detected with FUN-1 sooner after 3 h incubation.

Reference

1. Pina-Vaz, C. *et al.* (2001). *Clin Microbiol Infect* 7, 609–618.

P510 Paradoxical caspofungin effect: reduced activity against *Candida albicans* at high concentrations

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Objective: Resistance problems with caspofungin, an echinocandin inhibitor of fungal cell wall glucan synthesis, have been rare. We noted and investigated paradoxical turbid growth of *Candida albicans* isolates in some high, supra-MIC, concentrations of caspofungin.

Methods: Broth and agar dilution, checkerboard analysis of drug interaction, DNA sequencing, enzyme expression analysis.

Results: Among isolates submitted for susceptibility testing and screened to 12.5 mcg/mL, the frequency was 10%. Analysis of the turbid growth indicated slowing of growth in drug, but CFU up to 72% of drug-free controls. Clearing of growth again by the highest concentrations produces a quadri-phasic pattern in a tube dilution series. Cells growing at high drug concentrations were not resistant on retesting, but showed the paradoxical effect of the parent. Among a selected series of isolates tested to 50 mcg/mL, an additional 53% showed a 'mini effect': no turbid growth, but incomplete killing at high, supra-MFC concentrations. These effects were reproducible, medium dependent in extent, noted in macro- and micro-dilution and on agar containing drug (but not when drug concentrations were not constant, as in agar diffusion), not seen in other *Candida* species or with other echinocandins, and not due to destruction of drug in tubes with the effect or to mutations in resistance-associated regions of the glucan synthase complex. Co-operative enhancement of inhibition by a second drug could eradicate the effect. Extensive studies of relationship to azole resistance mechanisms suggest a weak association, at most. Occasional isolated clear tubes on subculture yielded a few viable cells in a ring-like pattern, suggesting random distribution, in some strains, of few cells with propensity to grow in the presence of drug.

Conclusion: We postulate high drug concentrations derepress or activate resistance mechanisms. The ability of subpopulations to survive at high drug concentrations could have *in vivo* consequences.

P511 Biofilms of *Candida albicans* on silicon catheters could be reduced by caspofungin *in vitro*

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Objectives: Some manifestations of candidiasis are associated with the formation of biofilms on inert surfaces, and the intrinsic resistance of *C. albicans* biofilms to the most commonly used antifungal agents has been demonstrated. We studied here the effect of caspofungin on biofilms of *C. albicans*.

Methods: Calibrated sections of silicone catheters were incubated with *C. albicans* yeasts to obtain biofilms of 2, 24 and 48 h of maturation. Ten strains of *C. albicans* were used: five strains susceptible to fluconazole *in vitro* and five strains resistant to this antifungal agent. We report on the effect of two concentrations of caspofungin (MIC and 2 mg/L) on these biofilms. The influence of caspofungin on *C. albicans* biofilms was determinated by evaluating a significant decrease or increase ($P < 0.0001$) in the metabolic activity of yeasts.

Results: The results showed that caspofungin (MIC) had no effect on *C. albicans* biofilms, whatever the strains and the maturation status of the biofilm. Caspofungin (2 mg/L) induced a significant decrease of the metabolic activity of all tested *C. albicans* biofilms. The efficiency of caspofungin (2 mg/L) was observed independently of (i) the susceptibility of yeasts to fluconazole, and (ii) the maturation status of fungal biofilms.

Conclusion: Caspofungin (2 mg/L) could represent a good candidate in the prevention of candidiasis associated with silicone medical devices.

P512 *In vitro* activities of fluconazole and voriconazole against Spanish bloodstream isolates of *Candida glabrata* and *Candida krusei*

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Objective: To evaluate the *in vitro* susceptibility to fluconazole and voriconazole of all the *Candida glabrata* and *Candida krusei* blood isolated during a 14-year period (1990–2003) at a tertiary care hospital (University Hospital of Cruces, Barakaldo, Spain).

Patients and Methods: Twenty-eight *C. glabrata* and 15 *C. krusei* blood isolates were tested. Disk diffusion was performed as described in NCCLS document M44-P in Mueller–Hinton agar (Difco Laboratories, USA) supplemented with 2% glucose and methylene blue. Fluconazole (25 μg) and voriconazole (1 μg) disks were obtained from Becton Dickinson (USA). Plates were inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard, incubated at 37°C and read at 24 h. Interpretive criteria for fluconazole were: (i) susceptible, zone diameter of 19 mm or more; (ii) susceptible-dose dependent, zone diameter of 15–18 mm; (iii) resistant, zone diameter of <14 mm.

Interpretive breakpoints have not yet been established for voriconazole an a zone diameter of <13 mm was considered as an indicator of *in vitro* resistance. Quality control was performed by using *C. albicans* ATCC 90028, *C. krusei* ATTC 6258 and *C. parapsilosis* ATCC 22019. E-test (AB Biodisk, Sweden) and Sensititre Yeast One (AccuMed International, USA) were used for testing all resistant and susceptible-dose dependent isolates and a representative number of susceptible isolates.
Results: 14.3% *C. glabrata* had a decreased susceptibility to fluconazole, as two *C. glabrata* were resistant and other two isolates were susceptible-dose dependent. All *C. krusei* were resistant to fluconazole. Voriconazole was active against 27 (96.4%) *C. glabrata* and 14 (93.3%) *C. krusei*. Both voriconazole resistant isolates were also resistant to fluconazole and were isolated one (*C. krusei*) in 1991 and other (*C. glabrata*) in 1993. The fluconazole and voriconazole susceptibility patterns were constant during this 14-year period. These susceptibility results to both triazoles were confirmed by the E-test and Sensititre YeastOne methods.
Conclusion: Voriconazole was very active *in vitro* against *C. glabrata* and *C. krusei* blood-stream isolates.
Acknowledgements: This work was financed in part by grant 1/UPV 00093.327-E-14645/2002 from the Universidad del País Vasco.

P513 Comparison of the *in vitro* activity of anidulafungin with amphotericin B, caspofungin, fluconazole, itraconazole and voriconazole against a panel of 780 yeast isolates obtained from five European centres

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Objectives: To compare the *in vitro* activity of the echinocandin agent anidulafungin with that of five other systemically active antifungal agents against a total of 780 yeast isolates obtained from five European countries.
Methods: Isolates of *Candida albicans* (505), *C. glabrata* (89), *C. krusei* (53), *C. parapsilosis* (41), *C. tropicalis* (65) and other *Candida* species (23) were obtained from superficial and deep infections of patients in the UK, France, Germany, Italy and Spain. All isolates were tested by the microtitre plate modification of method NCCLS M27-A2 with results recorded after 24 and 48 h. A subset of 50 isolates was also tested by the EUCAST method with reading after 24 h.
Results: The 24 and 48 h MIC50, MIC90 and range (mg/L) are presented for each drug against all isolates tested by the NCCLS method. Results of the NCCLS method read after 24 or 48 h and results obtained by the EUCAST method read after 24 h differed by no more than a doubling dilution. Anidulafungin was the most potent agent overall against the panel of yeasts tested. MICs of anidulafungin were similar for azole-susceptible and azole-resistant isolates.

Table 1. Table of 24 and 48h MIC50, MIC90 and Range (mg/L) for each drug against 780 isolates of *Candida* species

Drug	Range (mg/L) 24 h	Range (mg/L) 48 h	MIC50 (mg/L) 24 h	MIC50 (mg/L) 48 h	MIC90 (mg/L) 24 h	MIC90 mg/L 48 h
Anld	≤0.03–2.0	≤0.03–2.0	≤0.03	≤0.03	0.06	0.12
Caspo	≤0.125–2.0	≤0.125–8.0	0.25	0.5	1.0	2.0
AmB	≤0.03–1.0	≤0.03–2.0	0.25	0.5	0.5	0.5
Fiz	≤0.125–>64	≤0.125–>64	≤0.125	0.25	16	32
Itra	≤0.03–>16	≤0.03–>16	≤0.03	0.06	0.5	1.0
Vori	≤0.03–>16	≤0.03–>16	≤0.03	≤0.03	0.25	0.5

Conclusions: Anidulafungin was highly active *in vitro* against *Candida* isolates from five European countries. These data are consistent with previous findings in smaller studies and in a large US survey.

P514 The efficiency of fluconazole and amphotericin B in comparison with echinocandin micafungin and benzothiazole APB in *Candida albicans* and *C. dubliniensis* isolated from HIV and cancer patients

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Objectives: This work studied the efficiency of the experimental antifungal agent 6-amino-2-*n*-pentylthiobenzothiazole (APB), echinocandin micafungin and the conventional antifungal drug amphotericin B in fluconazole-resistant *Candida albicans* and *C. dubliniensis*. In addition to this, the work was focused on the study of synergy effect between APB and fluconazole in both mentioned *Candida* species.
Methods: For this study, 16 fluconazole resistant *C. albicans* and *C. dubliniensis* strains were selected out of 60 originally tested for susceptibility to fluconazole. Clinical isolates were obtained from patients with diabetes, different allergy and cancer diseases and oropharyngeal candidiasis of HIV infected patients from Slovakia, Brazil, Japan and Thailand. All clinical isolates were cultivated on CHROMagar Candida. Identification was performed with commercial set API 20C AUX. The discrimination between *C. albicans* and *C. dubliniensis* was carried out by PCR assay using set primer pair (Cd-oligo2/F and Cd-oligo2/R) specific only for *C. dubliniensis*. Standard *C. dubliniensis* CBS 7987 was used as positive control. Susceptibility to fluconazole, amphotericin B, APB and micafungin was tested by broth microdilution method according to NCCLS M27-A reference method. The synergy effect of APB with fluconazole was investigated in selected clinical isolates.
Results: The antifungal activity was determined at both concentrations, MIC80 and MIC95. For fluconazole, MIC80 was observed in range from 0.25 to 8 mg/L, but MIC95 was proved higher than 64 mg/L. Benzothiazole APB showed to be less active against *C. albicans* and *C. dubliniensis* (MIC80 = 8–32 mg/L; MIC95 = 16–64 mg/L) in comparison with clinical trial amphotericin B, which was efficient at concentration range from 0.125 to 2 mg/L. Efficiency of micafungin was very high with MIC80, and MIC100 <0.031 mg/L. In this study, the synergy effect of APB with fluconazole was confirmed in several selected *C. albicans* and *C. dubliniensis*. While MIC100 for single fluconazole was over 64 mg/mL, this agent was effective in the range from 16 to 2 mg/L in combination with APB. On the contrary, MIC100 for APB was 32 mg/mL in single application, but in combination with fluconazole, it was markedly lower (8–2 mg/L).
Conclusion: Results confirmed the high efficiency of echinocandin micafungin, as well as the synergy effect between APB and fluconazole in fluconazole resistant *C. albicans* and *C. dubliniensis* strains.

P515 Evaluation of a colorimetric antifungal susceptibility test by 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride

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Objectives: Reliable susceptibility testing of pathogenic and opportunistic fungi is of growing importance. Despite the considerable progress made by using the NCCLS standard method M27-A2 for broth dilution susceptibility testing of yeasts, problems still arise when determining the minimum inhibitory concentration (MIC). One of the most important is the trailing phenomenon. 2,3-Diphenyl-5-thienyl-(2)-tetrazolium chloride (STC) is an oxidation-reduction indicator that, in the presence of growing organisms, changes from colourless to red. This indicator has not been explored for antifungal susceptibility testing. The present study aimed to develop a colorimetric method of antifungal susceptibility testing using STC.
Methods: Five type strains (*Candida parapsilosis* ATCC 22019, *C. albicans* ATCC 90028, *C. albicans* ATCC 64550, *C. krusei* ATCC 6258 and *C. tropicalis* ATCC 201380) and 19 clinical strains of *C. albicans* were used. Antifungal susceptibility tests were

performed with NCCLS-recommended broth microdilution and STC-colorimetric methods in parallel. The colorimetric method using STC was identical to the broth microdilution method with two exceptions: that STC was added to RPMI 1640-MOPS medium with antifungal agents at a final concentration of 50 μg/mL, and that the solubilising agents were added at 48 h of incubation and then the plates were incubated for 2 h. The wells with fungal growth were pink to red after addition of the agent.
Results: Among 24 strains, 18 and nine strains, respectively, demonstrated the trailing phenomenon with ketoconazole and itraconazole in the broth microdilution method. In contrast, trailing growth was not seen in the STC-colorimetric method, and, for 22 (92%) and 20 (83%) of the 24 strains, the ketoconazole and itraconazole MICs, respectively, were within two dilutions of those obtained by the NCCLS method. Furthermore, the colorimetric method allows stringent endpoint designation, and there is no difference between visual observation before extraction and visual and spectrophotometric reading after extraction.
Conclusions: The colorimetric method using STC was objective and easy to interpret and showed high levels of agreement with the NCCLS method for ketoconazole and itraconazole. I think that the STC-based colorimetric method is easily applicable for the antifungal susceptibility test.

P516 Antifungal activity of *Juniperus turbinata* on species of *Candida* and dermatophytes

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Dermatophytosis and candidosis are common superficial infections that can be found all over the world. Recently, our group demonstrates that some essential oils (*Thymus* ssp., *Origanum* ssp. and *Lippia* ssp.) can be useful as antifungal agents (1–4).
Objectives: Continuing our research on the antifungal activity of essential oils, we report now the activity of *Juniperus turbinata* leaves and berries' essential oils in order to support its application as therapeutic agents in the treatment of superficial mycoses.
Methods: Two samples of leaves oils from plants collected at Algarve (A) and at Alentejo (B) and one sample of berries oil from Alentejo (C) were assayed. Essential oils were isolated from fresh material, by water distillation (3 h) in a Clevenger type apparatus (5) and their compositions investigated by GC and GC-MS, as previously reported (6). Leaves oils are dominated by monoterpene hydrocarbons (77.1–89.4%), but quantitative important differences were found in the main compounds (α-pinene 27.8% vs. 48.2% and β-phellandrene 28.8% vs. 23.1%, respectively, for samples A and B). The main constituents of the berries oil were α-pinene (66.7%) and β-phellandrene (8.4%). Antifungal activity on *Candida* and dermatophytes strains was evaluated by determination of the minimal inhibitory concentration (MIC), according to the NCCLS protocol, M 27-A and M 38-P, respectively.
Results: Important inhibitions of the growing of dermatophytes were observed, with MIC values ranging 0.08–0.32, 0.63–1.25 and 0.32–1.25 μL/mL for *J. turbinata* oils (A, B and C, respectively). For *Candida* strains the oils have low activity with MIC values ranging 0.32–2.5, 1.25–10.0 and 5.0–20.0 μL/mL for samples A, B and C, respectively).
Conclusion: The antifungal activity of *J. turbinata* essential oils on dermatophytes, may justify future clinical trials to validate their use as therapeutic alternatives for dermatophytosis treatment.
Acknowledgements: FCT, POCTI and FEDER (POCTI/40167/ESP/2001) for financial support.
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P517 The effect of onion extract on ultrastructure of *Trichophyton mentagrophytes* and *T. rubrum*

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Dermatophytes are a specialised group of fungi able to use the keratinised tissue of skin, nail and hair as the sole nutritional source. These fungi are classified in three major genera namely *Microsporum, Trichophyton* and *Epidermophyton*. The various species in each genus are the causative agents of dermatophytosis in human and animals. Dermatophytoses usually appear as chronic infections and do not respond well to current antifungal drugs. These drugs also have numerous side effects and their continued administration causes resistance against their therapeutic effects. So there have been several attempts to discover new agents with antidermatophytic effects and less side effects. This survey was conducted to evaluate the effect of onion extract on growth and ultrastructure of two important dermatophytes *T. rubrum* and *T. mentagrophytes*. The fungi were cultured in the presence of aqueous onion extract in sabouraud dextrose broth and the cultures were incubated for 5, 10 and 15 days. Mycelial dry weight was used as the index of fungal growth rate and a portion of mycelia was processed for electron microscopy as mentioned in the *Materials and Methods*. The results showed that aqueous onion extract can inhibit the growth of *T. rubrum* and *T. mentagrophytes* in a dose and time dependent manner. This inhibition is more revealed for *T. mentagrophytes* compared with *T. rubrum* and the maximum inhibition of growth was observed for both dermatophytes in 6.25% concentration of aqueous onion extract. Study of the effect of 3% (v/v) aqueous onion extract on fungal ultrastructure showed massive changes as deformation and swelling of mycelia, disruption of the mycelial cell wall, separation of filamentous material from mycelial cell wall, severe degeneration of mycelia and conidia and disruption of intracellular organelles especially nuclei and mitochondria. These morphologic changes were also greater for *T. mentagrophytes* than *T. rubrum*. It is assumed that there are some factors in *T. rubrum* causing its resistance to antifungal agents. On the whole it can be concluded that aqueous onion extract can inhibit the growth of *T. rubrum* and *T. mentagrophytes*. This effect is probably caused by disruption and deformation of the cell wall structure and intracellular organelles. Therefore, aqueous onion extract can be used in antifungal preparations with future determination of its effective substances.

P518 Effects of 5-hydroxytryptamine on virulence properties of *Candida albicans in vitro*

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Objectives: In human beings selective serotonin reuptake inhibitors (SSRIs) modify the concentration of 5-hydroxytryptamine (5-HT) and lead to an increase of 5-HT during therapy with SSRIs. Recently, we found that 5-HT has antifungal activity against *Candida* spp. *in vitro*. Therefore, we investigated the direct influence of 5-HT against clinical isolates of *C. albicans* (CBS 5982) with regard on direct effects on virulence properties of this fungal pathogen *in vitro*. We examined the influence of 5-HT on enzymatic activity with regard to extracellular phospholipases and the production of secreted aspartyl proteinases (SAPs).
Methods: Serial dilutions from 25–0.09 mg/mL 5-HT were used for testing extracellular phospholipase activity and SAPs. The extracellular phospholipase activity of *C. albicans* was measured by the egg yolk agar method. The assay for *Candida* secreted aspartyl proteinases was assessed by a modified version of the original protocol by Ollert *et al*.
Results: An interaction between 5-HT and virulence properties of *C. albicans in vitro* could be clearly demonstrated. A significant decrease ($P < 0.05$) on phospholipase activity and SAPs of *C. albicans* at 5-HT concentrations of 25–0.09 mg/mL compared with positive control was observed. At a range of 25–12.5 mg/mL 5-HT the most strongest effect on phospholipase activity was

determined, whereby the lowest concentration of 5-HT (0.09 mg/mL) had no effect on enzymatic activity. The highest reduction on SAPs was found to be at a range of 1.56–0.78 mg/mL 5-HT compared with positive control.
Conclusion: An influence of 5-HT on both virulence factors of *C. albicans* could be clearly observed. In conclusion, further studies are required to evaluate the potential role of 5-HT in antifungal host defence.

P519 Studies on *in vitro* antimicrobial activity of vitreous substitutes against *Candida albicans*

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Objectives: Vitreoretinal surgery is one of the most rapidly developing fields of ophthalmology. However, it may also be responsible for serious blindness as a result of complications including fungal endophthalmitis. Silicon oil (PDMS 5000), perfluorodecaline (PFCL) and perfluorohexyloctane (F6H8) have been used as internal tamponading agents in vitreous surgery. The aim of the study was to evaluate and compare possible antimicrobial properties of PDMS 5000, PFCL and F6H8 *in vitro* against *Candida albicans*, which is considered one of the major causative agents of postoperative fungal endophthalmitis.
Materials and Methods: The clinical isolate of *C. albicans* was selected. The fungus was separately inoculated into PDMS 5000 (produced by AcriMed, Germany), PFCL (produced by AcriMed, Germany) and F6H8 (produced by Fluoron, Germany). Control inoculations into physiological saline and sugar broth were performed. The fungal suspensions in each vitreous substitute, physiological saline and sugar broth were diluted according to serial dilution procedure and plated in Petri dishes with Sabouraud medium. After 48 h incubation fungal CFUs were counted.
Results: *Candida albicans* CFUs decreased significantly in all used vitreous substitutes. CFUs of *C. albicans* in PDMS 5000 decreased up to the fifth day of the study; afterwards no growth was observed. Fungal growth was inhibited on the medium inoculated with *Candida* suspension in PFCL up to the third day of the study but single colonies appeared after the fifth day. For fungal inoculations in F6H8 single colonies on Sabouraud medium were present during the whole period of the study. No total elimination of the fungal growth was observed for PFCL and F6H8. CFUs of *C. albicans* declined slightly in physiologic saline. A growth pattern similar to the growth curve of microorganisms was observed in sugar broth.
Conclusion: Our study indicates that silicon oil, perfluorodecaline and perfluorohexyloctane could have antifungal properties against *C. albicans*, which is considered one of the major causative agents of postoperative fungal endophthalmitis. Additionally, silicon oil seems to be the most efficient vitreous substitute inhibiting fungal growth.

P520 Antifungal resistance patterns among oral *Candida* species from patients receiving anticancer therapy

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Objectives: Oral fungal infections are frequent complications in immunocompromised patients. This study was conducted to understand the current status of yeast resistance to available antifungal agents among patients receiving anticancer therapy.
Materials and Methods: Oral swabs were collected from 216 hospitalised patients receiving chemotherapy or radiotherapy treatment for malignant disease. No patients in this series had previous episodes of oral candidiasis or had received any prophylactic antifungal therapy. Yeast isolates were tested for their susceptibility to five antifungal agents (amphotericin B, 5-flucytosine, fluconazole, itraconazole and ketoconazole) by the commercially available E-test, using RPMI + 2% glucose + MOPS agar inoculated with 0.5 McFarland yeast suspension in saline and incubated at 35°C/ambient in bag, for 24 and 48 h. For interpretation we used NCCLS M-27-A2, 2002 recommendation.
Results: At time of sampling, 46 (21.3%) patients were found to be colonised with yeasts, of which 42 (91.3%) were *Candida albicans* and only four (8.7%) non-albicans *Candida* species. Antifungal susceptibility patterns showed that 100% of isolates were susceptible to amphotericin B (mean MICs-0.098 μg/mL), 5-flucytosine (mean MICs 0.11 μg/mL) and fluconazole (mean MICs 1.64 μg/mL) while 91.3% were susceptible to itraconazole (mean MICs 0.058 μg/mL) and ketoconazole (mean MICs 0.029 μg/mL). Of five resistant yeasts, three were non-albicans species showing simultaneous resistance to both drugs.
Conclusion: The frequency of resistant *Candida* is still very low in cancer patients at the Clinical Hospital in Rijeka. However, it is important to follow continuously the distribution and susceptibility patterns of yeasts, which should contribute in developing optimal prophylactic strategies, as well as, in reducing clinically detectable oral candidiasis in this group of patients.

Antibiotic resistance in respiratory pathogens

P521 Changes in macrolide susceptibility of oral streptococci following administration of azithromycin or clarithromycin to healthy volunteers – a prospective randomised blinded trial

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Objective: Macrolides are the treatment of choice for many community-acquired airway infections, hence emerging resistance to these antibiotics, which may be transmitted from oral streptococci to pathogens such as pneumococci, poses a public health problem. Macrolides with long half-life and high concentrations in mucosal tissues (e.g. azithromycin, AZ) may increase compliance of patients. However, whether they cause changes in susceptibility of oral streptococci to macrolides more frequently than macrolides with shorter half-life and lower tissue concentrations (e.g. clarithromycin, CL) is not known.
Methods: To detect possible development of microbial resistance of oral streptococci to macrolides we performed a prospective, randomised, evaluator-blinded trial in healthy volunteers receiving standard courses of either AZ ($n = 20$) or CL ($n = 20$). Throat swabs for isolation of streptococci were taken before treatment and on days 1, 8, 16, 32, 48, 62 and 90 of the study. Compliance to treatment was monitored by analysis of drug concentrations in urine. Todd–Hewitt broth was inoculated with the swabs and 0.1 mL were transferred to Columbia agar containing either 0.01% pyridoxal and 10 mg/L gentamicin only (Col) or pyridoxal/gentamicin plus 2 mg/L erythromycin (Col + E) for incubation. CFUs on Col and Col + E were counted for each sample. Up to 10 morphologically different colonies were collected from Col and Col + E (day 1) or Col + E only (later time points) and stored at −70°C. Three isolates from each sample were randomly chosen

for determination of MICs for AZ and CL by E-test on Columbia agar, incubated for 24 h at 37°C in 5% CO_2.
Results: After exclusion of one subject in the AZ- and two in the CL-treated group who took antibiotics before completion of the trial and correction for baseline differences, the number of macrolide-resistant streptococcal isolates (expressed by the ratio Col + E/Col) did not differ significantly between the groups. Although higher MICs of isolates from CL-treated individuals compared with those from the AZ-treated group on days 32, 48 and 62 (but not earlier or later) were observed, these differences were not significant. Two subjects from the AZ-treated group were excluded from this analysis because no growth was found on Col + E at day 0 or 90.
Conclusion: The long half-life of AZ does not induce higher rates of antimicrobial resistance in oral streptococci compared with CL with a shorter half-life and lower mucosal concentrations.

P522 Unchanged susceptibility of key respiratory pathogens to telithromycin postintroduction in Germany

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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 third year. This analysis was undertaken to track and assess the susceptibility of community-acquired RTI isolates to the ketolide antibacterial telithromycin since its introduction in Germany in October 2001.
Methods: MICs of community-acquired RTI isolates collected within Germany as part of the PROTEKT programme over three consecutive respiratory seasons (year 1: 1999–2000; year 2: 2000–2001; year 3: 2001–2002) were determined centrally by NCCLS broth microdilution methods.
Results: Between 1999 and 2002, 3580 *Streptococcus pneumoniae* isolates, 2836 *Haemophilus influenzae* isolates and 691 *Streptococcus pyogenes* isolates were collected from a total of 11 centres (seven centres in year 1; 11 centres in years 2 and 3) and MICs were determined. Results show no major change in susceptibility of any of these pathogens to telithromycin over the 3 years of follow-up, with >99% of pathogens susceptible to telithromycin using breakpoints as approved by the NCCLS Antimicrobial Susceptibility Testing Subcommittee, January 2003. Telithromycin mode MIC and MIC90 for all three respiratory seasons were, respectively, 0.008 and 0.03 mg/L for *S. pneumoniae* and 1 and 2 mg/L for *H. influenzae*. MIC ranges for telithromycin against *S. pneumoniae* in years 1, 2 and 3 were 0.004–0.5, 0.004–2 and 0.002–0.5 mg/L, respectively. MIC ranges for *H. influenzae* were 0.06 or 0.12–4 mg/L. Mode MIC for telithromycin against *S. pyogenes* was 0.015 mg/L for all 3 years, with an MIC90 of 0.03 mg/L in year 1, 0.015 mg/L in year 2 and 0.12 mg/L in year 3, and an MIC range of 0.008–8.0 mg/L in years 1 and 2 and 0.004–0.5 mg/L in year 3.
Conclusions: The ketolide telithromycin is highly active against the major bacterial pathogens implicated in community-acquired RTIs, with no change in susceptibility noted in German clinical isolates over the 3 years of the PROTEKT study despite widespread use of this antibacterial since its introduction in 2001.

P523 Prevalence of antimicrobial resistance and activity of the ketolide telithromycin against *Haemophilus influenzae* isolated from Japanese children

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Objectives: Although β-lactamase (BL) production has been the primary mechanism of β-lactam resistance among *H. influenzae*, the prevalence of BL-nonproducing ampicillin-resistant (BLNAR) strains is increasing. This analysis – undertaken as part of the PROTEKT Kids surveillance programme – assesses the prevalence of BL-positive (BL+) and BLNAR strains among *H. influenzae* isolated from paediatric patients in Japan, and the activity of the ketolide antibacterial telithromycin (TEL) against these isolates.
Methods: Isolates of *H. influenzae* were collected over two 1-week periods (24–30 November 2002 and 19–26 January 2003) from children (aged <16 years) with community-acquired respiratory tract infections (CARTIs) attending 18 centres in Japan. Isolates were tested for BL production (by the chromagenic nitrocefin method) and nonsusceptibility to ampicillin (MIC = 2 mg/L). MICs for a panel of antibacterials were determined centrally by NCCLS broth microdilution methods and interpreted using NCCLS breakpoints.
Results: A total of 272 *H. influenzae* isolates were collected, of which 7.0% (19) were BL+, 18.8% (51) were BLNAR (MIC = 4 mg/L) and 12.9% (35) were BL-nonproducing ampicillin-intermediate (MIC = 2 mg/L; BLNAI). TEL showed good *in vitro* activity against *H. influenzae* with a mode MIC and MIC90 of 2 and 4 mg/L, respectively, irrespective of BL or BLNAR/I status. Overall, 99.3% (270/272) of *H. influenzae* isolates, including 85/86 BLNAR/I and 19/19 BL+ isolates, were susceptible to TEL (MIC = 4 mg/L), with the remaining 0.7% having intermediate susceptibility (MIC = 8 mg/L) (breakpoints approved by NCCLS SAST, January 2003). A total of 238 isolates of *S. pneumoniae* were also collected in this study, of which 73.1% (174) were resistant to macrolides (erythromycin MIC = 1 mg/L). TEL maintained high *in vitro* activity against *S. pneumoniae*, with 100% of isolates susceptible to TEL at concentrations 1 mg/L.
Conclusions: Approximately 19% of *H. influenzae* isolates collected from children in Japan were BLNAR (and therefore co-resistant to a number of other antibacterials including amoxicillin–clavulanate, ampicillin–sulbactam and most first and second generation cephalosporins), with a further 12.9% BLNAI and 7% BL+. TEL demonstrated good activity against *H. influenzae*, including BL+ and BLNAR/I strains, with a susceptibility rate of >99%. TEL has high *in vitro* activity against those pathogens targeted in the empiric treatment of CARTIs in Japan.

P524 Susceptibility of recent paediatric respiratory isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* in Spain

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Objectives: Resistance to penicillin and/or erythromycin in *Streptococcus pneumoniae*, and β-lactamase (BLA) production in *Haemophilus influenzae* are well-known predictor factors for treatment failure of acute otitis media in children. It is therefore critical to monitor rates of resistance in the community in order to tailor empiric therapeutic recommendations.
Methods: A prospective, multicentre (25 hospitals in 13 Autonomous Communities, CCAA) antimicrobial survey was carried out between November 2001 and October 2002. A total of 373 consecutive *S. pneumoniae* and 438 *H. influenzae* isolates from children with community-acquired respiratory tract infections were collected and sent to a central laboratory for further processing. Susceptibility testing was performed by a semiautomated microdilution method following NCCLS M100-S12 recommendations. Breakpoints for penicillin and erythromycin were ≥2 and ≥1 mg/L, respectively. Nitrocefin test was used to detect BLA production.
Results: Excluding those CCAA with less than 18 isolates (11 pneumococcal isolates: 36% penicillin resistant and 82% erythromycin resistant; and 38 *H. influenzae*: 21% BLA producers) for the sake of accuracy, mean penicillin resistance was 20% (95% CI 14–26), whereas erythromycin resistance was 48% (95% CI 36–60) for *S. pneumoniae*, and the MLSb phenotype was dominant (90%). As for *H. influenzae*, BLA production was 14% (95% CI 9–19). Rates by CCAA are given in the table.

	S. pneumoniae			*H. influenzae*		
CCAA	N	Penicillin resistance (%)	Erthromycin Resistance (%)	CCAA	N	BLA production (%)
Andalucia	40	20%	55%	Andalucia	34	12%
Cantabria	36	17%	17%	Cantabria	37	16%
Cataluña	41	20%	42%	Cataluña	92	17%
Galicia	20	25%	55%	Galicia	19	21%
Madrid	72	25%	46%	Madrid	57	18%
PaisVasco	95	7%	47%	PaisVasco	68	4%
Valencia	30	17%	53%	Valencia	47	19%
Murcia	28	29%	68%	Aragon	29	7%

Conclusions: BLA production in paediatric respiratory *H. influenzae* was below 20% and around 15%. Currently, resistance to penicillin does not seem to keep increasing among paediatric respiratory isolates and remains around 20%. However, resistance to erythromycin among paediatric pneumococcal isolates is alarmingly high, with regions showing more than 60%. Only Cantabria had <20%. Macrolides do not provide adequate coverage against the two key bacterial pathogens involved in infantile respiratory infections in Spain and therefore, in the absence of sounding reasons, empirical prescription of macrolides should be avoided.

P525 Susceptibility of *Haemophilus influenzae* respiratory isolates from adults in Spain (2001–2002)

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Objectives: The role of *Haemophilus influenzae* (and of *Streptococcus pneumoniae*) in exacerbations of chronic bronchitis, acute otitis media and community acquired pneumonia along with their capability to produce β-lactamase (BL) are the rationale to add clavulanate to aminopenicillins, to use second generation cephalosporins or a respiratory fluoroquinolone to treat these infections. Monitoring of its rate of BL production and of the phenotype BL-negative ampicillin resistant (BLNAR) is strongly recommended.
Methods: A prospective, multicentre (25 hospitals) antimicrobial survey was carried out between November 2001 and October 2002. A total of 2207 consecutive *H. influenzae* isolates from adult patients with community-acquired respiratory tract infections were collected and sent to a central laboratory for further processing. Susceptibility testing was then performed by a semiautomated microdilution method following NCCLS M100-S12 guidelines and breakpoints against antibiotics commonly used. Chromogenic nitrocefin was used to test BL production.
Results: *beta*-Lactamase production was detected in 466 of 2207 (21.1%) isolates (95% CI 17.5–23.4). Additionally, there was 4.0% of BLNAR isolates (95% CI 2.7–6.4). Coamoxiclav, cefuroxime, cefonicid, ciprofloxacin and azithromycin displayed an excellent *in vitro* activity. In contrast, full susceptibility to cefaclor and clarithromycin was found only in 82.1 and 72.3% of isolates, respectively.
Conclusions: β-Lactamase production seems to be decreasing compared with previous surveillances done in Spain and currently stands around 20%, although there are hot spots in the south-east of the peninsula (Valencia and Murcia) with rates around 35–40% and in the north (Vizcaya) with 30%. BLNAR phenotype appears stable around 4% of isolates, but there also were centres with rates between 15 and 19% (Ciudad Real in the centre and Vizcaya) that must be closely watched. The best-tested oral anti *H. influenzae* agents from an *in vitro* point of view were coamoxiclav, cefuroxime, azithromycin and ciprofloxacin. Clarithromycin and cefaclor displayed the worst susceptibility rates and should be avoided, as better options are available.

P526 Antibiotic sensitivity of streptococci isolated in ear, nose and throat diseases – development of resistance from 1999 to 2002/2003

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Objective: Streptococci belong to the most frequent causative agents of infections in the ENT area. In recent years, resistance of this group of organisms against penicillin and macrolides has increased worldwide. It was the aim of this study to monitor the resistance of β-haemolytic streptococci, pneumococci and viridans streptococci isolated from infected patients of the ENT department of the University Hospital in Leipzig.
Methods: Since 1999, all microbiological results for patients of the ENT department were recorded according to the clinical diagnosis. In addition, MIC determinations were made for all isolates and penicillin, cefuroxime and roxithromycin. MIC values were established employing E-test strips according to the recommendations of the manufacturer. In order to study possible trends in resistance development, results of strains tested in 1999 were compared with those obtained during 2002/2003.
Results: During the study periods, altogether 262 strains of streptococci were isolated, mostly from patients with peritonsillar and neck abscesses, acute otitis media and acute sinusitis and rhinitis (1999 $n = 112$; 2002/2003 $n = 150$). Fifty-three were identified as group A streptococci, 97 were pneumococci and 112 were other viridans streptococci.
Results: All group A streptococci and 99% of the pneumococci were susceptible for penicillin (MIC $\leq$ 0125 mg/L). An increasing resistance rate (MIC $\geq$ 2 mg/L) was observed for viridans streptococci (0% in 1999 vs. 3% in 2002/2003). All streptococci tested were susceptible for cefuroxime (MIC < 2 mg/L). Interestingly, the resistance rate for group A streptococci and roxithromycin (MIC $\geq$ 8 mg/L) was decreasing (15% in 1999 vs. 4% in 2002/2003), while an increase was observed for pneumococci (4.5% in 1999 and 15% in 2002/2003) and for viridans streptococci (2.5% in 1999 vs. 10% in 2002/2003).
Conclusion: We confirmed that there is no penicillin resistance in group A streptococci. In spite of worldwide reports on rapidly increasing resistance rates for penicillin and macrolides in pneumococci, we observed only limited alterations in the resistance rate of pneumococci and other viridans streptococci. Surprisingly, a large number of viridans streptococci were recovered from patients with abscesses. The pathogenic role of these isolates requires further analysis.

P527 The comparative *in vitro* activity of moxifloxacin against respiratory tract pathogens isolated during 2003 from Libra-targeted surveillance

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Objectives: To assess the antimicrobial agent susceptibility of *Haemophilus influenzae* (HI) and *Streptococcus pneumoniae* (SP) isolates causing community-acquired respiratory-tract infections from worldwide locations during 2003.
Methods: A total of 35 centres in seven countries submitted 1530 HI and 1541 SP. Bacteria were re-identified and their susceptibility to penicillin G (PEN, SP only), ampicillin (AMP, HI only), amoxicillin-clavulanate (AMC), azithromycin (AZI), ceftriaxone (CTX), levofloxacin (LFX), gatifloxacin (GFX) and moxifloxacin (MFX) was determined using the NCCLS broth microdilution method and breakpoints at a central laboratory.
Results: All HI were fully susceptible to AMC, CTX, LFX, GFX and MFX. AMP resistance in HI was: France (43.0%), Germany (20.2%), Italy (11.3%), Spain (13.8%), Mexico (27.5%), South Africa (6.4%) and USA (34.7%). Eleven AZI nonsusceptible HI strains were found overall (0.72%). SP resistance (number of centres, number of isolates per country) is shown below. Of the 1541

SP, 293 (19.0%) were resistant to two or more of PEN, AMC, CTX, LFX and AZI (GFX and MFX were omitted to avoid duplication). These multi-resistant (MDR) SP were 98.3, 92.5, 27.3, 10.9, 3.4 and 1.4 resistant to PEN, AZI, AMC, CTX, LFX and MFX, respectively.

% resistant	AZI	PEN	AMC	CTX	LFX	GFX	MFX
France (6, 224)	46.0%	28.6%	0.9%	3.6%	1.8%	1.8%	0.5%
Germany (3, 60)	6.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Italy (2, 125)	41.6%	4.8%	0.8%	1.6%	1.6%	1.6%	0.8%
Spain (3, 197)	23.9%	22.3%	6.1%	0.0%	0.0%	0.0%	0.0%
Mexico (3, 145)	20.7%	20.7%	0.0%	0.7%	2.8%	1.4%	0.7%
South Africa (2, 128)	46.1%	45.3%	15.6%	5.5%	0.8%	0.8%	0.8%
USA (16, 662)	28.9%	24.9%	6.8%	2.1%	0.8%	0.6%	0.3%

Conclusion: For HI, resistance to AMP was prevalent in many countries but full susceptibility was seen with MFX and other agents. With SP, AZI and PEN resistance was very high in many countries. Relatively high AMC and CTX resistance was also found in South Africa. Resistance to LFX and GFX was higher than MFX, where <1% resistance was seen in all countries. Virtually all MDR SP were resistant to PEN and AZI and over 1/4 resistant to AMC. MFX was the most active agent against both HI and SP causing community-acquired respiratory tract infection worldwide including MDR SP. MFX is therefore a valuable option for the treatment of community-acquired respiratory-tract infection.

New drugs and novel therapeutic aproaches

P528 *In vitro* activity of synthetic peptides and mechanisms of resistance to colistin in pan-resistant *Acinetobacter baumannii*

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Objectives: *Acinetobacter baumannii* colistin resistant constitute a severe chemotherapeutical threat for nosocomial infection. Membrane-active antibiotic peptides were proposed as a good alternative. Among them synthetic hybrids cecropin A-melittin hybrids (CAMEs) were reported among those with highest activity.
Objectives: To test the *in vitro* activity of CAMEs against pan-resistant *A. baumannii* (AbPr) as putative alternative to colistin, and to study the mechanism of AbPr colistin resistance.
Methods: Peptides: A = CA(1–8)M(1–18), B = CA(1–7)M(2–9), C = Octanoil-CA(1–7)M(2–9) and D = CA(1–7)M(5–9). MIC/MBC (NCCLS) against 13 AbPr clinical isolates. Bactericidal activity (time-killed curves using 1 MIC, 2 MIC and 4 MIC concentrations) with four strains (NCCLS): 208628, 201630, 183280R and 183280L. LPS-CAME affinity was determined by displacement of dansylpolymixin B bound to LPS. Spheroplasts from a colistin-susceptible (ATCC19606) and two colistin-resistant (208628 and 201630) strains were prepared according to Dathe *et al*. (2002); their lysis induced by the peptide, were monitored by decrease in A450.
Results: MIC50/MIC90 (mg/L) of colistin and peptides A, B, C and D: 32/64, 4/8, 2/4, 4/4 and 4/4, respectively. MBC50/MBC90 (mg/L) of peptides A, B, C and D: 4/8, 2/4, 4/4 and 4/8, respectively. Time-killing curves: the four peptides were bactericidal against the four strains; 'A' was bactericidal with all concentrations against the four strains; 'B' and 'D' were bactericidal with all concentrations against strains 183280R and 183280L, and from concentration > MIC against strains 208628 and 201630; 'C' was bactericidal with all concentrations against strains 208628, 201630 and 183280R, and from concentration > MIC against strain 183280L. LPS affinity for CAMEs were very similar for all of them and much higher than that for colistin, independently of the strain used to obtain LPS the same resulted with permeabilisation of inner membrane. Both, peptide A and colistin, permeabilised spheroplasts at a similar rate, independently of their colistin susceptibility. Thus, the differences in colistin activity were solely located at the outer membrane, mostly to LPS changes.
Conclusions: All CAMEs were bactericidal against pan-resistant clinical isolates of *A. baumannii*, being CA(1–8)M(1–18) the best one. Resistance to colistin is due to changes in outer membrane. CAMEs act by permeabilisation of the inner membrane, and it was achieved at much lower concentration than colistin.

P529 New antimicrobial peptide active against methicillin-resistant *Staphylococcus aureus*, multi-resistant coagulase negative staphylococci, and β-haemolytic streptococci

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Objectives: The main objective was to develop antimicrobial compounds active against methicillin-resistant *Staphylococcus aureus* and multi-resistant coagulase negative staphylococci and streptococci and to elucidate the mode of action of a new antibacterial oligopeptide.
Methods: The synthesis of Cystapep has been outlined previously and was prepared from Boc-L-valinol according to the general procedure. The antibacterial activity of Cystapep was tested by agar well diffusion. For determination of MBC and MIC concentrations, a broth dilution method was used.
Results: The derivative, here called Cystapep, displayed antibacterial activity against several clinically important Gram-positive bacteria. It displayed MIC and MBC of about 16 μg/mL for both *S. aureus* and *S. pyogenes*. In radial agar diffusion assays, groups A, B, C and G streptococci as well as staphylococci were generally susceptible to the action of Cystapep, whereas pneumococci and enterococci were less susceptible. Cystapep also showed high activity against methicillin-resistant *S. aureus* (MRSA) and multi-antibiotic resistant coagulase negative staphylococci (CNS), suggesting its mechanism of action to differ from those of most currently used antibiotics.
Conclusion: Cystapep was apparently as effective against various antibiotic resistant staphylococci and streptococci as against antibiotic susceptible strains of these species. In particular, in a large collection of MRSA comprising many strains with additional resistance properties, the susceptibility to Cystapep proved invariably high. Similarly, a substantial number of CNS clinical isolates involving many multi-resistant strains also showed high susceptibility to Cystapep. Presently, these staphylococci represent leading agents in nosocomial and biomaterial-associated infections posing significant therapeutic problems due to shortage of effective antibacterial agents. In addition, the susceptibility of β-haemolytic streptococci to Cystapep may prove important due to treatment problems both for invasive and superficially located infections. Although several hundreds of isolates have been studied, we have not so far observed any strains of staphylococci or β-haemolytic streptococci resistant to Cystapep. Furthermore, the possibility to select resistant mutants by repeated passages in media containing Cystapep is currently being investigated but no resistant mutants have so far been obtained.

P530 Comparison of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans

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Objective: To evaluate the effectiveness and side effects of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans.
Methods: Consecutive adult patients with typical erythema migrans were enrolled in a prospective study on early Lyme borreliosis at the Department of Infectious Diseases in Ljubljana during 1997. Patients receiving antibiotics at their first visit, having clinical evidence of disseminated *Borrelia burgdorferi* s.l. infection, and/or being pregnant were excluded. They were randomised to receive either azithromycin 500 mg b.i.d. for the first day, followed by 500 mg once a day for the following 4 days (AZT) or amoxicillin 1000 mg t.i.d. for the first 5 days, followed by 500 mg t.i.d. for the following 10 days (AMO). Basic epidemiological data were obtained by means of questionnaires. Serum IgM and IgG antibody titre against *B. burgdorferi* s.l. were determined by IFA without absorbtion. Titres equal and/or greater than 1:256 were interpreted as positive. In all patients skin biopsy had been accomplished prior to the institution of antibiotic treatment and specimen cultured in MKP medium.
Results: A total of 133 patients, 77 (57.9%) females and 56 (42.1%) males, aged 16–83 (median 49) years were included in this study. Sixty-five patients were evaluated in AZT group and 68 patients in AMO group. No differences in epidemiological and pretreatment characteristics were present comparing the two groups. Median duration of skin lesions after the institution of treatment was 7 (1–60) days in the AZT group and 7 (2–180) days in the AMO group ($P = 0.325$). During the follow-up of 12 months none of the patients developed major late manifestations of Lyme borreliosis but in six patients severe minor manifestations appeared: in two (3.1%) from AZT group and in four (5.9%) included in AMO group. Isolation rates of *B. burgdorferi* s.l. from skin before treatment (25/65 vs. 33/68; $P = 0.319$) as well as 2–3 months after therapy (0/25 vs. 0/33) were comparable for the two groups. Three (4.6%) AZT group patients and one (1.5%) patient from AMO group reported mild gastrointestinal discomfort ($P = 0.358$).
Conclusions: Treatment of adult patients with solitary erythema migrans with two different antibiotics exhibited equal effectiveness and comparable side effects. The outcome of borrelial infection after one year was favourable in both treatment groups.

P531 Comparative *in vitro* activity of ABT-492 and six other antimicrobial agents against anaerobic bacteria

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Objectives: ABT-492 is a new quinolone active against aerobic and anaerobic bacteria involved in respiratory tract infections, urinary tract infections, blood stream infections, and skin and soft tissue infections. ABT-492, 1-(6-amino-3,5-difluoropyridine-2-yl)-8-chloro-6-fluoro-(3-hydroxyazetidine-1-yl)-4-oxo-1,4-dihydroquinolone-3-carboxy acid, is more potent *in vitro* than other new quinolones. The present investigation determined the *in vitro* activity of ABT-492 against anaerobic bacteria recently isolated from human infections. The activity was compared with that of moxifloxacin, piperacillin, cefoxitin, imipenem, clindamycin and metronidazole.
Methods: The 369 anaerobic strains investigated were isolated from respiratory tract infections, gastrointestinal infections, gynaecological infections and skin infections. All strains were identified using morphological tests, biochemical tests and gas–liquid chromatography. The antimicrobial susceptibility tests were performed by the agar dilution method according to NCCLS. The testing was performed on Brucella agar supplemented with 5 mg haemin and 1 mg vitamin K per litre and 5% laked sheep blood. The plates were read after 48 h of incubation at 37°C in anaerobic jars. Four control strains were used for monitoring the antimicrobial susceptibility tests: *Bacteroides fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124 and *Eubacterium lentum* ATCC 43055.
Results: ABT-492 and imipenem were the most active antimicrobial agents tested: Peptostreptococci (52 strains) had the following minimum inhibitory concentrations: ABT-492, range 0.008–0.25 mg/L; imipenem, range 0.016–0.064 mg/L. *Propionibacterium acnes* (32 strains): ABT-492, 0.032–0.125 mg/L; imipenem, 0.032–0.064 mg/L. *Clostridium perfringens* (50 strains): ABT-492, 0.008–0.032 mg/L; imipenem, 0.016–0.5 mg/L. *Clostridium difficile* (50 strains): ABT-492, 0.008–0.5 mg/L; imipenem, 8 mg/L. *Bacteroides fragilis* (100 strains): ABT-492, 0.032–0.125 mg/L; imipenem, 0.064–0.25 mg/L. *Porphyromonas* and *Prevotella* species (55 strains): ABT-492, 0.008–0.5 mg/L; imipenem, 0.016–0.25 mg/L. *Fusobacterium nucleatum* (30 strains): ABT-492, 0.008–0.125 mg/L; imipenem, 0.008–0.064 mg/L.
Conclusions: ABT-492 is active against anaerobic bacteria and might be useful in the treatment and prophylaxis of anaerobic infections. Clinical trials are therefore warranted.

P532 Iclaprim, a novel diaminopyrimidine antibiotic: synergy studies with different classes of antibiotics

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Objectives: Iclaprim (formerly AR-100) is a novel broad-spectrum diaminopyrimidine antibiotic that exerts its antibacterial action through the specific and selective inhibition of bacterial dihydrofolate reductase. The *in vitro* synergistic potential of Iclaprim with thirty different antibiotics was evaluated using several Gram-positive and Gram-negative pathogens.
Methods: MICs were performed using the NCCLS micro dilution methods. Pathogens used were: *Staphylococcus aureus, S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Klebsiella pneumoniae*. Checkerboard experiments were used to determine the potential synergy or antagonism with 30 antibacterial agents. Synergism was calculated using the FIC index. [Synergy was defined whereby the SigmaFIC were <0.5, indifference (no synergy or antagonism) whereby SigmaFIC was >0.5 but <4 and antagonism whereby SigmaFIC was >4.]
Results: Iclaprim showed potent MIC against most of the pathogens used in this study with MICs ranging from 0.063 to 16 μg/mL. Iclaprim was also active against Trimethoprim-resistant strains of *S. aureus* and *S. pneumoniae*. In terms of the synergistic potential, Iclaprim was highly synergistic with the two sulphonamides tested, namely sulphamethoxazole and sulphadiazine against the majority of isolates used. By contrast, Iclaprim showed no synergy with the other 28 antibiotics including macrolides, aminoglycosides, quinolones, penicillins, trimethoprim, rifampicin, tetracycline and vancomycin. Importantly, no antagonism was observed between Iclaprim and the 30 antibiotics used in this study.
Conclusions: Iclaprim was synergistic with sulphonamides and showed neither synergy nor antagonism with other classes of antibiotics.

P533 Anti-pneumocystis activity of iclaprim, a reliable therapeutic alternative against *Pneumocystis pneumonia*

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Objective: Available drugs effective against severe forms of *Pneumocystis pneumonia* (PcP) are limited; often induce adverse reactions with treatment failure common. Moreover, recent reports suggest the emergence of *P. jirovecii* resistance to sulpha drugs, the most commonly used drugs to treat pneumocystosis. The unmet medical need is that no reliable therapeutic alternative to Trimethoprim/Sulfamethoxazole (TMP/SMX) is available to

physicians; pentamidine exhibits significant toxicity and atovaquone is currently used only against mild forms of PcP. Iclaprim (formerly AR-100), a novel broad-spectrum diaminopyrimidine compound that exerts its antimicrobial activity through inhibition of dihydrofolate reductase, may represent a new therapy for the treatment of this unmet medical need. For this reason the objective of the present work was to test the activity pf Iclaprim against *P. carinii* using highly efficient *in vitro* and *in vivo* models.
Methods: The activity of Iclaprim against *P. carinii* was tested both *in vitro*, using an axenic culture system, and *in vivo*, using *P. carinii* endotracheally inoculated corticosteroid-treated rats, the most reproducible PcP model available. Animals were orally administered with Iclaprim (5, 25 and 50 mg/kg/day), Iclaprim/SMX (5/25, 25/125, 50/250 mg/kg/day), TMP (50 mg/kg/day) or TMP/SMX (50/250 mg/kg/day) once a day for 10 consecutive days.
Results: Iclaprim showed *in vitro* a high anti-*Pneumocystis* activity, with an EC50 value of 20.3 μg/mL. Iclaprim/SMX combination (proportion 1/5) showed a significant synergistic activity with an EC50 value of 13.2/66 μg/mL. TMP/SMX was the least potent compound (EC50 of 51/255 μg/mL). *In vivo*, although Iclaprim and TMP showed a similar activity, the Iclaprim/SMX combination was more potent (98.5 + 0.9% of inhibition for 25/125 mg/kg/day) than TMP/SMX (86.6 + 7.1% of inhibition for 50/250 mg/kg/day).
Conclusion: These data suggest that Iclaprim may constitute a reliable therapeutic alternative for treating severe forms of PcP.

P534 *In vitro* plasma fibrin clot models vs. *in vivo* models of experimental endocarditis: case study for diaminopyrimidine antibiotics iclaprim and trimethoprim/sulfamethoxazole

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Background: Iclaprim (ICL) is a novel diaminopyrimidine antibiotic that exhibits potent broad-spectrum activity against many pathogens including MRSA. The drug recently completed a Proof of Concept Phase II clinical trial exhibiting efficacy in human beings similar to vancomycin (VAN). This study was aimed to determine the activity of ICL against MRSA AW6 in liquid medium, in infected plasma-fibrin clots *in vitro*, and in rats with experimental endocarditis.
Methods: MICs for ICL, trimethoprim/sulfamethoxazole (TMP/SMX) and VAN were performed in MHB (under NCCLS conditions) and in MHB supplemented with 10–50% of either human or rat serum, or 0.1 U/mL of thymidine phosphorylase (TP). Plasma-fibrin clots were made with human or rat plasma-containing MRSA and treated with homologous serum containing either ICL (6 mg/L), TMP/SMX (8/40 mg/L), VAN (40 mg/L) or saline for 18 h. Rats with experimental endocarditis were treated with i.v. ICL or VAN every 12 h or TMP/SMX every 8 h at doses mimicking expected serum kinetics in human beings.
Results: In MHB, the MICs of ICL, TMP/SMX and VAN for the test strain were 0.12, 1 and 1 mg/L, respectively. However, addition of 10% of rat but not human serum, selectively increased the MIC of ICL and TMP/SMX by 2x. Addition of TP decreased MICs by 2x. Serum did not affect the MIC of VAN. In bactericidal tests (MBC), concentrations of ICL and TMP/SMX above the MIC inflicted a loss of 0.5–1 log 10 CFU in pure MHB. However, addition of 50% of human serum increased this effect to 3 log 10 CFU. In contrast, addition of 50% of rat serum totally abolished the antibacterial effect of both ICL and TMP/SMX. Addition of TP to pure MHB or to rat serum restored the bactericidal activity of both ICL and TMP/SMX. In human plasma clots, ICL and TMP/SMX significantly killed MRSA within 18 h ($P < 0.005$ vs. untreated clots), whereas they failed in clots made with rat plasma and *in vivo*. Addition of TP to clots made with rat plasma re-established drug activity. By contrast to ICL and TMP/SMX, VAN was active both in terms of clot and rat endocarditis.
Conclusions: Although ICL and TMP/SMX showed good activity in the '*in vitro* endocarditis model' such activity was absent in the *in vivo* endocarditis study in rats. These data suggest that rodent models of endocarditis are not suitable for determining the potential of diaminopyrimidine antibiotics due to significant antagonism and that *in vitro* human plasma clot models may prove to be better predictors for human use.

P535 Antimicrobial activity of the novel cephalosporin LB11058 tested against pathogens commonly associated with bacterial meningitis

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Background: The increasing prevalence of multi-drug resistance (MDR) pathogens has jeopardised the use of extended-spectrum cephalosporins for empiric treatment of community-acquired bacterial meningitis. LB 11058 is a novel cephalosporin, which has shown broad-spectrum activity and high potency against penicillin (PEN)-R streptococci. Additionally, the activity of LB 11058 against isolates from bacterial species most frequently associated with bacterial meningitis was further assessed.
Methods: A total of 573 organisms were tested, including 63 *N. menigitidis* (NM), 205 *Streptococcus pneumoniae* [SPN; 103 penicillin (PEN) nonsusceptible], 203 *Haemophilus influenzae* [HI; 100 β-lactamase (BL) producers]. LB 11058 MIC values were determined by methods recommended by NCCLS (M7-A6).
Results: LB 11058 was highly active against NM with all isolates being inhibited at <0.008 μg/mL (see table). All PEN-R SPN (MIC >2 μg/mL) were very susceptible to LB 11058 (MIC90, 0.12 μg/mL; range 0.06–0.25 μg/mL). LB 11058 was eightfold to 16-fold more potent than ceftriaxone (CRO) or cefepime (CPM) against PEN-I and PEN-R strains. LB 11058 activity against HI (MIC90, 0.25–0.5 μg/mL) was not affected by BL production, and it was similar to that of CPM (MIC90, 0.12–0.25 μg/mL), but inferior to CRO (MIC90, 0.008–0.015 μg/mL).
Conclusions: LB 11058 showed excellent activity against the most significant pathogens causing community-acquired bacterial meningitis. This compound may represent an excellent option for empiric therapy of this infection, especially in areas with high rates of β-lactam resistance among SPN.

P536 Gram-negative bacteria producing characterised β-lactamases: *in vitro* activities of BAL 9141 and comparators

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Objectives: BAL 9141 is the first of a new class of anti-MRSA cephalosporins, which also possesses broad activities towards most clinically relevant bacterial pathogens. This study aimed at evaluating *in vitro* activities of BAL 9141 and comparators towards β-lactamase-producing Gram-negative bacteria.
Methods: 57 strains (33 *Escherichia coli*, 10 *Klebsiella pneumoniae*, nine *Pseudomonas aeruginosa*, three *Klebsiella oxytoca*, one *Proteus mirabilis* and one *Aeromonas hydrophila*) with well-characterised β-lactamases were studied. Two strains produced more than one β-lactamase. MICs of BAL 9141, cefepime, ceftazidime, ceftriaxone, aztreonam, piperacillin, piperacillin-tazobactam and imipenem were determined by broth microdilution according to NCCLS guidelines.

Table 1. *In vitro* activity of LB1 1058 and selected agents against invasive NM strains

Antimicrobial	MIC_{50}	MIC_{90}	Range
LB1 1058	≤0.008	≤0.008	≤0.008
Ceftriaxone	≤0.25	≤0.25	≤0.25
Penicillin	≤0.015	0.25	≤0.015-0.25
Gatifloxacin	≤0.03	≤0.03	≤0.03
Rifampin	≤0.25	≤0.25	≤0.25

Results: The MICs of BAL 9141 for strains producing β-lactamases of groups 1 (CMY-2, CMY-7, DHA-1, FOX-1, FOX-2 and LAT-1), 2b (HMS-1, LXA-1, SHV-1, TEM-1, TEM-2 and TEM-90) and 2br (TEM-30 to TEM-36) as defined by Bush *et al*. (1) ranged from 0.06 to 2 mg/L. However, the MIC of LAT-2 producing *K. pneumoniae* strain N10 was 32 mg/L. BAL 9141 displayed inconsistent activity against extended spectrum β-lactamase (ESBL)-producing strains (group 2be β-lactamases) of *E. coli*, *Klebsiella* and *P. mirabilis*. The MICs of BAL 9141 for *E. coli* strains harbouring ESBLs SHV-2, SHV-4, SHV-5, TEM-3, TEM-21, or TEM-50 as well as those of *P. mirabilis* strain 33 producing TEM-52 and *K. oxytoca* hyperproducers of K1 β-lactamase (range of MICs 8–64 mg/L) exceeded those for *E. coli* strains producing ESBLs SHV-3, TEM-5 to TEM-10, or TEM-20 (range of MICs 0.06–2 mg/L). BAL 9141 had also inconsistent activity against *E. coli* producing OXA enzymes. MICs were 4–8 mg/L for OXA-5 and OXA-7 compared with 0.06–0.125 mg/L for OXA-1 to OXA-4. *Pseudomonas aeruginosa* strains producing carbenicillin- or oxacillin-hydrolysing enzymes (group 2c and 2d) were associated with MICs ranging from 1 to 64 mg/L, whereas an MIC of 0.06 mg/L was recorded for the *Aeromonas hydrophila* strain producing AER-1. In general, MICs of BAL 9141 were comparable with those of aztreonam.
Conclusions: BAL 9141 displayed good activity against Gram-negative bacteria producing various types of β-lactamase. In addition to its anti-MRSA activity, BAL 9141 maintains the potent activity of third generation cephalosporins and aztreonam.

Reference

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P537 MIC determination of the anti-pneumococcal activity of BAL 9141 compared with other agents

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Background: Pneumococcal drug resistance has become a worldwide problem.
Objective: This study examined the anti-pneumococcal activity of BAL9141, a new broad-spectrum intravenous cephalosporin, compared with those of amoxicillin, imipenem, ertapenem, cefepime, ceftriaxone, cefotaxime, cefuroxime, cefdinir, levofloxacin, moxifloxacin, azithromycin, clarithromycin, linezolid, quinupristin/dalfopristin, daptomycin, vancomycin, teicoplanin and telithromycin. Strains included 30 PSSP, 60 PISP and 209 PRSP ($n = 299$); of these, 152 (51%) were macrolide-resistant and 39 (13%) were levofloxacin-resistant (both groups with defined resistance genotypes).
Methods: Agar dilution MIC with cation-adjusted Mueller–Hinton agar + 5% sheep blood and inocula of 104 CFU/spot were used. The following MIC50/MIC90 values (μg/mL) were obtained:

Drug	Pen S	Pen I	Pen R	Macrolide R	Quinolone R
BAL9141	0.016/0.016	0.06/0.5	0.5/1	0.5/1	0.5/1
Amox	≤0.016/0.03	0.25/2	2/8	2/8	2/4
Imipen	≤0.004/0.03	0.03/0.12	0.25/0.5	0.25/0.5	0.25/0.25
Ertapen	≤0.004/0.12	0.12/0.5	0.5/1	0.5/1	0.5/1
Cefipime	0.03/0.12	0.5/1	1/2	1/2	1/2
Ceftriax	0.016/0.12	0.25/1	1/2	1/4	1/4
Cefurox	0.016/0.25	0.5/4	4/16	4/16	4/16
Cefotax	0.016/0.03	0.25/1	1/2	1/2	1/2
Cefdinir	0.06/0.25	0.5/8	8/16	8/16	8/16
Levo	0.5/32	1/2	1/4	1/2	16/32
Moxi	0.06/4	0.12/0.25	0.12/0.5	0.12/0.5	2/8
Azithro	0.06/>64	0.25/>64	4/>64	>64/>64	0.12/>64
Clarithro	≤0.016/>64	0.06/>64	1/>64	>64/>64	0.06/>64
Linez	0.25/2	1/2	1/2	1/2	1/2
Quin/Daf	0.12/1	0.5/1	0.5/1	0.5/1	0.5/1
Dapto	0.06/0.25	0.12/0.25	0.12/0.25	0.12/0.25	0.12/0.25
Vanco	0.12/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5
Teico	≤0.016/0.12	0.03/0.06	0.06/0.12	0.06/0.12	0.06/0.12
Telithro	0.008/1	0.03/0.12	0.03/0.5	0.12/1	0.016/0.25

Although MICs of all β-lactams rose with those of penicillin G, BAL9141 had the lowest MICs of all cephalosporins tested. Using NCCLS IV cephalosporin nonmeningeal pneumococcal breakpoints, 98.3% of strains were S, 1.3% I, and 0.3% R to BAL9141; compared with 73.2% S, 20% I, and 6.7% R with ceftriaxone and 70.9% S, 24% I, and 5% R with cefepime. All strains were S to quinupristin/dalfopristin, daptomycin, vancomycin and teicoplanin, and 93.3% S to telithromycin (breakpoint 0.5 μg/mL).
Conclusions: BAL 9141 had the lowest MICs (similar to carbapenems) of all IV cephalosporins tested against pneumococci irrespective of a strain's β-lactam, macrolide or quinolone susceptibility.

P538 Efficacy and safety of pharmacokinetically enhanced amoxicillin/clavulanate 2000/125 mg in adult patients with acute bacterial sinusitis in Hungary

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Objectives: Recent increases in the prevalence of antimicrobial resistance among common respiratory pathogens have caused concern worldwide. Pharmacokinetically enhanced amoxicillin/clavulanate 2000/125 mg was designed using pharmacokinetic/pharmacodynamic principles to achieve eradication of common respiratory pathogens, including penicillin-resistant *Streptococcus pneumoniae* (PRSP, penicillin MICs ≥2 mg/L) with amoxicillin ± clavulanate MICs of up to and including 4 mg/L.
Methods: In this multicentre, international, open-label, noncomparative study, patients with acute bacterial sinusitis (ABS) were given amoxicillin/clavulanate 2000/125 mg as two 1000/62.5 mg tablets twice daily for 10 days. Diagnosis of ABS was based on clinical and radiological findings. Patients were required to have sinus aspiration for bacteriological assessment at screening and, for patients in whom treatment failed, at the time of treatment failure. Treatment success was based on eradication of the initial infecting pathogen or, in the absence of an evaluable repeat sample, clinical evidence of eradication. Data from a subset of patients recruited in Hungary are presented here.
Results: A total of 222 patients received study medication. The mean age of patients was 40.2 years and the majority of patients were female (59.5%). Overall, 127 isolates were cultured from 109 patients [bacteriology intent-to-treat (bITT) population]. *Streptococcus pneumoniae* was the most frequently isolated pathogen, identified in 40.4% (44/109) of patients in the bITT population. Success in the bITT population at follow-up (days 17–28, primary efficacy endpoint) was 87.2% (95/109). Of 14 patients who were not successes, seven were confirmed bacteriological failures and seven had a response of 'unable to determine'. In patients with *S. pneumoniae* infection, 93.2% (43/44) were successes at follow-up. Two patients had PRSP identified at screening, and both patients were successes at follow-up. Adverse events (AEs), due to any cause, were reported by 25.2% (56/222) of patients. Diarrhoea was reported as an AE by 12.6% (28/222) of patients. The majority of AEs were mild to moderate in severity. Only 2.7% (6/222) of patients withdrew from the study due to AEs.
Conclusion: Amoxicillin/clavulanate 2000/125 mg was highly effective in treating patients with ABS, particularly cases caused by *S. pneumoniae*, including two patients with PRSP infection, and was generally well tolerated.

P539 An *in vitro* evaluation of the antimicrobial activity of a novel fluoroquinolone

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Background: Since the introduction of the fluoroquinolones, ciprofloxacin and ofloxacin over 10 years ago, there has been a small but significant increase in the number of resistant clinical

isolates of species previously susceptible to these agents. More recent fluoroquinolones have better activity against Gram-positive species while retaining activity against Gram-negative species. The comparative *in vitro* activity of the novel fluoroquinolone WCK 1152A was determined against a range of clinical isolates.
Methods: MICs were determined for 1007 clinical isolates, the majority using the NCCLS agar dilution method. The microbroth method was performed when testing isolates of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*.
Results: MICs of WCK 1152A, moxifloxacin and ciprofloxacin for respiratory tract pathogens are summarised below. Generally, the *in vitro* activity of WCK 1152A was similar to that of moxifloxacin. Against isolates of *S. pneumoniae* of defined genotype, and 'atypical' respiratory pathogens, WCK 1152A was some twofold to fourfold more active than moxifloxacin.
Conclusion: Improved activity against multi-resistant isolates of *S. pneumoniae* and 'atypical' pathogens indicate a potential role for WCK 1152A in the treatment of respiratory tract infections.

	MIC(mg/L					
	WCK1152A		Moxifloxacin		Ciprofloxacin	
Species (n)	50%	90%	50%	90%	50%	90%
S. pneumoniae multi-resistant (86)	0.03	0.06	0.12	0.12	1	2
S. pneumoniae FLQ I/R (51)	0.25	1	2	4	16	64
S. pyrogenes(20)	0.06	0.06	0.12	0.12	0.5	0.5
H. influenzae (24)	0.015	0.03	0.015	0.03	0.0008	0.015
M. catarrhalis (14)	–	0.06	–	0.06	–	0.06
L. Legiomella spp. (25)	0.015	0.03	0.03	0.03	0.015	0.015
M. pneumoniae (12)	–	0.03	–	0.12	–	2
C. pneumoniae (5)	range 0.03–0.06		range 0.12–0.25		All at 4	

P540 Intracellular penetration and activity of cethromycin against *Legionella pneumophila* in a monocytic cell line (Mono Mac 6)

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Objectives: Antimicrobial intracellular penetration and activity are important parameters in the therapy of infections due to intracellular pathogens. The penetration of the ketolide cethromycin into the mature monocytic cell line Mono Mac 6 and its intracellular activity compared with telithromycin against *Legionella pneumophila* was evaluated.
Methods: Uptake of radiolabeled cethromycin by Mono Mac 6 cells was determined by a radiometric assay. The cells were incubated with different extracellular concentrations of cethromycin (1–25 mg/L) and after different incubation times separated by a velocity gradient centrifugation technique. Extracellular susceptibility (MIC) was determined by microdilution susceptibility testing in BYEalpha agar after 48 h of incubation. The activity of cethromycin and telithromycin against intracellular *L. pneumophila* ATCC 33152 into Mono Mac 6 cells in a 48 h assay was also evaluated. Mono Mac 6 cells containing intracellular *L. pneumophila* were incubated with different ketolide extracellular concentrations (0.1–10 mg/L). After 48 h of incubation, intracellular bacteria were released and plated on BCYEα agar.
Results: The uptake of cehromycin by Mono Mac 6 cells was rapid and not saturable. At extracellular concentration of 2 mg/L, the intracellular concentration of cethromycin was 40 times higher than extracellular one. Cethromycin was rapidly released from loaded Mono Mac 6 cells (after 30 min incubation in antimicrobial-free medium, only 20% of accumulated-drug remained cell associated). MIC of cethromycin and telithromycin against *L. pneumophila* ATCC 33152 were 0.008 and 0.015, respectively. At the extracellular concentrations evaluated, cethromycin impaired significantly the intracellular growth of *L. pneumophila*. At an extracellular concentration of 0.1 mg/L (10XCMI), the percentage of bacterial inhibition for cethromycin was 90%. Only at higher extracellular concentrations (around 1 mg/L), a similar bactericidal effect against intracellular bacteria was achieved by telithromycin.
Conclusions: Cethromycin penetrates into the mature monocytic cell line Mono Mac 6, reaching high intracellular concentrations, while it remains active intracellularly. The intracellular activity of cethromycin against *L. pneumophila* was significantly higher than that of telithromycin.

P541 Phase 2 trial comparing four regimens of oritavancin vs. comparator in the treatment of patients with *S. aureus* bacteraemia

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Objectives: The efficacy and safety of four dose regimens of oritavancin, an investigational semisynthetic glycopeptide with bactericidal activity *in vitro* against gram-positive pathogens, was evaluated in the treatment of patients with *S. aureus* bacteraemia.
Methods: In this Phase 2, open-label, randomised trial, patients with *S. aureus* bacteraemia were randomised to receive oritavancin (5, 6.5, 8, or 10 mg/kg IV qd for 10–14 days) or comparator (vancomycin 15 mg/kg IV q12 h for 10–14 days or a B-lactam agent if the organism was sensitive). Efficacy and safety were assessed at End of IV Therapy, Early Follow-Up (EFU, 5–12 days post-therapy) and Late Follow-Up (LFU, Day 42). The primary endpoint was the composite (combined clinical and bacteriologic) outcome in the Evaluable population (EVAL) at EFU.
Results: 125 patients enrolled; 123 received study drug (ITT population) and 84 met criteria for the EVAL population. The efficacy data for the EVAL population at EFU are presented. Composite outcome success rates for the 5, 6.5, 8, and 10 mg/kg oritavancin groups were five of six (83%), five of seven (71%), 16 of 24 (67%), and 16 of 20 (80%), respectively, and for the comparator group, 19 of 27 (70%). Clinical cure rates were five of six (83%), five of seven (71%), 17 of 24 (71%), 16 of 20 (80%) for oritavancin and 20 of 27 (74%) for comparator. Bacteriologic eradication rates were five of six (83%), six of seven (86%), 19 of 24 (79%), 17 of 20 (85%) for oritavancin and 21 of 27 (78%) for comparator. Rates of deaths, SAEs, AEs, and discontinuations from IV therapy because of AEs were similar across all treatment groups. The most common AEs seen in the oritavancin vs. comparator groups, respectively, were diarrhoea (17% vs. 19%), injection site phlebitis (19% vs. 14%), pyrexia (19% vs. 14%), hypotension (16% vs. 16%), vomiting (10% vs. 22%), nausea (12% vs. 14%), and hypokalaemia (10% vs. 13%). No increase in AEs was seen with increasing dose levels of oritavancin.
Conclusions: Oritavancin for 10–14 days was as effective as comparator in the treatment of patients with *S. aureus* bacteraemia with higher Clinical, Bacteriologic and Composite outcomes seen in the 10 mg/kg cohort. Safety results were comparable across all treatment groups with no evidence of increasing AEs with increasing dose levels of oritavancin.

P542 Linezolid in the treatment of skin and soft tissue infections caused by methicillin-resistant *Staphylococcus aureus*

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Objectives: Linezolid, the first member of a new class of synthetic antibacterial agents (the oxazolidinones), has demonstrated Gram-positive spectrum of activity, involving all strains of staphylococci, including methicilin resistant *Staphylococcus aureus* (MRSA) and methicilin resistant *Staphylococcus epidermidis* (MRSE), enterococci, including vankomycin resistant enterococci (VRE), and pneumococci (including penicillin-intermediate and penicillin-

resistant strains). To assess the efficacy, safety and tolerance of intravenously and orally administered linezolid in the treatment of patients with documented MRSA skin and soft tissue infections (SSTI) our department participated in the international multicentric clinical trials.
Methods: The study was conducted in 16 patients with confirmed MRSA surgical SSTI. Patients were randomised to receive one of the following regimens: linezolid iv 600 mg every 12 h for the entire treatment period or switched to linezolid orally 600 mg every 12 h; vancomycin iv 1 g every 12 h for the entire treatment period (dose may have been adjusted by serum level determination to maintain therapeutic level) Along with linezolid the surgical procedures were performed, when necessary. Clinical and microbiological assessments were performed throughout the study. After completion of therapy all patients were short-term followed-up.
Results: The treatment with linezolid resulted in resolution of clinical signs and symptoms as well as laboratory signs of infection in all patients enrolled. No serious adverse events related to the medication were noted. The efficacy of linezolid was comparable with that of vancomycin, while its microbiological success rate at the end of treatment was significantly higher and on the follow-up.
Conclusion: Our trials suggest that linezolid is an effective, safe and well-tolerated option for the treatment of SSTI caused by methicilin resistant *Staphylococcus aureus*. It may be regarded as a valuable antimicrobial agent to control the increasing occurrence and spread of infections caused by resistant Gram-positive strains or in patients who cannot tolerate standard antimicrobial therapy.

P543 Linezolid tolerance among multiresistant nasopharyngeal isolates of *Streptococcus pneumoniae*

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Objectives: Linezolid, a synthetic compound belonging to a new class of antibiotics called the oxazolidinones, shows antipneumococcal activity. Nasopharyngeal isolates of *S. pneumoniae* from healthy children are useful for predicting antimicrobial susceptibility of pneumococci in a given population. We assess *in vitro* activity of linezolid against multiresistant strains of *S. pneumoniae* isolated from nasopharynx of healthy children.
Methods: 58 strains of *S. pneumoniae* were tested, including 22 isolates sensitive to penicillin (PSSP) and 36 – relatively resistant to penicillin (RRSP). All strains were resistant to at least three antimicrobials, including erythromycin, clindamycin, tetracycline, chloramphenicol or cotrimoxazole. Minimal inhibitory concentraion (MIC) and minimal bactericidal concentration (MBC) values for linezolid were determined by broth microdilution method. The bacteriostatic or bactericidal effects of linezolid were assessed by a time-kill assay. Sensitivity of isolates to optochin and their bile solubility were tested by conventional methods.
Results: Linezolid inhibited all isolates tested with MICs ranging from 0.25 to 1 mg/L, showing similar activity against PSSP and RRSP strains. This indicates that all strains, including those resistant to all antimicrobials used, were sensitive to linezolid. Although the majority of the tested isolates were sensitive to bactericidal effect of linezolid with MBCs = 8 mg/L or less, three of them (1 RRSP, 2 PSSP) were killed at high concentrations of linezolid – MBC = 16 or 32 mg/L and had a high MBC/MIC ratios 32 or 64. Linezolid tolerance was confirmed by monitoring viability of these strains during exposure to 20 mg/L of this antibiotic, that is similar to its maximal serum concentration after standard dosing. All tolerant strains were sensitive to optochin and lysed with desoxycholate, which indicates autolysin production. The prevalence of linezolid tolerance among the tested multiresistant pneumococci was 5.17%.
Conclusions: Although it has not been found linezolid resistance in *S. pneumoniae*, some pneumococcal strains were insensitive to bactericidal effect of this antibiotic. Linezolid tolerance in clinical isolates may represent a potential risk, especially in patients with suppressed immune response.

P544 New penams (6-APA) and cephems (7-ACA) with bulky T-shaped side-chains: synthesis and antibiotic activities

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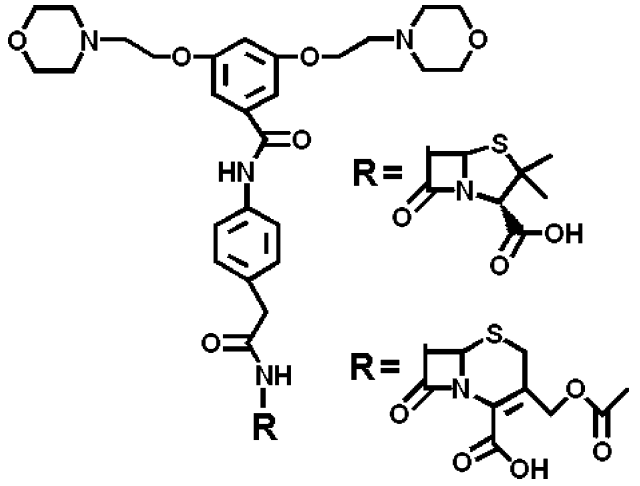
Objectives: Discovered more than 50 years ago, beta-lactam antibiotics remain of large interest because of their potentially large spectrum and low intrinsic toxicity. A large number of semi-synthetic derivatives have been obtained, but few have explored the possibility to use bulky side chains in an attempt to more fully block the catalytic crevice in PBPs. In this context, we have synthesised penam and cephem derivatives bearing two morpholine rings attached to their side-chains. We report here on two typical compounds (DEMO-Pen and DEMO-Cef; see structures in the Figure) modelled after benzylpenicillin and the corresponding methoxy-acetyl-cephem.
Methods: DEMO-Pen and DEMO-Cef were formed via the mixed anhydride method. The whole side chain was first obtained by bisalkylation of methyl 3,5-dihydroxybenzoate with N-chloroethylmorpholine, and made into a pentafluorophenyl ester. The latter was coupled with 6-APA and 7-ACA to give the corresponding penam and cephem derivatives. MIC's were determined by common agar dilution method in comparison with ampicillin and cefadroxil against both collection strains and clinical isolates.
Results: The structures and stabilities of DEMO-Pen and DEMO-Cef were confirmed by 1H NMR, 13C NMR, IR, and high-resolution mass spectrometry. DEMO-Pen was active against Gram (+) organims (*S. aureus, S. pneumoniae, S. pyogenes*). DEMO-Cef was more active (8x) than cefadroxil against *S. pneumoniae.*



Conclusion: Bulky side-chains do not prevent penams and cephems to reach the active site of PBPs and may provide increased activity. This opens the way to renewed efforts in the synthesis of novel beta-lactam derivatives.

P545 *In vitro* and *in vivo* activities of novel compounds active against multiresistant strains of *Mycobacterium tuberculosis*

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Objectives: During the past two decades the WHO indicated an increasing number of TB patients infected by strains of *M. tuberculosis* resistant to most of the available drugs. In contrast it has been nearly 30 years since the introduction of a novel compound for the treatment of TB. We synthesised series of analogues of our newly discovered class of antimycobacterial compounds to enhance *in vitro* and *in vivo* activity by structure activity relationship studies.
Methods: Molecules were derived by specific methods of classical synthesis. *In vitro* activity was determined in a first screen against

a variety of microorganisms (Gram-positive and Gram-negative bacteria, yeast, fungi) including fast growing mycobacteria, than against sensitive strains and drug resistant clinical isolates of *Mycobacterium tuberculosis*. *In vivo* activity was tested in a murine model of TB infection. Acute and chronic toxicity were checked in mice.
Results: While starting compounds were active against Gram-positive bacteria, mycobacteria, fungi and yeast, in the course of the investigations activity was focused on bacteria and mycobacteria. Various analogues demonstrated high *in vitro* activity against *M. tuberculosis* including clinical isolates and MDR strains. MIC's of the most advanced compounds for *M. tuberculosis* H37Rv and clinically isolated MDR strains were <0.78 to <0.063 μg/mL. The compounds were therapeutically active after oral application in mice infected with *M. tuberculosis* H37Rv with 100% survival rates. The LD50 in mice after oral application was >500 mg/kg for all compounds. Additionally, synthesis of the compounds is efficient and inexpensive.
Conclusions: Considering the activity of the novel compounds against MDR strains of *M. tuberculosis* the mechanism of action must be different to that of the existing therapeutics. Because of the narrow spectrum of activity and the low toxicity this new class of antimycobacterial compounds represents a promising lead candidate for low cost drugs to overcome MDR-TB with reduced side effects.

P546 Basidiomycete metabolites attenuate virulence properties of *Candida albicans in vitro*

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Objective: The previously used stratagem of targeting a singular cellular metabolic or biosynthetic process in antifungal drug design has proven inadequate, especially with regard to the increasing drug-resistance. We therefore studied the recently discovered basidomycete protease inhibitors Aureoquinone and Laccaridiones A and B for their ability to block fungal adhesion and to inhibit candidial secreted aspartate protease (Sap) release.
Methods: Inhibition of adhesion was tested on endothelial and epithelial cells, Sap antigen concentrations by specific ELISA and their activity by enzymatic cleavage of bovine serum albumin.
Results: The inhibition of *C. albicans* adhesion to the epithelial cell line Hela S3 was shown to be dose dependant and highly significant (24% inhibition with Aureoquinone, 35% with Laccaridione A and 56% with Laccaridione B at 10 μg/mL, respectively), clearly marking Laccaridione B as the front runner. The inhibitory effect of Laccaridione B on candidial adherence to the endothelial cell line EAhy 926 was an even greater (highly significant 66%). Concerning Sap-release Laccaridione B also proved to be the most effective among the substances tested, showing a significant 50% reduction in concentration and a reduced activity at 10 μg/mL. The inhibitory effects observed were shown to be the result of an inhibition of Sap-production and/or -release and not due to a direct interaction of the basidiomycete metabolites with Sap. For both Sap-release and activity a single application of the drug resulted in a much less pronounced effect than regular drug addition over a period of 8 days.
Conclusion: Animal studies will show whether Laccaridione A and especially Laccaridione B, or derivatives thereof, may also represent potent antifungal drugs *in vivo* targeting fungal virulence factors such as adhesion and protease release, without being fungicidal.

P547 Search and assessment of novel substances active against Epstein–Barr virus

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Objectives: The aim of the study was to assay the anti-EBV activity of several substances prepared from the raw material of the plant origin, namely Proteflasid in the miscellaneous modified forms (drug ^{1}1 and drug ^{1}2). Proteflasid (Ecopharm Research and Production Company, Kyiv) represents the quercetin-containing herbal extract of wild grasses *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L.
Methods: PCR. An inhibition of reproduction of EBV in cell culture by Proteflasid was determined by reduction of number of genome equivalents of EBV DNA on a cell in treated vs. untreated cells. To determine it, a quantitative PCR was applied using primes and reagents of 'AMPLY-Senc-100R' (Russia) and programme 'Biotest A'.
Results: The search for the novel antiviral substances active against Epstein–Barr virus (EBV) is a topical problem since the persistent EBV infection alters immune status promoting the development of adenocarcinomas and lymphoproliferative diseases. Moreover, EBV like other herpesviruses affects central and peripheral nervous system being involved in the pathogenesis of meningoencephalitis, arachnoencephalitis and meningitis. Anti-EBV activity of the substances was assessed in EBV-infected lymphoblastoid Raji cells. The substances were assayed within broad concentration ranges. The activity of drugs was studied at its depositing simultaneously with to infected, 24 h prior to infected and in 24 h after to infected. The maximally tolerable concentrations for the cell line being assayed amounted to 150 mg/mL. Is rotined, that the depositing drug ^{1}2 in a syrup gives the best outcomes as contrasted to by drug ^{1}1 – about it the values chemotherapeutic indices of an indices testify. Specially good outcomes were obtained on medical operating preparations in two solvents chemotherapeutic indices 350 (1) and 1250 (2). But also preventive the processing lymphoblastoid of cages by a drug (24 h prior to contamination) gives high enough parameters – 250 (1) and 400 (2). The inhibition of EBV reproduction was assessed by PCR technique estimating the number of EBV DNA genomic equivalents. Minimal effective concentrations amounted to 0.1 mg/mL for Proteflasid for both modifications.
Conclusion: To summarise, the substances under study possess the significant anti-EBV activity and may be the advantageous for the therapy of EBV-associated diseases.

P548 A phase 2 study of the toxin binding polymer tolevamer in patients with *C. difficile* associated diarrhoea

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Background: Tolevamer sodium is a novel, nonabsorbed, nonantibiotic polymer that binds *C. difficile* toxins A and B, and is being developed to treat *C. difficile*-associated diarrhoea (CDAD). *C. difficile* is the most common cause of infectious nosocomial diarrhea, affecting ~1% of hospitalised patients. *C. difficile* proliferates when normal colonic flora are altered, typically by antibiotics. Pathogenic strains of *C. difficile* produce two toxins, A and B, that induce colonic inflammation and fluid loss. CDAD treatment with metronidazole or vancomycin also disrupts normal flora, and CDAD recurs in 20% of treated patients. We have completed a phase 2 trial demonstrating that tolevamer and vancomycin have similar efficacy in the treatment of mild-moderate CDAD.
Primary objective: To demonstrate noninferiority vs. vancomycin with respect to time to resolution of diarrhoea (TTROD).
Methods: A randomised, double-blind, double-dummy, active-controlled phase 2 trial was conducted to determine the safety and efficacy of monotherapy with 1 or 2 g tolevamer TID vs. a standard oral dose of 125 mg vancomycin QID. 289 patients with a first episode or recurrent CDAD were enrolled at 58 US, UK and Canadian sites.
Results: Preliminary data showed that the median TTROD in the per protocol population (PP) was 2.0 days with vancomycin, and 2.5 days with 6 g tolevamer. Noninferiority testing demonstrated that these response times were comparable ($P = 0.015$), and that the 6 g tolevamer TTROD risk ratio relative to vancomycin was 0.98 (95% CI: 0.68–1.41). In the ITT population, the median TTROD was 3.0 days for both groups. There was no statistically significant difference in TTROD between vancomycin and 6 g tolevamer in either primary or recurrent CDAD patients. The 3 g TTROD was 4.0 days, showing a tolevamer dose response. In the

PP, the definitive recurrence rate (DRR: confirmed by a positive toxin assay) was 19% with vancomycin and 10% with 6 g tolevamer, but this advantage did not achieve statistical significance ($P = 0.185$). In the PP with recurrent CDAD at enrollment, the DRR was 27% with vancomycin and 0% with 6 g tolevamer ($P = 0.07$). Overall rates of serious and nonserious adverse events were similar between groups.
Conclusion: Tolevamer dosed at 6 g/day rapidly resolved CDAD similarly to vancomycin and demonstrated a trend towards reduced recurrence. Tolevamer may provide a nonantibiotic alternative for the treatment of this antibiotic induced disease.

P549 A novel antimicrobial system to treat methicillin-resistant and glycopeptide-resistant staphylococci

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) pose a worldwide public health problem. It has long been a major cause of nosocomial infections and strains causing serious community-acquired infections have recently emerged. MRSA and indeed coagulase negative staphylococci (CNS) are often multidrug resistant and can rapidly acquire resistance to new antimicrobials, such as linezolid, making infections difficult to treat, costly and life-threatening. Hence, there is a real need to develop alternative treatments. We have previously shown that MRSA strains can be killed by photodynamic therapy (PDT) using targeted photosensitizer conjugates. We describe the use of non-strain-specific phage-photosensitizer conjugates to target and kill MRSA, glycopeptide resistant MRSA and CNS.
Methods: The *S. aureus* phage 75 was conjugated to the photosensitizer tin (IV) chlorin e6. The conjugate was then added to a suspension of MRSA and this was exposed to red light (l 633 nm). This activated the photosensitizer, producing highly reactive singlet oxygen which can exert a bactericidal effect. Following irradiation, survivors were enumerated.
Results: 99.99% of MRSA in suspensions containing 1×10^7 cfu/mL were killed by the treatment using a conjugate/bacteria ratio of 1:1. Furthermore, the phage-photosensitizer conjugate could kill bacteria in exponential and stationary phases of growth. By increasing the ratio of conjugate to bacteria to 10:1, it was possible to achieve 100% kills. Controls showed that at the concentrations used phage, photosensitizer or laser alone did not produce significant kills. We were also able to kill Glycopeptide resistant MRSA and CNS. Calcium neutralisation showed that these effects were because of nonspecific phage adhesion.
Conclusions: The phage-photosensitizer conjugate was very effective at killing multidrug-resistant MRSA and CNS. Because *Staphylococcus aureus* phages have the ability to adsorb to any staphylococcus, they make ideal targeting systems for PDT. Therefore, phage-targeted PDT is an excellent candidate for a new treatment against such infections. By selecting different phages and photosensitizers, it may be possible to use this technique against many species of bacteria.

P550 Novel 'ethiological' therapies for AIDS-related Kaposi's sarcoma, and possible laboratory monitoring

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HAART changed the natural history of HIV disease, but AIDS-related malignancies still occur, including Kaposi sarcoma (KS), whose multifactorial pathogenesis include a definite pathogenetic role of HHV-8, HIV infection itself, and underlying immunodeficiency. Five consecutive male homosexual patients (p) diagnosed with AIDS because of cutaneous disseminated and/or visceral KS, received HAART combined with an alternating weekly schedule of liposomal doxorubicin or daunorubicin, and the antiviral drug cidofovir at 5 mg/kg (preceded by probenecid administration). Our p received four to 13 cycles of alternating chemotherapy-cidofovir administered every other week, which led to a significant improvement of number, size, and activity of KS lesions, obtained in all treated p at all involved body sites, since the second-third therapeutic cycle. However, the role of concurrent HAART-related immune recovery (45–395% increase of CD4+ lymphocyte count, compared with baseline), has to be taken into account, as well as the hypothetic direct or indirect activity of antiretroviral therapy on KS evolution. Only one p with a prior, recent myocardial infarction received only four cycles of daunorubicin, because of contraindications indicated for p suffering from coronary heart disease; he continued cidofovir alone, administered every other week. Four p needed concurrent rHuG-CSF administration to control drug-related incoming neutropenia. A drop of quantitative plasma HHV-8 viraemia was demonstrated in four treated p, and strictly paralleled clinical improvement. Both liposomal doxorubicin and daunorubicin were approved for the treatment of HIV-related KS, demonstrating a comparable effect (but less toxicity), of combined chemotherapy based on vincristine, adriamycin, and bleomycin. The confirmed pathogenetic role of HHV-8 in KS prompts the introduction of cidofovir (an antiviral drug with extensive activity towards all Herpesviruses), in the ethiological KS treatment, but neither validated schedules of administration of both drugs are available, nor criteria for selection of a specific drug over the other one, because of the lack of data from controlled studies. After paying careful attention to heart-kidney toxicity of doxorubicin-daunorubicin, and cidofovir too, this cytotoxic-antiviral combination therapy may become the first-line therapeutic choice for HIV-associated KS, waiting for controlled data. The decay of HHV-8 viraemia needs further investigation, as a predictor of response to combined cytotoxic-antiviral therapy.

P551 A novel antiviral based on oral antibodies: clinical benefits in paediatric upper respiratory infections

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Objectives: High prevalence of viral upper respiratory infections (URI) and inefficacy of anti-influenza vaccination for noninfluenza and mixed URIs reveal an unmet need in managing URI, most urgent in pediatic population. A novel antiviral and immunomodulating drug developed and currently marketed in Russia – Anaferon (AF) – contains antibodies to interferon gamma (IFNg) in ultra-low doses intended for oral use. Animal studies showed its therapeutic and preventive action in influenza and herpetic infection. On course oral treatment AF significantly (three to six times) enhanced IFNg *ex vivo* secretion in peripheral mononuclears.
Methods: Efficacy and safety of AF for influenza and other URI was studied in a multicentre randomised placebo-controlled trial that involved over 400 pediatric in- and outpatients aged 6 months–14 years. AF (oral tablets) was given three to seven times daily starting on day 1–2 of URI onset, as add-on therapy to symptomatics and/or antibiotics (if indicated). Major clinical signs of URI and possible adverse drug reactions were monitored daily.In a randomised study of AF as a prophylactic for URI, over 400 patients (aged 6 months–4 years) received AF/placebo, 1 oral tablet daily for 3 months. In cases of URI, AF was given according to treatment schedule. Points to consider were occurrence and severity of URIs, rate of complications, tolerability.
Results: Treatment with AF provided considerable ($P < 0.05$) reduction in duration and severity of major signs of URIs, and rate of complications. Duration of fever reduced by 35–40%, of intoxication – by 40–50%, of coryza – by 20%, of cough – by 30%. None of AF patients showed any adverse drug-related events (ADREs). Prophylactic use of AF caused a 2-2,3-fold drop in occurrence of URIs. The part of children who avoided URI within 3 months of treatment rose from 3% in placebo to 24,7% in AF group. The use of AF provided a 1.5–2-fold reduction in duration of major signs of URI. Typical pediatric URI complications (otitis and purulent rhinitis) were 2.3 and 2.1 times less frequent (correspondingly). No ADREs were registered on AF.

Conclusion: AF is effective and safe in prophylaxis and treatment of influenza and other URIs in children aged 6 months and older. Moreover, pilot studies have shown AF efficiency in infectious mononucleosis and haemorrhagic fever with renal syndrome. Russian Health Ministry recommends AF as one of choice remedies for pediatric URIs.

P552 Disruption of the interaction between the HCMV DNA polymerase subunits: towards new anti-HCMV inhibitors

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Objectives: The human cytomegalovirus DNA polymerase is composed of a catalytic subunit, UL54, and an accessory protein, UL44. The observations that both UL54 and UL44 are essential for HCMV DNA replication, and that antisense inhibition of UL44 synthesis in HCMV-infected cells strongly inhibits viral DNA replication raises the possibility that the UL54/UL44 interaction might be a valid target for antiviral drugs.

Methods and Results: To investigate this possibility, overlapping peptides spanning residues 1161–1242 of UL54 were synthesised and tested for inhibition of the interaction between purified UL54 and UL44 proteins. A peptide, LPRRLHLEPAFLPYSVKAHECC, corresponding to residues 1221–1242 at the very C-terminus of UL54, disrupted both the physical interaction between the two proteins and specifically inhibited the stimulation of UL54 by UL44. Moreover, to define individual residues in UL44 and UL54 that are crucial for interacting with each other, we have engineered several mutations both in the C-terminal region of UL54 and in a region of UL44 identified in the crystal structure as the 'connector loop'. Substitution of alanine for Ile135 in UL44 or for Leu1227 or Phe1231 in UL54 greatly and specifically impaired the UL54–UL44 interaction in both pull-down assays and assays of long-chain DNA synthesis, identifying these residues as crucial for subunit interaction.
Conclusions: Thus, a few specific side chains appear to be crucial for UL54/UL44 interaction, suggesting that small molecules targeting the relevant side chains could interfere with this interaction. This information may aid in the discovery of new drugs for the treatment of HCMV infection.

MRSA and staphylococci: epidemiology

P553 MRSA outbreaks in nursing homes in Central Norway

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Introduction: Norway has a low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), with a rate of less than 1% of all staphylococcal infections. Reports from the Norwegian Notification System for Infectious Diseases (MSIS) show an increase in the number of reported cases of MRSA from 63 in 1998 to 142 in 2002. Most cases are now contracted in Norway and outside hospitals. Although there have been several outbreaks of MRSA in hospitals, this is the first report of MRSA outbreaks in Norwegian nursing homes (NH).
Material/methods: Since March 2003 there has been outbreaks of MRSA at two different NH in Central Norway located 111 km from each other. The initial detection of MRSA was performed at a local hospital. Positive strains were sent to St Olav University hospital for confirmation and further investigation. Strains from 24 persons (Both infection and carrier strains) were analysed by Pulsed Field Gel Electrophoresis (PFGE), and one strain from each NH was analysed by Multi Locus Sequence Typing (MLST).
Results: NH A had 10 cases among inmates and five among health care workers. NH B had nine cases, all among inmates. MRSA strains were isolated from nostrils or wounds, in some case from both locations. The nine cases in NH B had an identical PFGE pattern. The strains from NH A were also similar except from one band difference. The strains from NH A differed by one or two bands from the NH B strains, thus all strains were closely related using PFGE. By MLST both strains were found to be ST 45. The SCCmec gene has not yet been determined. (The international strains Berlin is ST 45 type IV).
Discussion: No exchange of personnel or inmates had occurred between the two NH, and no obvious cause of spread was found. The close relation of the strains makes us suspect a common source. Although both NH are served by the same hospital, this hospital had not experienced any outbreak of MRSA. Our results show the potential for spread of MRSA within NH. Several authors report MRSA within NH to be an increasing problem, and NH residency has been proven to be an independent risk factor for MRSA carriage upon hospital admission. It is important that health care workers at NH are aware of this. Proper infection control must be implemented not only in hospitals but also in NH.

P554 Prevalence and risk factors for colonisation with methicillin-resistant *Staphylococcus aureus* (MRSA-C) in residents of long-term-care facilities in Greece

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Objectives: LTCF are becoming a major component of the health care delivery system. The high prevalence of antimicrobial-resistant organisms in some LTCF has been documented for many years. The prevalence of colonisation or infection with MRSA may be as high as 20–30%. MRSA-C in LTCF often precedes invasive infection and represents a reservoir for dissemination in other residents or facilities. A survey was conducted to determine the prevalence and main risk factors for MRSA-C in residents of LTCF in Greece.
Methods: 18 LTCF were randomly selected from the public sanitation list of Attic province. Nasopharyngeal (N) and wound (W) samples were collected from 561 residents; from each LTCF, we chose randomly 30% of the existing population (minimum sum 25 residents). Cultures and susceptibilities were performed, following NCCLS guidelines. Information was collected on facility and resident demographic data. Univariate and multivariate analyses were performed.
Results: 587 samples were collected and 22 (3.75%) MRSA were isolated, 18 (2%) from N and 4 (0.6%) from W specimens. The most common site was W (four of 26, 15.4%), followed by N (18 of 561, 3.2%). Variables associated with MRSA-C by univariate analysis: recent (previous 30 days) antimicrobial use (RR 3.8, $P = 0.017$), indwelling urinary catheter (RR 2.2, $P = 0.02$), recent (previous 120 days) hospitalisation (RR 2.5, $P = 0.011$), poor functional status (RR 3.6, $P = 0.013$) and feeding tube (RR 4.4,

$P = 0.001$). In multivariate analysis, only recent hospitalisation (RR 2.5, $P = 0.01$), poor functional status (RR 3.6, $P = 0.01$) and feeding tube (RR 4.4, $P = 0.01$) were independently associated with MRSA-C. This model had a sensitivity of 95% and an area under the ROC of 76%.
Conclusions: MRSA-C is relatively low in Greek LTCF. According to our survey, independent risk factors for MRSA-C in LTCF are hospitalisation, poor functional status and usage of feeding tubes.

P555 Risk factors for nasal carriage of methicillin-resistant *Staphylococcus aureus* among patients in long-term care facilities in Korea

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Objective: As populations in Korea age, increasing numbers of older individuals reside in long term care facilities (LTCF) such as nursing homes and chronic disease hospitals. As antimicrobials are frequently subscribed in LTCF, the emergence of antimicrobial resistant organisms is a serious problem. But, little is known about anitmicrobial resistance of LTCF in Korea. Among various antimicrobial organisms, Methicillin resistant *Staphylcoccus aureus* (MRSA) is one of the most common pathogens causing nosocomial infections and widely prevailing in Korean hospitals. We designed this study to investigate the prevalence of MRSA and risk factors associated with nasal carriage of MRSA in LTCF.
Methods: The study was performed among 632 residents in eight geriatric care hospitals from July to August 2002. Samples were obtained by swabbing both nares. Mannitol salt agar containing 6 mg/mL of oxacillin was used for isolation of MRSA. The antimicrobial resistance of isolated strains was determined by the disk diffusion method, according to the recommendation of NCCLS. We reviewed all medical records of 632 residents to analyse variables as risk factors including demographic, hospitalisation-related factors, antibiotic usage and comorbid conditions.
Results: Overall prevalence of nasal carriage of *S. aures* was 50.2% (317 of 632). Among 317 isolated *S. aureus*, 64.1% was resistant to oxacillin. Recent infections (OR, 2.79, $P = 0.01$) and use of antimicrobials (OR, 2.787, $P = 0.01$), indwelling devices such as foley catheters and tracheostomy tubes ($P < 0.001$) and the existence of bedsores or wounds (OR, 2.508, $P = 0.02$) were associated with the isolations of MRSA. And the history of previous hospitalisation and the duration of hospitalisation were also associated with the isolation of MRSA but not significant statistically.
Conclusion: We found that MRSA were spreading widely in Korean LTCF. Multiple risk factors including recent infections and the use of antimicrobials, indwelling devices and the existence of wounds or bedsores were associated with the nasal carriage of MRSA in geriatric care hospitals in Korea.

P556 The prevalence of nasal colonisation with MRSA among residents of long-term care facilities in South Korea

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Objectives: The long-term care facilities (LTCFs) patients are those with serious underlying disease, poor functional status, wounds such as pressure sores, invasive devices of urinary catheters. Residents of LTCFs are at risk for colonisation with multidrug-resistant bacteria including methicillin resistant *S. aureus* (MRSA). More than 70% of *S. aureus* isolated in tertiary hospitals in Korea was methicillin resistant. But the prevalence of antimicrobial resistance data in elderly population has not been known yet, in Korea. To determine the prevalence of nasal MRSA colonisation in LTCFs, we investigated the rates of methicillin resistance among the nasal isolates of *S. aureus* isolated from provincial hospitals for the elderly.
Methods: Nasal swab specimens were obtained from 632 patients of eight provincial hospitals for elderly from July to August 2002. Swab specimens were cultured on staphylococcal broth for enrichment, and plated on mannitol salt agar and mannitol salt oxacillin agar which was supplemented with 6 μg/mL of oxacillin. Species were identified by PCR and biochemical test. Antimicrobial susceptibility tests to 12 antibiotics were performed by disc diffusion method, MIC of oxacillin was confirmed by agar dilution method. mecA and mupA were detected by multiplex PCR. PFGE and coagulase typing were performed for molecular epidemiological analysis of isolates.
Results: On admission to the LTCFs, 317 *S. aureus* (50.2%) were isolated from the specimens and were identified resistant to oxacillin in 64% of them range from 36.6 to 80.0% depends on hospitals. mecA gene was detected in all tested 233 MRSA isolates with MICs of 16 to >128 μg/mL. Twenty-three isolates of MRSA were mupirocin resistant with mupA gene and MICs of >1024 μg/mL. The majority of those showed closely genetic relatedness with more than 75% in PFGE and coagulase type II.
Conclusions: Methicillin resistance of *S. aureus* isolates in provincial hospitals for the elderly was very high (64%). This study suggests that the importance of infection control programmes in preventing the spread of MRSA in LTCFs should be encouraged.

P557 Basic hospital infection control methods reduced the isolation rate of methicillin-resistant *Staphylococcus aureus*

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Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is highly prevalent in hospitals in Korea. Hospital infections by MRSA are causing serious problems, so every hospital is trying to control MRSA by applying effective methods. Seoul Paik Hospital, tertiary teaching 430 bed hospital, applied relatively stricter infection control methods than previous ones in March 2002. This study was designed to evaluate the difference of the isolation rate of MRSA from all clinical specimens before and after the application of the infection control methods.
Methods: Each month, data of the number of MRSA was gathered and sorted; the results of the study were reported to every ward. All wards were supplied with Microshild (Johnson & Johnson, Australia), Clean N' Fresh (Carroll, USA), and standard paper towels. Health care workers were educated about hospital infection control. The use of antibiotics was then restricted. We retrospectively compared MRSA isolation rate based on results reported by the Department of Clinical Microbiology before (September 2001–February 2002) and after (March 2002–August 2003), applying the new infection control methods.
Results: After starting restricting system of antibiotics, glycopeptides and carbapenems were prescribed 15% (81 vs. 69 vials/1000 patient-days, $P < 0.01$) and 35% (37 vs. 24 vials/1000 patient-days, $P < 0.01$) less respectively, during the same period (September 2001–May 2002 vs. September 2002–May 2003). Methicillin resistance rate of *S. aureus* was reduced from 78 to 69% during the period of study ($P = 0.02$). The isolation rate of MRSA was reduced by 39% from 2.3 to 1.6/1000 patient-days ($P = 0.002$) during the same period (September 2001–February 2002 vs. September 2002–February 2003).
Conclusions: This study showed that the isolation rate of MRSA was reduced by applying hospital infection control methods in the hospital.

P558 Molecular surveillance of clinical methicillin-resistant *Staphylococcus aureus* isolates in neonatal intensive care units

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Objects: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an important nosocomial pathogen in our neonatal intensive care units (NICUs) and accounts for almost 100% of all

S. aureus clinical isolates. To assess the relatedness of these MRSA strains, we conducted this molecular surveillance study.
Materials and methods: During 1998 and 2000, a total of 125 MRSA clinical isolates, 10 isolates in 1998, 59 isolates in 1999 and 56 isolates in 2000, from 107 infants hospitalised in our NICUs were collected. Fifteen infants were with multiple isolates (two to four isolates). The sources of specimens included blood (75), pus (23), sputum (15), body fluids (3), and catheter tip (9). The genotyping method used was pulsed-field gel electrophoresis with Sma I digestion.
Results: A total of six genotypes with 23 type-subtypes were identified. Subtypes could be identified in genotypes A, C and D. There were two genotypes in 1998, five genotypes in 1999 and four genotypes in 2000. Seventy-seven isolates (61.6%) were shown to belong to a major type (Genotype A, 7 type-subtypes), while 43 isolates (34.4%) belonged to genotype C (11 type-subtypes). Both types could be identified in each year and were the two predominant clones in each year. The other four types were minor. Among the 15 infants with multiple isolates, the genotype was usually same if the isolates were from the same episode of MRSA infection, while the genotype was different if the isolates were from distinct episodes.
Conclusion: There were two predominant MRSA clones prevailing in our NICUs between 1998 and 2000. Infection control measures should be implemented to try to control the spread of MRSA.

P559 Methicillin-resistant *Staphylococcus aureus* bacteraemia in neonatal intensive care units: genotyping analysis and case–control study

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Objects: To assess the relatedness of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and to identify the risk factors for the acquisition of MRSA bacteraemia in the infants hospitalised in the MRSA endemic neonatal intensive care units (NICUs).
Methods: Twenty-one isolates from the bloodstreams of 21 infants hospitalised in NICUs were genotyped by pulsed-field gel eletrophoresis (PFGE) and infrequent-restriction-site polymerase chain reaction (IRS-PCR). Other 21 infants stayed in the same NICUs with a same gender, a similar gestational age and a similar birth weight but without MRSA bacteraemia were matched for a case–control study.
Results: Of the 21 MRSA isolates, two genotypes (A and C) were identified by PFGE while three genotypes (I, II and III) by IRS-PCR. Genotype C-III in nine isolates and genotype A-I in seven isolates were the two most common genotypes. With multiple logistic regression analysis, presence of skin infection at onset and prior sepsis were the two significant risk factors of MRSA bacteremia with odd ratios of 20.8 (95% CI 2.95–145.4, $P = 0.002$) and 7.97 (95% CI 1.33–47.7, $P = 0.02$), respectively. Both prolonged prior duration of central venous catheter (CVC) indwelling and prolonged hospital stay were also significantly associated with MRSA bacteraemia in these infants.
Conclusion: Two major clones of bacteraemic MRSA prevailed in our NICUs in 1999 and the presence of skin infections, particularly the insertion site of CVC, and prior sepsis were the two risk factors for the acquisition of MRSA bacteraemia in these infants.

P560 Delineation of the endemic and sporadic clones among methicillin-resistant *Staphylococcus aureus* strains in a Czech hospital

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Objectives: To define the clones among methicillin-resistant *Staphylococcus aureus* (MRSA) strains collected between September 2001 and February 2003 at the regional hospital of Nový Jièín, Czech Republic.
Methods: The isolates were characterised by susceptibility tests, *Hin*dIII ribotyping, and pulsed-field gel electrophoresis. Representatives of each clonal type were analysed by multilocus sequence typing and staphylococcal cassette chromosome mec (SCCmec) typing. The prevalence of the major macrolide (ermA, ermB, ermC and msrA) and aminoglycoside (aac6′-aph2′′, aph3′ and ant4′) resistance genes was evaluated as well.
Results: The presence of two international MRSA clones was documented in the Czech hospital: (i) the Iberian clone (ST247: SCCmec IA: PFGE A: ribotype H2) endemic in the hospital and associated with a single multiresistant phenotype and (ii) clone EMRSA-15 (ST22: SCCmec IV: PFGE H: ribotype H7) detected since the beginning of 2002 and associated with three phenotypes. These two clones could be distinguished by the distribution of macrolide and aminoglycoside resistance genes (ermA, aac6′-aph2′′, ant4′ and ermC plus msrA in a few isolates, respectively) and the presence of enterotoxin A (in the Iberian clone).
Conclusions: Two clones could be delineated among the strains studied. The combination of the molecular characterisation with chronological epidemiological data enabled following the spread of EMRSA-15 clone in the hospital.

P561 MRSA – emerging issue in a university hospital, Hradec Kralove

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Introduction: Although the incidence of strains of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and other MRS varies from country to country and from hospital to hospital, it has been steadily increasing worldwide in the last decade.
Objectives: In the year 2003 one of the troublesome bacteria of the last decade, MRSA, became the emerging issue also for many departments of the University Hospital in Hradec Králové. Thus we decided to perform analysis of MRSA incidence.
Methods: Retrospective analysis of MRSA prevalence from University Hospital databases.
Results: Since 1 January till 8 December 2003 was 5530 strains of *Staphylococcus aureus* isolated in the Department of Clinical Microbiology from various samples. Eighty-nine strains resistant to oxacilin (MRSA) were identified in 21 patients. The positive findings from sputum and/or tracheal cannulas were most frequent, in 12 individuals, followed by skin isolates including the decubituses and surgical wounds. Positive haemocultures and isolations from abscesses were rare. Regarding the common results of mixed cultivation from above-mentioned samples, it was difficult to specify MRSA in all cases as a primary cause of invasive diseases. The diagnosis related to the cultivation results of MRSA – nosocomial bronchopneumonia, sepsis, infection of wounds, decubitus and abscess – were determined in patients with other underlying disease or shock. We have found no strain resistant to vancomycin, linezolid and quinupristin/dalfopristin. Sensitivity to the other antibiotics were rather changing in follow up each patient, it was mostly present also for lincomycin and chloramphenicol.
Conclusion: Therapy of particular invasive disease caused by MRSA was successful in most of cases. One patient aged 81 died on septic shock (nosocomial bronchopneumonia), where we could not exclude MRSA as causative agent. Nevertheless long term results are left to assess, including the practice in isolation precautions of these patients.

P562 Epidemiological analysis of the incidence of MRSA infections in hospitals in the Czech Republic

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Objectives: Infections caused by MRSA (methicillin-resistant *Staphylococcus aureus*) have been historically considered as hospital infections. Nevertheless, MRSA is becoming increasingly involved in community infections as well. The incidence of MRSA infections varies widely with the regions and population groups.
Methods: Since 2000 the Czech Republic has been taking part in the European Antimicrobial Resistance Surveillance System monitoring the incidence of five pathogens in invasive isolates from blood. One of the causative agents under surveillance is *Staphylococcus aureus*. The incidence of MRSA has been monitored in blood specimens from 89 hospitals of the Czech Republic. Data on patients, hospitals and phenotypes of antibiotic resistance in the bacterial strains isolated have been recorded. Basic statistical methods are used for epidemiological analysis.
Results: Between 2000 and 2002, 2804 *Staphylococcus aureus* strains were analyzed. Out of these strains, 5.5% were MRSA (see Table). The incidence of MRSA varied with types of hospitals, hospital wards, age groups and time. Geographical distribution of MRSA strains in the Czech Republic was mapped. Thanks to collaboration of 45 laboratories, highly valid data covering 82% of the Czech population have been available.

Year	*S. aureus*	MRSA
2000	525	20 (3.8%)
2001	109	53 (5.8%)
2002	1166	70 (5.9%)
Total	2804	153 (5.5%)

Conclusion: Surveillance of MRSA strains as a basis for active antibiotic policy has become of increasing concern to both health care providers in hospitals and community general practitioners. There is a need for better awareness of MRSA infections among both health care professionals and the public. The incidence of MRSA infections in the Czech Republic shows a slightly increasing trend. The development of the incidence of MRSA infections and factors involved in their spread will be the subject of further study.

P563 Evolution of two different clones of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in Italy

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Objectives: The origins of the major MRSA clones are still poorly understood. Previous reports have suggested a common origin for all MRSA from a single ancestral *S. aureus* strain that acquired the mec complex. Recent studies have shown that some MRSA strains are very divergent, implying that mecA has been transferred among *S. aureus* lineages at different times in the past.
Methods: *S. aureus* MLST, together with BURST analysis and SCCmec-typing, has been used to probe population biology of bacteria and to predict ancestral genotypes and evolutionary descendents within groups of related genotypes.
Results: We explored the origin and the evolution of two novel MRSA clones: the Rome and the Italian clones. The Rome clone, circulating in a Rome hospital from 1997, had a characteristic clonal type (II::NH::C) and was susceptible to erythromycin, clindamycin, spectinomycin, vancomycin and teicoplanin. During the last few years, a variant of this clone has appeared and undergone evolution, consisting in the acquisition of two copies of Tn554, the integration of the pUB110 plasmid downstream of the mecA gene with the evolution of the SCCmec type I to IA and the consequent modification of mecA polymorph II to I, maintaining the sequence type ST247 (3-3-1-12-4-4-16; CC8) and the PFGE C pattern. The acquisition of extra-resistance genes determined erythromycin, spectinomycin and clindamycin resistance; moreover, these strains showed heteroresistance to glycopeptides. On the contrary, the Italian clone has always shown the same phenotypic (susceptible to tetracycline and rifampin) and genotypic features (II-E-E; ST228: 1-4-1-4-12-24-29; SCCmec I), suggesting the immediate success of these strains in the environment.
Conclusions: Our results unambiguously indicate that the Rome clone and its variant derive from the same MRSA clone as the 'Archaic', the 'Iberian' and the 'Brazilian' ones (CC8). Moreover, the 'Italian' clone closely correlates with that of several *S. aureus* including MSSA, MRSA and GISA strains (CC5), confirming the horizontal mecA transfer among different *S. aureus* ancestral lineages. This ancestral MRSA became the successful clone that is spreading in Italy.

P564 Methicillin resistance in Staphylococci: results of a survey in a Northern Italian region

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Objectives: Methicillin-resistance (MR) in staphylococci represents a major therapeutic treat in the management of nosocomial Gram-positive infections. This parameter therefore, need a periodical evaluation.
Methods: In this study of the Italian Clinical Microbiologist Association (AMCLI), MR was assessed in nine Centres localised in Ligurian area (Northern Italy). During April 2003, 476 staphylococcal strains were collected from hospitalised patients in Medical Department (64.5%), Surgery (12.2%) and high risk of infection wards (23.3%). Samples were obtained from wound swabs (22.3%), infections of upper (17.0%) and lower respiratory tract (15.8%), blood (11.6%), vascular devices (8.0%) and other (25.3%). MR was evaluated by NCCLS suggested guidelines.
Results: The strain collection included 358 *S. aureus* (75.2%) and 118 CNS (24.8%). *S. epidermidis* were 56.8% of this latter group of strains. MR *S. aureus* (MRSA) accounted for 63.7% and in CNS this trait was 66.1%. Many isolates were also resistant to other classes of antibiotics. In particular, some representative pathogens (among MRSA) exhibited together with MR, unsusceptible to aminoglycosides, MLS, fluoroquinolones, and tetracycline (46.5%). All strains resulted susceptible to vancomycin and teicoplanin. Among CNS, concomitant resistance to methicillin and other antibiotics were detected in 33.3% of these pathogens. No strain was found resistant to vancomycin and teicoplanin.
Conclusions: The present findings indicate that antibiotic resistance in nosocomial staphylococci is largely diffused and need a continuous programme of surveillance.

P565 Methicillin-resistant Staphylococci in a surgical hospital: incidence, antimicrobial susceptibility, molecular typing

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Objectives: Monitoring of the incidence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative Staphylococci (MR CoNS) in hospitalised patients in a surgical hospital, characterising of their multiresistant nature, slime production in CoNS and molecular types in MRSA.
Methods: A total of 165 strains of *S. aureus* and coagulase-negative Staphylococci collected from clinical specimens of different hospitalised patients within 2002 were analysed. Microbial strains were

isolated and characterised by conventional methods. An automated Crystal system was used for species' identification. Antimicrobial susceptibility was tested by the disk diffusion method (NCCLS) and detection of the minimal inhibitory concentration (MIC) in SCEPTOR panels. Methicillin resistance was tested by oxacillin disks with the potency 1 μg of oxacillin and confirmed by detection of the mec A gene by PCR, the latex agglutination test and the E-test (Bio Disk). Slime production in CoNS was tested using the tube test with tryptic soya broth (TSB) and glucose. Molecular typing was performed by the randomly amplified polymorphic DNA test (RAPD).
Results: The incidence rate of MRSA and MR CoNS in 2002 was 1.64 and 30.6%, respectively. MRSA were resistant to erythromycin (99%), clindamycin (71.5%), trimethoprim (35.8%), gentamicin (25%), ciprofloxacin (17.9%). MR CoNS were resistant to clindamycin (94%), erythromycin (92%), trimethoprim (66%), gentamicin (40%), ciprofloxacin (28%). The result among different species of CoNS varied. 74 CoNS strains (52 *S. epidermidis sensu* stricto, 12 *S. haemolyticus*, 4. *S. hominis*, 3 *S. capitis*, 1 *S. cohnii*) were tested for slime production. Twenty-three of them were methicillin-sensitive, 51 – methicillin-resistant. Fifty-one (69%) of the investigated strains did not produce slime, 10 strains (13.5%) produced slime with a high intensity, 13 strains (17.5%) – with a moderate intensity. Slime production in MRS was more intensive than in MSS strains. Thirty-six strains of MRSA were examined by the RAPD typing method, and the main genetic groups were differentiated.
Conclusions: The incidence rate of methicillin resistance in Staphylococci in our hospital is not high. MRS are multiresistant. 13.5% of CoNS are active producers of slime, 17.5% are moderate producers, 69% do not produce slime. Methicillin-resistant Staphylococci produce slime more actively than methicillin-sensitive strains. The RAPD method is a sensitive and reliable molecular typing method.

P566 Initial molecular characterisation of the first MRSA outbreak at a clinical university hospital

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Objectives: Meticillin resistant *S. aureus* (MRSA) is a well known pathogen which causes severe nosocomial infections. First confirmed case of MRSA at P. Stradins Clinical University Hospital (CUH) was registered in March 2003. The aims of this study were comparison of bacteriological and molecular identification of staphylococcus isolates as well as their initial characterisation and typing.
Methods: *Staphylococcus* isolates were collected at CUH in period March–September 2003. Presence of the cflA, femB, mecA and PVL genes were detected by PCR amplification. rep-PCR and RFLP were used for MRSA typing.
Results: Screening of 86 isolates revealed 16 MRSA (cflA+, femB+, mecA+). This result was in concordance with the one obtained by bacteriological methods. Twelve of these strains were typed by rep-PCR and RFLP. Application of these methods generated three groups including nine, two and one isolates, respectively. The last, single isolate was the only one positive for Panton-Valentine leukocidin toxin.
Conclusions: The local ICU outbreak was registered and confirmed by molecular methods. Maintenance of vigorous surveillance and prevention measures are strongly required.

P567 Clonal spread of borderline oxacillin-resistant *Staphylococcus aureus* in a dermatological hospital unit

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Objectives: The aim of this study was to describe and investigate a clonal spread of mecA gene-negative *Staphylococcus aureus* strains with decreased sensitivity towards oxacillin (BORSA) among patients in a dermatological hospital unit.
Methods: The medical records from patients in the dermatological hospital unit and clinical samples received in the Department of Clinical Microbiology were reviewed, retrospectively, from November 2000 to October 2001. Susceptibility to oxacillin (1 μg disk, Oxoid) was examined with either the disc diffusion method or Etest on Iso-Sensitest Agar or Columbia agar (with 4.5% NaCl) at 35°C. The presence of the coa gene and absence of the mecA gene was examined with a polymerase chain reaction (PCR) method. BORSA was defined as phenotypic oxacillin-resistant *S. aureus* being mecA-negative. All isolates were phage-typed and genotyping was performed with pulsed field gel electrophoresis ('enzym').
Results: Fifteen isolates from fifteen patients were evaluated. All the strains carried the coa gene, were of phage-type 95u and the PFGE results confirmed that all the isolates constituted a clone. The median zone size from disc diffusion testing was 6 mm (range 6–19 mm) while the median oxacillin MIC was 3.0 mg/L (ranged from 0.25 to 6.0 mg/L). None of them carried the mecA gene. Ten of the patients received systemic immuno-suppressive medications, and four others used topic immuno-suppressive agents (chlormethine and group III steroids). At least 10 of the patients received low-dosis penicillinase-stable penicillins for longer periods of time.
Discussion: We describe for the first time a clonal outbreak of BORSA among patients in a dermatological hospital unit. The clonality was confirmed by means of both phenotyping (phage-type 95u) and geno-typing (same PFGE type). It could not be clarified whether the 'BORSA clone' spread directly from one patient to another or whether it was spread by a health care worker or through shared objects. One of the patients had a history with visits to the out-patients clinic only which may suggest indirect transmission. We believe that the spread of BORSA in our dermatological hospital unit was facilitated by longtime (low-dosis beta-lactam) antibiotic pressure, close relationships between patients during admission, and immuno-suppressive treatment. The BORSA clone seems to have disappeared after changes in hygienic measures and discussions of the antibiotic policy in the department.

P568 The use of molecular epidemiology to monitor the nosocomial fissemination of methicillin-resistant *Staphylococcus aureus* in a tertiary care university hospital during a 10-year survey: 1991–2001

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a severe clinical threat for patients worldwide and has been the cause of major outbreaks and epidemics among hospitalised patients, with high mortality and morbidity rates.
Objectives: To study the genomic diversity of MRSA strains isolated from patients with nosocomial infection assisted in a tertiary care university hospital during a 10-year survey (1991–2001).
Methods: The study comprised two periods: the period I from 1991 to 1993 and period II from 1995 to 2001. The DNA typing analysis was performed by pulsed field gel electrophoresis and the similarity among the MRSA strains was determined by cluster analysis.
Results: In the period I, 73 strains presented five distinctive DNA profiles: A, B, C, D and E. Profile A was the most frequent DNA pattern and was identified 55 (75.3%) strains; three closely-related and four possibly-related profiles were also identified. During period II, 80 (68.8%) of 117 strains had the same endemic profile A identified in period I, 18 (13.7%) closely-related profiles and 18 (13.7%) possibly-related profiles and only one strain presented an unrelated profile. Cluster analysis showed a 96% coefficient of similarity between profiles A from period I and profile A from period II and they were considered to be from the same clone. The molecular monitoring of MRSA strains permitted to

determine the clonal dissemination and maintenance of a dominant endemic strain during a 10-year period and the presence of closely and possibly related patterns to the endemic profile A.
Conclusion: Further studies on virulence factors of this MRSA endemic profile and the reinforcement of strict measures of hands hygiene and environment cleaning are necessary to improve the understanding and control of the dissemination of the endemic profile in our hospital.

P569 Epidemiology of methicillin-resistant *Staphylococcus aureus* at a German university hospital

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Background: Many risk factors for acquisition of methicillin-resistant *Staphlococcus aureus* (MRSA) infections or colonisation during hospital stay are described in the literature. In our study we evaluated the risk factors of patients identified as MRSA positive at the University hospital Aachen.
Methods: During January–November 2003 all medical records of inpatients identified as MRSA positive from clinical species were reviewed prospectively for demographic data and risk factors. Whenever possible the patients were interviewed also about hospital stay in the last 6 months, being a nursing home resident, being known as MRSA positive, previous antibiotic therapy and underlying diseases. Division for infection or colonisation was determined by GP.
Results: During the study period 148 *S. aureus* strains (7.8%) were identified as MRSA. Fifteen isolates were detected in outpatients; 133 strains in inpatients, 20 (15%) of which were known as MRSA positive at time of admission. Until now the data of 114 (85.7%) inpatients could be evaluated. The major sites of detection of nosocomial acquired MRSA included the lower respiratory tract (19%), wound (21%), urinary tract (7%), skin (17%), bloodstream (13%) and tip of central vascular catheter (19%). Seventy per cent of patients were colonised and 23% infected, no information was available for 7%. Lethality rate was 30%. The demographic data were: male: 70%, female: 30%, age 60 years (mean 64); hospital service: surgery 48%, internal medicine 31%, other wards 21%; 44% were detected in any ICU; major admission diagnosis: trauma (25%), acute infection (21%), previous surgery (13%), cardiovascular disease (11%), malignancy 10%; known chronic diseases (malignancy, cardiovascular disease, diabetes etc.) on admission 67%; previous antibiotic therapy 79%; central venous catheter 59%; hospital stay days 47 (mean 39), time until MRSA detection 26 days (mean 20), hospitalisation in the last 6 months 34%, transmitted from other hospitals 29%, nursing home residents 6%.
Conclusions: The MRSA-rate of 7.8% in our hospital is low. The late detection of MRSA after average hospital stay of 26 days shows that most patients were colonised or infected during their hospitalisation either by strain transmission or strain selection because of shown risk factors.

P570 Nosocomial meningitis due to methicillin-resistant *Staphylococcus aureus* (MRSA): review of eight cases

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Objectives: To evaluate MRSA meningitis cases in our hospital between 1999 and 2003. Patients and method: We evaluated the hospital charts of eight patients who had culture proven MRSA meningitis retrospectively.
Results: Patients were six men, two women, aged 4–70 years (mean 39). All had postneurosurgical state and two had shunt infections. All patients were evaluated as hospital-acquired meningitis. Fever, leucocytosis, disturbances in the consciousness were the most common clinical and laboratory findings. One patient had mixed infection (MRSA + *Enterococcus* spp.) whereas seven were infected only with MRSA. One patient was treated with vancomycin alone and three with teicoplanin alone. One patient was treated empirically with cefazolin and died during this treatment while awaiting the CSF culture results. One patient was treated with vancomycin followed with teicoplanin + meropenem because of tubulo interstitial nephritis. The last two were treated with combined regimens one with vancomycin + chloramphenicol, and one with teicoplanin + chloramphenicol. Mean duration of treatment was 27.5 days (range 3–60 days). Mortality rate was 12.5%.
Conclusions: MRSA meningitis is a rare but hard to manage nosocomial infection. Although IV vancomycin is the mainstay of therapy, the fact that five of these eight cases were successfully treated with teicoplanin (alone or in combination) shows that it may be an alternative treatment option.

P571 Effect of an intervention programme on the MRSA outbreak in an Aberdeen infirmary

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Objectives: Aberdeen Royal Infirmary (ARI) has experienced an outbreak of Methicillin resistant *Staphylococcus aureus* (MRSA) since 1997. We previously reported the relationship between hospital use of third generation cephalosporins (3GC), macrolides (MAC) and fluoroquinolones (FQU) and the emergence of MRSA. In May 2001, an intervention programme was introduced in the intensive care unit (ICU) involving admission screening and body decontamination. This study evaluated the effect of this programme on the overall hospital outbreak.
Methods: ARI is a 1200 bed teaching hospital with 16 ICU beds. Monthly nonduplicate MRSA data and antibiotic use data were collected for the ICU beds and the non-ICU beds, for the period January 96 to March 03. Time series dynamic regression models were adjusted to evaluate the intervention effect.
Results: ICU-MRSA evolution preceded the non-ICU MRSA by a 1-month lag. ICU-MRSA was dependent on past ICU-MRSA values as well as lagged ICU use of MAC, TGC and FQU. The intervention decreased the per cent monthly ICU-MRSA by the value 10.6. The impact of the ICU intervention on the non-ICU MRSA was 5.6%, thus breaking the increasing trend of the MRSA epidemic.

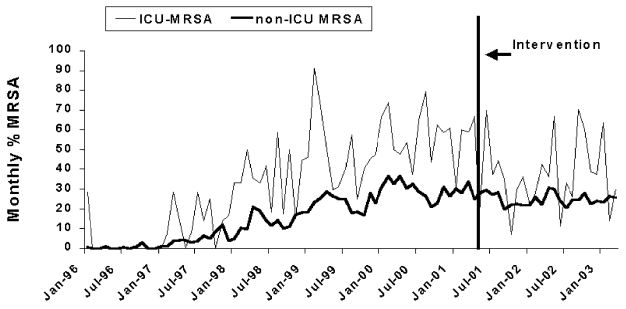

Figure 1. Monthly %MRSA for ICU and Non-ICU beds. Aberdeen Royal Infirmary, January 1996-March 2003

Conclusion: The ICU can influence the prevalence of nosocomial infections in the rest of the hospital because of the continuous flux of patients. This study promotes the benefits of interventions other than reducing antimicrobial use in the control of MRSA.

P572 How dangerous is the environment of a patient with respect to transfer of MRSA

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Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is a dangerous and persistent hospital pathogen. It is accepted that the major source of this organism within the hospital environment is infected and colonised patients' themselves. However, there is little documented fact on the relative importance of the patients' environment in the spread of this organism.
Objectives: To assess the degree of contamination of the patient's immediate environment and to estimate the likelihood of spread of MRSA from this environment to Health Care Workers and via them to other patients.
Method: A 5-week prospective study was carried out on a variety of wards, The environments of 29 colonised patients were sampled, including 29 curtains both sides, 59 hard surfaces, and 58 samples of the gloved hands of the investigator after samples had been taken. Curtains were sampled by direct indentation of the curtains on to selective agar. Hard surfaces were swabbed and hands were sampled using the finger streak method. Ten control environments containing patients who were not known to be colonised were also sampled.
Results: MRSA was isolated in 15 of the 29 environments. It was found on the sampler's hands on eight occasions out of the 29.
Conclusion: MRSA is a frequent contaminant of the patients' environment especially soft furnishings such as curtains. It is readily transferred to the hands after minimal contact. These findings need to be taken into consideration when cleaning protocols are devised and are especially important in terminal cleaning after patients' infected or colonised with MRSA have been discharged.

P573 Antibiotic usage and environmental reservoirs maintain methicillin-resistant *Staphylococcus aureus* on an intensive care unit

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Objectives: To determine the rate of colonisation and the incidence of transmission of methicillin resistant *Staphylococcus aureus* (MRSA) on an Intensive Care Unit (ICU) and control MRSA transmission.
Methods: A nine bed ICU was studied for an initial observation period of 8 months, all patients admitted to ICU for >24 h were screened for MRSA within 48 h then three times a week. Demographic data and antibiotic usage was recorded. An intervention period of 8 months when antibiotic prescribing was restricted followed. A second observation period (6 months) was instituted. Monthly environmental screening (29 sites) with swabs from three areas in each bed space (bed floor, monitor and workstation) plus two from the nurses workstation took place. Patient and environmental isolates were typed using pulse field gel electrophoresis (PFGE).
Results: Fifty-seven of 215 patients (26%) were colonised with MRSA during the initial phase, 50% of these acquired MRSA on ICU. Eighty-eight per cent of patients received >1 antibiotic, cefuroxime and metronidazole being the most heavily prescribed primarily as prophylaxis. During the intervention period prophylactic antibiotics were restricted to one dose and the need for treatment antibiotics reviewed daily. Postintervention the total antibiotics used was reduced from 132.5 to 104.1 DDD/100 patient days and a reduction in metronidazole/cefuroxime from 33.1 to 12.0 and 20.6 to 3.8 DDD/100 patient days respectively. Despite this there was no reduction in the number of patients colonised with MRSA on ICU, but the percentage of patients acquiring MRSA on ICU fell to 43.2%. Results from 19 environmental screens of 29 sites in the ICU yielded MRSA from 1 to 11 sites on every screen (mean 4.6). On two occasions no patients on ICU were colonised with MRSA, but MRSA was isolated from two and five environmental sites respectively. Typing showed EMRSA-15 variants were predominant in patients and environment with the environmental isolates reflecting both the current patient types and previously discharged patients.
Conclusion: Despite reducing the total antibiotic usage the rates of MRSA colonisation remained the same. The continuous isolation of MRSA from the environment together with our typing data indicates that environmental sources of MRSA have a role to play and are important in controlling the endemic MRSA infection in hospitals.

P574 Monitoring outbreaks of MRSA in a university hospital, Innsbruck by automated ribotyping

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Objectives: Methicillin-resistant *Staphylcoccus aureus* (MRSA) is an important cause of hospital- and community-acquired infections. The aim of this epidemiological study was to elucidate the spread of MRSA clones by means of molecular typing in the University hospital of Innsbruck.
Methods: Positive MRSA cultures collected from clinical specimens, such as blood cultures, cerebrospinal fluids, sputum, drains and various swabs isolated from patients admitted to the University hospital of Innsbruck were investigated from March to November 2003. All MRSA strains (initial isolate per patient) were typed by automated ribotyping according to manufacturer instructions using EcoRI as restriction enzyme.
Results: 116 patients acquired MRSA in the hospital and 49 were in intensive care units. Seventy-one MRSA strains were investigated and classified in 14 different ribotyping patterns (RP 1–RP 14) using EcoRI. RP 10 was identified in 24, RP 1 in 18, RP 3 in 10, RP 7 in 7, RP 4 and RP 2 in two patients. Further eight MRSA isolates yielded unique RPs. In the neurology intensive care unit RP 1 strains and in the medical intensive care unit RP 10 strains occurred constantly over a period of seven and four months, respectively. These strains were related to colonisation ($n = 22$) and infections ($n = 20$). Overall, 41 and 30 patients showed colonisation and infections because of MRSA, respectively.
Conclusions: Automated ribotyping successfully fulfilled our aim to study epidemiological aspects of MRSA spread. The majority of MRSA strains were limited to three clonal groups. RP 1, 3 and 10 strains were predominant in intensive care units, yet spread and persistence was also found within other wards. Furthermore this study shows the importance of stringent infection control measurements and the necessity of guidelines for antibiotic use to avoid selection of antibacterial resistance.

P575 MRSA acquisition on an intensive care unit (ITU)

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Objectives: The aim of this study was to investigate MRSA acquisition within a seven-bedded ITU in a tertiary hospital over a period of 5 months.
Methods: Data was collected from all patients admitted into ITU, including microbiological results and dependency scores. This enabled the distinction to be made between patients who were admitted with MRSA and those who acquired it whilst in ITU. Staffing levels for trained, auxiliary and agency nurses were plotted against bed occupancy rates and acute admissions; student attendance and nurses from other wards were also included. From these, nurse–patient ratios, workload and MRSA colonisation pressures were calculated and modelled against the timing of MRSA clusters. Standardised environmental screening was performed throughout the study using commercial dipslides.

Results: Of 162 patients admitted into ITU, 28 (17%) were found to have MRSA. Twelve of 28 (43%) acquired MRSA on the unit in four discrete clusters involving three patients. Each cluster occurred within a 5-day period and was preceded by enhanced workload, because of a shortage of trained nurses and increased bed occupancy. There was also an association with surface level hygiene throughout the study. Of 160 sites screened, 37 (23%) produced quantitative growth of 2.5–12 cfu/cm^2 and 26 of 37 (70%) were from hand touch sites. MRSA was found in the environment during the most intense period of activity. Some of the strains appeared to be related within and between clusters, and were particularly associated with upper respiratory sites.
Conclusions: Over a 5-month period, 12 of 162 (7%) patients acquired MRSA in this ITU, less than half of the patients shown to have MRSA overall. Clusters of MRSA acquisition were associated with shortages of trained nurses, increase in workload and hygiene failures predominantly involving hand-touch sites.

P576 Control of methicillin-resistant *Staphylococcus aureus* transmission in an intensive care unit: evaluation of the efficacy of control practices

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Objectives: Fighting against the dissemination of methicillin resistant *Staphylococcus aureus* (MRSA) infections depends on the understanding of the use of antibiotics and also the prevention of cross contamination. We evaluate the importance of hygienic measures in order to prevent MRSA spreading in a surgical intensive care unit.
Methods: We compared the incidence of MRSA carriage and infections before and after starting preventive measures among ill patients who often experience infections caused by MRSA. Environmental measures (technical and geographical isolation, hand washing) and decontamination of ill patients carrying MRSA may help improve the care of many at risk patients.
Results: Incidence of cases of nasal MRSA is relatively decrease from 28 to 5% and 30 to 2% for acquired MRSA infections over 10-year period. Risk factors to develop pneumonia because of MRSA is stable for chronically ill carriers through the time despite of nasal and skin decontamination.
Conclusion: Observance of hygienic measures by the medical team is a key to the prevention and control of the hyperendaemic estate of MRSA. However, it seems useful a global strategy to fight against MRSA, but the benefit of each measure of a global control programme is difficult to evaluate.

P577 Emergence of a VISA strain in a patient with osteomyelitis: first isolation reported from Austria

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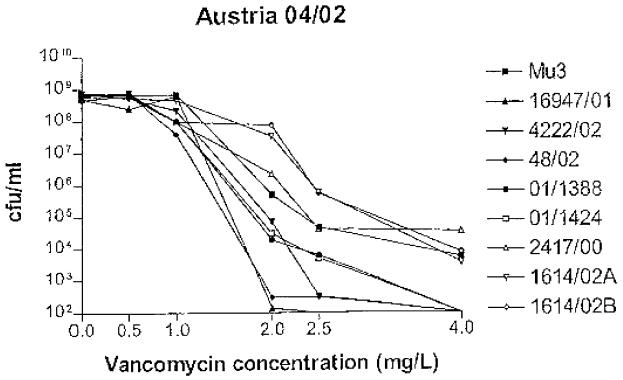
Introduction: MRSA with reduced susceptibility to vancomycin was first described 1997 by Hiramatsu in Japan. So far, no MRSA strains with intermediate resistance to glycopeptides have been reported from Austria. We present a patient with osteomyelitis caused by MRSA developing intermediate resistance to glycopeptides during therapy.
Case report: In June 1998 W.P., a male, aged 59, presented with a polymicrobial diabetic foot infection requiring the amputation of two toes of the right foot. After initial clinical improvement MRSA fully susceptible only to glycopeptides and fusidic acid was isolated repeatedly from the wound as well as from the bone in the autumn of 1998. As the patient was willing to comply with a long-term outpatient intravenous therapy teicoplanin was administered three times a week, which resulted in improvement clinically and by MRI investigations. Additionally, in May 2000 a bone graft impregnated with vancomycin was implanted resulting in local healing and decreased inflammatory activity in MRI controls. After a traumatic injury 2001 amputation of the right lower leg had to be performed because of a massive infection with MSSA and Acinetobacter.
Methods: In 2001, 40 MRSA strains isolated from 1-6/2000 were retested for vancomycin resistance with Va 5 μg disks on Mueller-Hinton agar using McF 0.5. For all strains with a zone-of-inhibition diameter 14 mm MICs were determined by E-Test on BHI agar using McF 2. The MICs for vancomycin and teicoplanin for the MRSA-strain 2417/00 recovered from patient W.P. in January 2000 had increased to 12 and 16 μg/mL, respectively, thus fulfilling the NCCLS-criteria for intermediate glycopeptide resistance. This strain was subjected to population analysis.
Result: The population analysis performed by Dr T. Walsh confirmed the E-test result for strain 2417/00: Figure 1.



Conclusion: Detection of VISA is a challenge for the routine clinical laboratory and requires a high level of suspicion. Prolonged use of glycopeptides promotes the development of glycopeptide resistance as seen in this case and in many others and should prompt further investigation in order to identify VISA and to enable adequate isolation precautions and efficient antimicrobial treatment.

P578 Six lethal cases of community-acquired methicillin-resistant *Staphylococcus aureus* infections in young adults in Montevideo, Uruguay

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Objective: To describe six severe sepsis (in 6-month period) in young immunocompetent hospitalised at ICU during an outbreak of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections.
Methods: Retrospective study between July and December 2003 of CA-MRSA severe sepsis syndrome cases. Two haemoculture sets (HEM) at admission (FAN Bact-Alert), culture of traqueal aspirate, pleural fluid(PF), drains(D) and skin lesions if present. *Staphylococcus aureus* identified by standard methods. 'MRSA screen' (Oxoid) for PBP2a. Susceptibility testing NCCLS (M2-A8, M100 S13): Oxacillin(OXA), Erythromycin(E), Clindamycin(CLI), Gentamycin(GM), Co-trimoxazol(SXT), Ciprofloxacin(CIP), Vancomycin(VA). Genomic typing at Juntendo University of the first three cases.
Results: Three females (16, 16, 45 years) and three males (15, 20, 37 years) except 1, none presented health care associated risk factors. Four patients with necrotizing pneumonia and respiratory sepsis (RS) three complicated with empyema and during period of more incidence of Influenza virus. One sepsis secondary to skin infection (SI) and one surgical site infection (SSI) sepsis. Four of them with previous superficial SI. All had severe respiratory distress syndrome and haemoptysis that require ventilatory assistance, refractory septic shock, kidney and haematology failure, four liver failure, two coagulopathy. Initial empiric antimicrobial

therapy (AT) for respiratory sepsis: Ceftriaxone and Azythromycin iv; for skin associated sepsis: Cefradine iv and for SSI: Cefradine and Gentamycin iv. Outcome: four died in the first 48 h: three RS and SI before bacteriologic culture results were available and in the other two (RS and SSI) AT change to Vancomycin 2 gr iv bid. These patients died 8–45 days after. CA-MRSA (PBP2a positive) were isolated from all patients HEM, three PF from four RS and from one D. All susceptible to VA, CIP, SXT, GM, CLI, and resistant to OXA, E. Gene mecA positive, SCCmec type IV, Panton Valentine leucocidine gene + (LPV), enterotoxin A-B gene – and PFGE indistinguishable and closely related.
Conclusions: In our country like others, in a short period CA-MRSA infections were associated with unusual rate of severe and rapidly mortal cases mainly respiratory sepsis in young people, probably clonally related all LPV + that challenges the current empiric antimicrobial guidelines. Because their high virulence, this unsuspected emergent pathogen, constitutes a health care problem nonresolved yet.

P579 *S. aureus* community-acquired infections. Antibiotic resistance rates and macrolide resistance phenotypes

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Objectives: To estimate the antibiotic resistance rates of *S. aureus* strains isolated from community-acquired infections and to determine the macrolide resistance phenotypes.
Methods: We examined retrospectively 152 *S. aureus* strains isolated over a 2-year period (2001–02) from 152 clinical specimens received from patients with community-acquired infections as follows: 99 strains (65.1%) from abscesses, 18 strains (11.8%) from wound infections, 15 strains (9.9%) from skin and soft tissue infections and 20 strains (13.2%) from various other infections. Conventional methodology was used for identification to the species level and the susceptibility tests were performed using the disk diffusion method according to the NCCLS procedure (2000). MRSAs detection was achieved by oxacillin 1 μg disk and detection of PBP 2a by a slide latex agglutination assay. The macrolide resistance phenotypes were determined by the double disk method using erythromycin and clindamycin disks.
Results: A total of 44 (28.9%) MRSA strains were found, the penicillin resistance rate was 86.8% (132 strains) and we found no vancomycin or teicoplanine resistant *S. aureus* isolates. The resistance rates to other antibiotics were as follows for MRSA and MSSA respectively: ciprofloxacin 22.7–3.7%, erythromycin 20.5–13.9%, clindamycin 15.9–3.7%, cotrimoxazole 29.5–13.9%, fusidic acid 84.1–25.9% (CA-FSM 1996 guidelines), gentamicin 11.4–1.8%. Resistance to gentamicin was clearly associated ($P < 0.05$) with methicillin resistance. The macrolide resistant phenotypes for MRSA were cMLSB 78% – iMLSB 22% and for MSSA were cMLSB 27% – iMLSB 73%. Multiresistant strains were isolated among MRSA (10 strains, 22.7%).
Conclusions: In our area aminopenicillins are not useful in the empirical treatment of community-acquired *S. aureus* infections and their combinations with beta lactamase inhibitors must used with caution. Erythromycin, clindamycin and cotrimoxazole can be used in selected cases. The high prevalence of inducible macrolide resistant phenotype in MSSA raises questions about the use of clindamycin in infections caused by these *S. aureus* strains.

P580 An epidemic European fusidic acid resistant strain of *Staphylococcus aureus* carries the fusB determinant

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Objectives: Fusidic acid-resistant epidemic clonotypes of *Staphylococcus aureus* causing impetigo have recently been reported in several European countries. The genetic basis of fusidic acid resistance in these strains has not been determined, and it is unknown whether they constitute a single epidemic strain undergoing inter-country spread. To address this, representative epidemic strains were typed by Pulsed-Field Gel Electrophoresis (PFGE) and the genetic basis for their reduced susceptibility to fusidic acid was established.
Methods: PFGE-typing was performed according to the HARMONY protocol. Strains were examined for fusidic acid resistance polymorphisms in the fusA drug target by PCR amplification and DNA sequencing. For detection of the acquired staphylococcal fusidic acid-resistance determinant, fusB, southern hybridisation was employed to probe both total DNA and purified plasmid DNA preparations. Conjugational transfer capabilities were examined by filter-mating.
Results: PFGE-analysis of epidemic fusR clonotypes established that strains from Sweden, Norway, the United Kingdom, Denmark and Ireland represent a single clone. No mutations were detected in the fusA genes of this clonotype. Strains were positive for fusB in both total and purified plasmid DNA preparations, indicating that fusB is associated with a plasmid. Further characterisation of this replicon revealed that it was ~42 Kb in size, and incapable of transfer by self-mobilisation. Development of a sensitive and specific PCR-based assay for probing strains for fusB enabled rapid detection of the fusB determinant in further members of this clonotype.
Conclusions: The epidemic fusidic acid-resistant clonotypes of *S. aureus* described in several European countries actually constitute a single clonotype that is spreading in Europe. Fusidic acid resistance in this clonotype is mediated by carriage of the fusB resistance determinant on a large, nonconjugative plasmid.

MRSA and staphylococci: laboratory aspects

P581 Susceptibility of pulse field characterised methicillin-resistant *Staphylococcus aureus* to daptomycin

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Vxjö, S

Objectives: Daptomycin, the first antibiotic in the lipopeptide class, is in clinical development for the treatment of serious infections caused by Gram-positive pathogens. Daptomycin possesses potent *in vitro* and *in vivo* bactericidal activity against *Staphylococcus aureus* isolates, including those strains resistant to methicillin (MRSA), vancomycin (VRSA), and linezolid. This study is the first to test the potency of daptomycin against a set of clinical MSSA and MRSA isolates in Sweden.
Methods: The strains comprised 100 *S. aureus* from a reference collection at the Swedish Institute for Infectious Disease Control (SMI), 25 of which were MSSA (mecA-) and 75 of which were MRSA (mecA+). The MRSA strains were clinical isolates from single cases or minor outbreaks in Sweden (1998–99) and had had their Harmony-type and pulse field patterns characterised. Susceptibility testing was performed by the NCCLS broth microdilution methodology using commercial lyophilised panels.
Results: Daptomycin had a MIC90 of 1 mg/L for all strains tested regardless of their susceptibility to methicillin. No strain exhibited an MIC above 2 mg/L.
Conclusion: The results of this study demonstrate that daptomycin has potent activity against Swedish *S. aureus* isolates, regardless of the presence of the mecA gene. This suggests that daptomycin

Summary of results

MIC (mg/L)	MRSA	MSSA
0.25	1	0
0.50	39	14
1.00	33	11
2.00	2	0
Total	75	25

may provide an alternative to the limited antimicrobial agents available for the treatment of serious *S. aureus* infections.

P582 Usefulness of mec-associated dru sequences in monitoring the spread of highly clonal epidemic methicillin-resistant *Staphylococcus aureus* isolates in Scotland

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Objectives: The epidemic methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and -16, initially observed in England in the early 1990s, spread to Scotland by the mid 1990s. Since that time, reports of MRSA in Scotland have risen dramatically from 565 in 1995 to over 12 000 in 2001. EMRSA-15 and -16 account for 70 and 20% of these isolates, respectively. While PFGE typing has identified a number of EMRSA-15 and -16 clonal variants, epidemiological tracking is difficult because *c*. 50% of EMRSA-15 and 35% of EMRSA-16 isolates are indistinguishable by PFGE (pulsotypes PF15a and PF16a, respectively) and other typing methods. We evaluated the usefulness of mec-associated dru sequences as a more sensitive approach to tracking the persistence and spread of these 'clonal' epidemic MRSA isolates in Scotland.
Methods: EMRSA-15 and -16 cultures were collected from hospitals throughout Scotland. Sixty-nine isolates with PFGE pulsotypes PF15a and PF16a were selected for analysis of the mec-associated dru region. DNA sequences were aligned and interrelationships analysed using BioNumerics v. 3.5 (Sint-Martens-Latem, Belgium).
Results: Analysis of dru sequences allowed separation of the 69 PF15a and PF16a isolates into 19 specific subtypes. While some types were found in multiple hospitals, dru sequence comparisons identified instances of specific strain movement between hospitals in a given geographic region (i.e. hospital-specific types).
Conclusions: The mec-associated dru region has the potential for extensive variability both in sequence and in number of 40-bp tandem repeats. However, specific sequence types appear to be very stable over time. Analysis of dru sequences thus appears very promising as a means of identifying and tracking specific subtypes of otherwise indistinguishable epidemic MRSA isolates in Scotland. The ability to potentially monitor the specific movement of these epidemic MRSA within and between hospitals is a welcome addition to ongoing public health and infection control efforts to control the persistence and spread of these problem organisms.

P583 Detection of methicillin resistance in clinical isolates of *Staphylococcus* isolates: a comparative study between rapid PBP-2a test, conventional phenotypic tests and mec A gene detection by PCR

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Objectives: The study was conducted to delineate the prevalence of methicillin-resistant staphylococcal (MRS) nosocomial infections particularly coagulase-negative staphylococci (CoNS) among debilitated subjects using indwelling foreign bodies or subjected to repeated invasive medical procedures. Also the performance characteristics of several laboratory tests for detection of MRS, which is often heterogeneous, were evaluated.
Methods: The tests performed were the conventional phenotypic oxacillin disk diffusion, the oxacillin-salt agar dilution using modified National Committee for Clinical Laboratory Standards (NCCLS) guidelines, and a novel rapid latex agglutination test that detects penicillin binding protein (PBP-2a LA Oxoid), the gene product of mec A. Detection of mec A gene by PCR was used as the 'gold standard' for comparing the assays.
Results: Methicillin-resistance (MR) was detected in 40% of 98 staphylococcal clinical isolates (38% in 55 *S. aureus* strains and 42% in 43 CoNS). Among the *S. aureus* isolates the PBP 2a LA test and the oxacillin-salt agar dilution test showed higher sensitivity and specificity in detection of MRSA compared with disk diffusion test (sensitivity 95, 90 and 85% and specificity 100, 100 and 88% respectively). However, among CoNS the PBP2a LA showed a significantly lower sensitivity but a higher specificity for detection of MR compared with the two phenotypic tests that demonstrated equivocal results (sensitivity 50, 83 and 83% and specificity 88, 80 and 80% respectively). The PBP2aLA test also correctly identified six heterogeneous strains of *S. aureus* isolates, which were not likewise detected by one or both of phenotypic tests.
Conclusion: The PBP2aLA test is recommended for reliable accurate and rapid identification of MRSA and can be easily incorporated as a routine laboratory test when PCR is not feasible. Further evaluation is recommended for its utilisation in detecting MR among CoNS, which is better diagnosed, by conventional phenotypic test as well as molecular methods.

P584 Use of polyvalent anti-staphylococcal bacteriophages for the biocontrol of methicillin-resistant *Staphylococcus aureus* and other staphylococci

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Objectives: The emergence of drug resistant staphylococci has prompted the need for alternate controls other than antibiotics. The objectives of this study were to isolate and characterise anti-staphylococcal bacteriophages and to test their host-range against a broad range of staphylococci including antibiotic-resistant *S. aureus* such as MRSA and VRSA.
Methods: General bacteriophage isolation procedures including enrichment, filtration, plaque assay and broth-culture bacterial challenge assays were performed. This was followed by restriction analysis and electron microscopy according to standard protocols. Challenge experiments were carried out *in vitro* and anti-microbial efficacy was evaluated by plate count.
Results: A number of anti-staphylococcal phages, which effectively kill documented typed isolates of MRSA, GISA, VISA, VRSA and teicoplanin resistant *S. aureus* were successfully collected and characterised. Those phages exhibiting broadest host range and largest plaque size were retained for further use. These termed 'polyvalent' phages were phage K, phage CS1 and phage DW2. In the case of phage K, the host range extends well beyond the species *S. aureus* to include the coagulase-negative staphylococci *S. caprea, S. hyicus, S. epidermidis, S. captis, S. haemolyticus* and *S. chromogenes*. On the basis of genomic and electron microscopic analysis, the three phages fall into the myoviridae family in the classification scheme of the International Committee on Virus Taxonomy. As expected, these phages have no effect whatsoever on bacteria outside of the genus Staphylococcus nor do they affect eukaryotic cells. In general, phage K had the broadest host range. In cases where phage resistance occurred in staphylococci, it was demonstrated that this was because of indigenous staphylococcal restriction-modification systems. Phages could be modified to circumvent these systems. Infusion of the phage into a bismuth-based cream resulted in strong anti staphylococcal activity from

the cream. Similarly phages were incorporated into handwash where they also exhibited a strong anti-staphylococcal activity.
Conclusions: The results indicate that the phages used in this study are capable of significantly reducing the numbers of recently-emerged antibiotic-resistant staphylococci from Irish hospitals.

P585 Evaluation of cefoxitin MIC determination to detect low-level methicillin-resistant *Staphylococcus aureus* (MRSA) by the automatic system Phoenix

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Background and objectives: Phenotypic detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA) may fail when relying only on oxacillin susceptibility, regardless of whether determined by disk diffusion or by broth dilution. In a previous study, testing of cefoxitin by disk diffusion proved to be a powerful tool to detect MRSA. The aim of this study was to evaluate the possibility to identify a cefoxitin concentration which would enhance the ability to detect MRSA.
Methods: Seventy-seven *Staphylococcus aureus* (SA) isolates and seven SA reference strains were tested by oxacillin disk diffusion methods and by the Phoenix System panel PMIC/ID25 for staphylococci which measures broth dilutions MICs including cefoxitin MICs (FOX-MIC) in the range of concentrations from 1 to 64 mg/L. Isolates were classified as being MRSA or not according to the presence or absence of the mecA gene.
Results: Eleven SA were mecA negative: nine were methicillin-susceptible and two borderline. Oxacillin MICs (OXA-MIC) and FOX-MICs ranged respectively from $\leq$0.25 to 1 mg/L and from 2 to 4 mg/L. Seventy-three SA were mecA positive: 38 with OXA-MICs $\geq$4 mg/L and 35 with low-level OXA-MICs from 0.5 to 2 mg/L. For 70 of 72, FOX-MICs ranged from 8 to >64 mg/L, for two of 72 FOX-MIC was 2 mg/L. Therefore, a 8 mg/L cefoxitin cut-off value allowed detection of 100% of the MRSA detected by OXA-MICs and of 94% of those with low-level OXA-MICs (<4 mg/L).
Conclusions: Cefoxitin MIC determination increases the rate of detection of low-level MRSA. A >4 mg/L cefoxitin cut-off MIC is 100% predictive of MRSA in low-level OXA-MICs SA according to a yearlong practice in our laboratory.

P586 The stability of mecA genes and MIC values of methicillin in passage-selected vancomycin-resistant EMRSA strains isolated in the UK

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Objectives: Nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA) strains are associated with significant morbidity and mortality. Such strains have been characterised by their phage type and antibiogram and, in the UK, are termed epidemic MRSA (EMRSA). Seventeen epidemic MRSA strains (EMRSA 1-17) have been documented. The purpose of the present study was to ascertain if representatives of strains of EMRSA 1-17 could grow in increasing concentrations of vancomycin and whether this was associated with consistent changes in genotype and phenotype.
Methods: Vancomycin susceptible isolates previously reported and identified to be EMRSA (1-17) and their vancomycin-resistant derivatives were examined. Vancomycin-resistant derivatives were obtained by serial passage of the parental strains in nutrient broth with increasing concentrations of vancomycin producing vancomycin- resistant isolates. Antibiotic sensitivities (vancomycin, methicillin) were determined by *E*-test performed according to the manufacturers recommendations. The stability of mecA genes was examined by using PCR. Cell wall changes were demonstrated by transmission electron microscopy.
Results: Six strains became vancomycin resistant, three became vancomycin intermediate and seven remained susceptible. The vancomycin MICs for the vancomycin resistant clones ranged from 24 to 32 μg/mL, and were associated with decreased methicillin susceptibilities and increased cell wall thickness. Four out of the six vancomycin-resistant derivatives became sensitive to methicillin (MIC 0.75–1 mg/mL) ($P < 0.05$) and the mecA gene could not be detected using PCR.
Conclusions: In conclusion, we found that, in vitro, decreased susceptibility to vancomycin was readily inducible following exposure to sub-inhibitory concentrations of vancomycin. This may be a strain specific phenomenon. Development of vancomycin resistance affects resistance to other antimicrobial agents, including methicillin and affects the stability of the *S. aureus* mecA gene. Southern hybridisation of mecA should be investigated to confirm the points and size of the deletion.

P587 Susceptibity of methicillin-resistant strains of *S. aureus* isolates from nasal carrier to mupirocin and bacitracin in a Tehran hospital, Iran

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Objective: The aim of this study was to determine prevalence of nasal colonisation with *Staphylococcus aureus* and susceptibility of isolates to mupirocin and bacitracin.
Methods: Of 1000 Health Care workers (HVWs) of Milad hospital 774 participated in our study nasal swabs were taken from both of nose of all participants. All specimen were processed in microbiology laboratory within 2 h. Culture performed on Manitol salt gar. Suspected colonies of *S. aureus* were subcultured on sheep blood agar. Identification of *S. aureus* was based on the morphology of colonies, a positive coagulase test and other tests. We performed susceptibility testing by disk diffusion method as recommended by National Committee for Clinical Laboratories Standards. (NCCLS).
Results: We screened 774 HCWs for nasal carriage of *S. aureus*. Among HCWs 241 (31%) were colonised by *S. aureus*. We found significantly more male HCWs with *S. aureus* (34.9% vs. 27%, $P < 0.05$). In some department there was a high frequency for nasal carriage of *S. aureus*. For example in Postintensive Care Units (PICU) and general operating room 53 and 37% were carriage of *S. aureus* respectively. About 7% of all isolates of *S. aureus* were resistant to Methicillin. All strains of *S. aureus* were susceptible to mupirocin (Mast Diagnostic, Mupirocin5) and bacitracin. Resistance to other antibiotics was: penicillin 97% erythromycin 9.5% gentamycin 9% tetracycline 40% clindamycin 5% co-trimoxazol 7.5%, choloramphenicol 1% ciprofloxacin 5.4% and vacomycin 0%.
Conclusion: This study reveals that prevalence of MRSA is not high in HCWs and all MRSA were susceptible to mupirocin and bacitracin. Mupirocin could be used as nasal ointment for eradication of MRSA.

P588 Aminoglycoside-resistance genes and phenotypes in multiresistant nosocomial strains of *Staphylococcus aureus*

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Objectives: Aminoglycosides are potent bactericidal agents that play a role in chemotherapy of serious staphylococcal infections. In this study, multidrug-resistant (MDR) nosocomial strains of *Staphylococcus aureus* from an Italian hospital, were analysed for susceptibility to several aminoglycosides and for the presence of aminoglycoside resistance genes.
Methods: Studied strains: a collection of 28 MDR strains of *S. aureus*, isolated from nosocomial infections at the Siena University Hospital (Italy), that included representatives of genotypically

different (as per results of spa and coa typing) *S. aureus* strains circulating in the hospital (some of which had caused nosocomial outbreaks). *In vitro* susceptibility testing was determined by a microdilution method according to NCCLS guidelines. Aminoglycoside resistance genes were detected by dot-blot hybridisation and PCR analysis.

Results: Of the 28 MDR strains of *S. aureus* included in this study, 18 were methicillin-susceptible (MSSA) and 10 methicillin-resistant (MRSA). The resistance rates to aminoglycosides were: gentamicin and tobramycin, 50% (22% in MSSA and 100% in MRSA); amikacin, 18% (11% in MSSA and 30% in MRSA); netilmicin 7% (0% in MSSA and 20% in MRSA). All the 14 aminoglycoside-resistant (AR) strains harbored the aac(6′)-Ie-aph(2′′) resistance gene, encoding the bifunctional AAC(6′)-APH(2′′) enzyme; of them, 10 (71%) also carried the aph(3′)-IIIa aminoglycoside phosphotransferase gene, and 4 (29%) the ant(4′)-Ia aminoglycoside nucleotidyl-transferase gene. Multiple resistance genes were present in most AR strains (three of four MSSA AR strains, and all 10 MRSA strains). Resistance genes were never detected in aminoglycoside susceptible strains.

Conclusions: High resistance rates to gentamicin and tobramycin were observed in MDR nosocomial strains of *S. aureus*, especially in MRSA strains. Netilmicin was the most effective anti-staphylococcal aminoglycoside, and retained activity against most gentamicin- and tobramycin-resistant strains, including MRSA. The aac(6′)-Ie-aph(2′′) gene was the most common resistance determinant, followed by the aph(3′)-IIIa and ant(4′)-Ia. The latter genes were never observed alone. The distribution of aminoglycoside resistance genes revealed differences in comparison with other epidemiological settings.

Acknowledgements: This work was supported by a research grant from Essex Italia S.p.a.

P589 Frequency of glycopeptide intermediate and hetero-glycopeptide intermediate *Staphylococcus aureus* among methicillin-resistant strains isolated in 2002 in a Warsaw university hospital

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Objectives: The problem of glycopeptide intermediate *Staphylococcus aureus* (GISA) and hetero-GISA was first found by Hiramatsu group. They showed, that in the case of infections caused by GISA strains, glycopeptide antibiotics were not effective. The inefficacy of glycopeptides against h-GISA strains was not fully confirmed, but they were presumably precursors of GISA. Because the usage of glycopeptides in hospitals is still very high, it seems that monitoring of both types of *S. aureus* strains is necessary.

Methods: In the presented work, the 103 methicillin-resistant strains of *S. aureus* (MRSA) isolated during 1 year (2002) were examined. All strains were isolated from patients from different wards of the one of Warsaw University hospitals, the Center of Injuries Treatment. The reference Keiichi Hiramatsu strains Mu50 and Mu3 were also used, as well as reference susceptible strain FDA 209P. First, the preliminary selection was performed, using a sector of BHI-agar plate with 4 mg/L of vancomycin and undiluted suspension of strain. For all strains that showed growth, MICs for vancomycin were examined, performing *E*-tests. Strains with MIC values of vancomycin <8 mg/L were examined if they are h-VISA. The population analysis and modifications of the method were performed. The consecutive dilutions of bacterial strain were plated on the growing concentrations of vancomycin. At the same time reference strains were examined.

Results: From investigated 103 MRSA only 18 strains grew in the preliminary selection. The MICs of vancomycin for them were lower than 8 mg/L, but higher than 1 mg/L. For all of them the population analysis was performed. The course of population analysis curve in the case of five of the examined strains suggested that they are h-VISA. In the case of some clinical as well as standard strains (susceptible, GISA and especially h-GISA) the start inoculum significantly influenced a shape of the growth curve obtained as a result of the population analysis. The obtained results of frequency of VISA and h-VISA in 2002 were compared with results obtained for *S. aureus* strains obtained in former years in the same laboratory.

Conclusions: The method of detection of h-VISA still requires improvements. There was observed no increase in the frequency of VISA and h-VISA in the investigated hospital in last years, despite that consumption of glycopeptides was not reduced.

P590 Methicillin-resistance expression mediated by subinhibitory concentrations of fluoroquinolones in MRSA

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Objectives: To know how minimal fluoroquinolone concentrations modify the expression of methicillin resistance in mecA (+) *S. aureus*.

Methods: Two mecA (+) *S. aureus* clinical strains were grown for 10 days in Mueller Hinton broth containing norfloxacin (NFX) at 1/8 MIC and in NFX free Mueller Hinton broth. Aliquots were obtained from the cultured broth and inoculated to a fresh broth every 24 h. Hundred millilitres were obtained every 24 h from broths with and without NFX, diluted and spread onto Mueller Hinton agar plates with 6 and 32 μg/mL of oxacillin and oxacillin-free.

Results: The proportion of microorganisms expressing methicillin-resistance (colonies on oxacillin-containing agar/colonies on oxacillin-free agar), when the microorganism was grown in NFX-free both remained stable along the whole study (3×10^{-5}–3×10^{-6}). When the microorganism was grown in NFX-containing broth, methicillin resistance expression increased during the experiment (5×10^{-2} the second day, 1.5×10^{-1} the fourth day and 50% the fifth day. The level of resistance also increased in the second strain. In NFX-free broth, colonies growing on Mueller Hinton agar with 32 μg/mL of oxacillin remained $<5 \times 10^{-6}$. In NFX-containing broth, proportion was $<5 \times 10^{-6}$ the first day, 2×10^{-3} the fourth day and 3×10^{-1} the fifth day.

Conclusions: Previous studies suggest the high frequency of fluoroquinolone and methicillin co-resistance derives from the use of fluoroquinolones against MRSA, so co-resistant clona have remained and expanded. This study show that this high frequency can also derive from the increased expression of methicillin resistance in MRSA.

P591 Screening criteria for glycopeptide susceptibility of methicillin-resistant *Staphylococcus aureus* revisited for two periods of blood cultures isolates

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Objectives: Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates exhibiting intermediate susceptibility to glycopeptides (GISA) is challenging for clinical microbiology laboratories.

Methods: We compared three different screening assays for evaluating trends in decreased glycopeptide susceptibility during two periods. Ninety-four and 95 consecutive MRSA blood isolates from 189 bacteremic patients collected during periods A (1989–94) and B (1999–2001), respectively, were screened in parallel for vancomycin or teicoplanin susceptibility by glycopeptide-containing Brain-Heart Infusion agar (BHIA) tests, Etest MICs performed

at a standard (0.5 McFarland) inoculum on Mueller-Hinton (MHA), or high (2.0 McFarland) inoculum or BHIA, respectively. Any MRSA isolate yielding <50 CFU (representing $<10^{-6}$ of the plated inoculum) on either BHIA containing 2 mg/L of vancomycin (V2-BHIA) or 5 mg/L of teicoplanin (T5-BHIA) at 48 h at 37°C was considered as fully susceptible to vancomycin or teicoplanin, respectively. All MRSA isolates were typed by pulsed-field gel electrophoresis (PFGE).

Results: The proportion of MRSA isolates yielding >50 CFU on either V2-BHIA or T5-BHIA significantly ($P < 0.01$) increased from seven of 94 (7.4%) or eight of 94 (8.5%) in period A to 16 of 95 (16.8%) or 14 of 95 (14.7%) in period B, respectively. Vancomycin Etests MICs on MHA were of lower sensitivity (<65%), but high specificity (99%) on period B isolates compared with those on V2-BHIA. Vancomycin Etests MICs on BHIA were of a higher sensitivity (81%) but lower specificity (65%) compared with those on V2-BHIA, because of an unexpectedly high number of false positive isolates (28/95). Eighty-three per cent of period B isolates (including the potential GISA isolates) analysed by PFGE belonged to a single predominant MRSA clonotype that essentially replaced all four major MRSA clonotypes formerly present during period A.

Conclusions: Screening of decreased susceptibility to vancomycin or teicoplanin on V2-BHIA or T5-BHIA, respectively, may represent simple low-cost alternatives to Etest MICs, minimising the risk of missing potential GISA isolates.

P592 Sequence analysis of the polymorphic region X of protein A in a methicillin-sensitive *Staphylococcus aureus* population isolated from the airways of cystic fibrosis patients

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Objectives: Recently, we demonstrated a high prevalence and long-term persistence of methicillin-sensitive *Staphylococcus aureus* (MSSA) in the airways of cystic fibrosis (CF) patients. Pulsed-field gel electrophoresis (PFGE) distinguished six prevalent clonal lineages (Dice similarity coefficient >85%; 36 isolates) and 35 individual clones. Single locus DNA-sequencing of the *S. aureus* polymorphic region X of protein A (spa) was used to evaluate a faster and more feasible method. The region X consists of a variable number of 21–27 bp repeats. The goals of the present study were: (i) to determine the spa-types of persistent MSSA strains in a defined patient group and (ii) to compare PFGE and spa-typing as a tool for molecular typing for long-term observations.

Methods: Seventy-one MSSA isolates collected during a 6-year longitudinal study from 50 patients were analysed. The following primers were used for amplification and sequencing: spa-1113f and spa-1496r. Spa-types were determined with the Ridom StaphType? software (Ridom GmbH Würzburg, Germany), which automatically assigns numeric spa-repeat and -type codes. The software synchronizes with an accompanying website (http://www.ridom.de/spaserver) to ensure a uniform terminology code.

Results: In total, 48 spa-types were distinguished by sequencing all 71 MSSA isolates. Twenty-four spa-types occurred within the 6 -by PFGE- defined prevalent clonal lineages (36 isolates). The remaining 35 individual isolates showed 24 different spa-types. As the overall composition of spa-types within a clonal lineage was very similar, it is conceivable that the differing spa-types could be explained by micro-evolution of the spa-region: deletion of single or several repeats (11 strains), duplication of repeats (three strains) or point-mutations (three strains). However, four strains displayed totally different spa-types.

Conclusions: Spa-typing showed a high diversity in a MSSA population isolated from a defined patient group. The discriminatory power of spa-typing was comparable with PFGE results, and overall the same clonal lineages were detected. However, gains, losses of repeats, and point-mutations occurred in strains within the six prevalent clonal lineages indicating micro-evolution of the spa-region. Therefore, if spa-typing is used as a molecular typing method, micro-evolutionary events have to be taken into account when analyzing the data, especially if isolates from long-term observations are to be compared.

P593 Ridom StaphType software: facing the challenge of inter-laboratory evaluation of sequence-based MRSA typing

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Objectives: *Staphylococcus aureus* is a major pathogen that causes a wide range of infectious diseases. Since its first identification in the early 1960s, methicillin-resistant *S. aureus* (MRSA) has become a major concern. In order to manage increasing MRSA numbers effective typing protocols have to be applied. Typing of MRSAs by analysis of protein A (spa) gene repeat sequences is reproducible; it has a high discriminatory power; the data generated are highly portable (digital data management), and delivers results congruent to other typing methods (e.g. PFGE). One major drawback of repeat typing is that automatic Internet based repeat code assignment has not been available until now.

Methods: In our study, we used the recently developed Ridom StaphType software (Ridom GmbH, Würzburg, Germany; version 1.0) which meets these requirements. The performance of the software was evaluated using spa sequences obtained from 220 independent MRSA isolates, which were collected at the two German university hospitals in Würzburg ($n = 107$, collected from 7/2001–6/2002) and Münster ($n = 113$, 1/2002–12/2002).

Results: Assignment of spa-types by Ridom StaphType was possible for all 220 isolates tested. In total, three predominant spa-types, two of them identical in both institutions, accounting for about 50% of all isolates were observed. The remaining isolates showed sporadic and different spa-types in both hospitals. The use of Ridom StaphType greatly reduced time needed for generation of data. Furthermore, the software allowed for easy sequence chromatogram editing and flexible data management and retrieval.

Conclusion: In conclusion, automatic repeat assignment by Ridom StaphType overcomes shortcomings of current spa-typing and will promote wide-spread use of the method and inter-laboratory exchange of data. Finally and most important the software helps to take evidence based actions (e.g. infectious disease control measurements) in hospital settings.

P594 Intracellular killing of drug-resistant *Staphylococcus aureus* by different antibiotics

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Objectives: The emergence of epidemic multiple resistant *Staphylococcus aureus* (EMRSA) and vancomycin intermediate susceptible (VISA) strains has heightened concerns about the treatment of associated infections. It has also been suggested that intracellular survival of *S. aureus* in phagocytic cells play an important role in the pathogenesis of associated infections, moreover it may contribute to failure of antimicrobial chemotherapy. Therefore intracellularly active antibiotics may be of value in treatment. This study was aimed to investigate the effect of linezolid, moxifloxacin and vancomycin on the intracellular survival of *S. aureus*.

Methods: Two clinical isolates of methicillin resistant *S. aureus* (EMRSA 16 also resistant to macrolids and VISA 3759.v with intermediate susceptibility to vancomycin) were tested in J774 macrophage cell line. Susceptibility of the strains was determined by NCCLS broth microdilution method. Cells were infected with bacteria opsonised with 10% normal pooled human serum in Hank s balanced salt solution supplemented with 1% gelatine. After 2 h of incubation the supernatant was discarded and cells were washed three times in the buffer. Thereafter, antibiotics were

added to the cells at the following concentration: 0 × MIC, 1/2 × MIC, 1 × MIC, 2 × MIC in triplicate. Samples were taken 1, 2, 3, 4 h after antibiotic addition. Cells were washed three times with phosphate buffered saline, then lysed with distilled water. Bacterial count of the cell lysate was determined by the microdilution followed by blood agar plating. Each test was performed three times.
Results: In the presence of vancomycin, intracellular killing was not enhanced, as linezolid and moxifloxacin facilitated the clearance of live intracellular bacteria even at sub-MIC concentrations.
Discussion: Whether this enhancement is due to inhibition of intracellular bacterial multiplication, or due to an effect on the host cells' killing mechanisms, or both remains to be seen. According to our observation linezolid and moxifloxacin have been shown bioactive intracellularly against MRSA.
Acknowledgement: The project was supported by the FEMS.

P595 MRSA with Panton-Valentine-Leukocidin in Germany

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MRSA containing lukS-lukF (Panton-Valentine-Leukocidin) have been reported as community acquired MRSA from US, Australia and Europe (France and Switzerland). The European cMRSA are clearly different by genomic background from isolates from other continents. Here we report on emergence and of lukS-lukF MRSA in Germany.
Methodology: Typing of MRSA from hospital and community all over Germany as National reference center by means of SmaI-macrorestriction patterns, MLST (according to www.mlst.net) and spa-sequence (www.ridom.de). PCR demonstration of lukS-lukF, of resistance genes and the agr locus.
Results: From autumn 2002 until December 2003 there were nine sporadic infections in hospitals and 10 cases of deep seated skin infections with lukS-lukF MRSA in the community in different geographical areas of Germany. The isolates exhibited an unique typing pattern with regard to SmaI-macrorestriction, MLST (ST80) and spa (type 46). The exhibited resistance to oxacillin, ciprofloxacin, oxytetracycline (tetM) and fusidic acid (far-1 coded efflux). They were negative for the agr-locus.
Conclusion: The typing pattern of lukS-lukF MRSA from until now sporadic cases of infections corresponds to that known for MRSA from France and Switzerland. Further characteristics are far-1 mediated fusidic acid resistance (not in other MRSA from Central Europe) and deletion of agr.

P596 Characterisation of methicillin-resistant *Staphylococcus aureus* isolated at a policlinic in Bari, Italy

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Objectives: To evaluate several methods for the detection of methicillin resistance of *Staphylococcus aureus* (MRSA) isolated in Bari, South Italy and to characterise the strains by genotyping methods.
Methods: Forty-eight strains of *S. aureus* isolated from clinical samples and different wards of the Policlinico Hospital (Bari, South Italy) were evaluated for methicillin resistance by the PBP latex agglutination test (Oxoid, Milan, Italy), the oxacillin-salt agar screen test, the results of an automated system (Microscan Pos-Panels, Dade Behring, Milan, Italy), the determination of MIC values to methicillin by the agar dilution and the detection of the gene MecA by PCR. The strains were also analysed by PCR for the mec-associated hypervariable region (HVR-PCR) and by Random Amplified Polymorphic DNA (RAPD) analysis. Thirty-one strains were also tested for the production of enteroxins A, B, C and D by the Reverse Passive Agglutination Assay (RPLA, Oxoid).
Results: All the 48 strains of *S. aureus* resulted MRSA by the oxacillin agar screen test, the PBP2a latex agglutination test, the automated system and by the mecA-PCR. The MIC90 for oxacillin was >256 mg/L. Both RAPD and HVR-PCR clustered the strains in three main genotypes. Eight different resistotypes were found. The strains isolated in the surgical units belonged to the same RAPD and HVR group and displayed the same resistotype. Fifteen of the 31 strains (48.4%) of *S. aureus* tested resulted enterotoxins producers (11 were producers of the enterotoxin A, three of the enterotoxin B and one of the enterotoxin D).
Conclusion: In previous studies MRSA accounted for the 40.1% of the *S. aureus* isolated at Policlinico hospital. In this survey all the methods used for MRSA detection produced concordant results with mecA-PCR that is considered the gold standard for methicillin detection. In addition RAPD and HVR-PCR resulted well correlated to each other in order to cluster the strains and to correlate the groups to the different hospital wards. The methods described and the pattern of antimicrobial susceptibility may be useful for a rapid and inexpensive typing of MRSA in the hospital, especially for the comparison of the strains isolated from different wards and for identification of clonal spread within a hospital.

Parasitic diseases

P597 Detection of *Giardia lamblia* in stool samples by enzyme immunoassays

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Background: *Giardia lamblia* (GL) protozoa type is the most frequent parasite in the digestive tract. Transmission of GL is faecal-oral, and interhuman contact has the greatest significance in bad hyginenic and sanitary conditions. We made comparison of two methods for determination of GL cyst in stool.
Methods: Three successive stool samples were examined by direct microscopy of native preparations with Lugol and after applying of formaline-ethyl acetate concentracion technique. Second method for detection of GL antigens in stool specimens was enzyme immunoassays (EIAs) (Ridascreen Giardia, R-Biopharm, Germany). Investigation was performed in July 2003 on risk group of patients in Special hospital for retarded children in Kulina. Stool samples of 104 patients were examined.
Results: stool samples of all patients were examined by conventional microscopy examination (CME), and by EIAs. From total score of examined samples 101 was negative to GL, using both methods. GL was detected at three stool samples using both methods. Three stool samples were positive using EIAs, and negative using CME. In repeated examination of stool samples using CME, there was no change in results. We conclude that EIAs method is more sensitive for determination of GL cyst in stool. In risk group of retarded children is hard to perform examination of at least three successive stool samples using CME on presence of GL. By using a more sensitive method one can obtain results examining one stool sample, which has great diagnostic and epidemiological importance.

P598 Epidemiological features of intestinal parasitosis in a children's hospital in Athens

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Background: Intestinal parasitosis is a major problem in children, which is responsible for diarrhoea and nutritional deficiencies. Environmental, socioeconomic, demographic and health related behavior is known to influence the transmission and distribution of these infections.
Objective: Our goal was to determine the prevalence of intestinal parasitic infections among patients in a children's hospital in Athens and its possible association with demographic and socioeconomic parameters.
Material and methods: During a period of 6 years (27/9/97–27/9/03) a total of 3022 samples were examined in our laboratory (1720 of stool specimens and 1302 of scotch tests). The study population (both Greeks and immigrants from developing countries) was children between 3 and 15 years old, which either examined in the outpatient's clinic or hospitalised. These patients had one or more of the following symptoms: diarrhoea, abdominal pain, eosinophilia, pruritus. All specimens were examined in direct microscopy. In addition for all stool specimens the formalin – ether sedimentation technique and trichromic stain were used.
Results: Of the 3022 children examined, 212 (7%) were found positive for various intestinal parasites. Six (6) different species of helminthes and protozoa were found among the samples. By far the highest frequency 162 cases (76.5%) was noted for *Enterobius vermicularis*, followed by *Giardia lamblia* 30 cases (14.2%), *Entamoeba histolytica* 10 cases (4.7%), *Ascaris lumbricoides* six cases (2.8%), *Trichuris trichiura* two cases (0.9%) and *Taenia saginata* two cases (0.9%).
Conclusion: The frequency of intestinal parasitic infection in Greece, although it is relatively low, it is not rare. Fifty-five per cent of the 212 cases that were found positive belonged to immigrants coming from developing countries. The above results indicate that education policies should be taken and domestic and personal hygiene should be improved as well.

P599 Survey of intestinal parasitic infections among physical and mental retardees in a maintenance centre, Taft

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Introduction: There are more than 453 070 individuals suffering from physical and mental retardation in Iran, those who need expensive health services and maintenance facilities. Because of their mental, physiological and physical problems in handling personal health care and also living in mass population, they are always at high risk in acquiring contagious infections. In order to decrease their infection rate and then to treat them, it seems that searching their intestine for parasitic infection is highly necessary. The general purpose of this descriptive study was to determine the prevalence of intestinal parasitic infections among the physical and mental retardation's to encourage their sponsors for controlling and more health care on them.
Material and methods: The current descriptive and cross-sectional study was performed on 86 mental and physical retardation patients. Following completion questionnaire form of cases, three stool samples were collected from each case for direct examination using wet-mount and formaline–ether concentration methods. Data was analysed using SPSS software.
Result: 54.7% of cases included female, 34.9% had more than 20 years old and 65.8% needed the camping observation. 45.3% of cases had previous history of infectious disease, that highest ratio was related to cutaneous fungal disease (25.6%). In total, 48.8% were infected with different types of intestinal parasites and 18.6% had more than one parasite. The parasite frequency, which were detected is followed: *Entamoeba coli* 32.6%, *Giardia lamblia* 18.6%, *Chilomastix mesnili* 11.6%, *Lodamoeba butschlii* 8.1%, *Blastocystis hominis* 1.2% and oxyour 1.2%. There was not seen any statistical significant differences of infection rate between male and female cases. The 20-year-old and more age group showed the highest rate of parasitic infection (16.5%). There was seen a statistical significant differences in infection rates between camping and noncamping individual ($P < 0.05$).
Conclusion: Fifty per cent infection suggest needs of control and supervision for health care services and facilities to improve their personal health and also prevention of contagious contacts.

P600 Intestinal parasites in a paediatric hospital population in Madrid (Spain), January 2002 to October 2003

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Madrid, E

Objectives: Parasitic diseases are important causes of chronic diarrhea and low growth in childhood. The aim of this study was to analyse and present the incidence of fecal parasites in pediatric population in our area.
Methods: From January 2002 to October 2003 in our laboratory 2710 faecal samples were examined for the presence of parasitic pathogens. We studied 721 consecutive outpatients (mean age 4.8 years, range 0.1 months–16 years) who were referred to our Paediatric Department and considered for recruitment into this analysis.
Results: The following parasites were detected. *Blastocystis hominis* (105); *Chilomastix mesnilii* (10); *Dientamoeba fragilis* (3); *Endolimax nana* (88); *Entamoeba coli* (103); *Entamoeba hartmanii* (24); *Entamoeba histolytica* (34); *Enterobius vermicularis* (3); *Giardia intestinalis* (156); *Schistosoma mansoni* (1); *Hymenolepis nana* (89); *Iodamoeba butschlii* (12); *Isospora belli* (1); *Paragonimus* sp. (2); *Strongyloides stercoralis* (10); *Taenia* sp. (2); *Trichuris trichiura* (41); Hookworms (5); *Ascaris lumbricoides* (22). Parasitologic findings were proved in 19.37% of all examined samples (29.54% of total patients). No significant differences was observed when we analysed per sex. In spite of the greater number of samples was found in the range of 2 years old or less, the highest positive rate was found in the range of 7–10 years old. Of the total number of faecal samples, in 383 (53.7%) pathogen parasites were detected, while in 330 (46.2%) nonpathogen parasites were found. *Giardia intestinalis* was the most commonly pathogen isolated, followed by *Hymenolepis nana* and *Entamoeba histolytica*, whereas *Blastocystis hominis* and *Entamoeba coli* were the main nonpathogen parasites detected.
Conclusion: From these results, we could conclude intestinal parasitic diseases are important causes of morbility in childhood. We observed discrepancies between age ranges in which diarrhoeas because of parasites are mainly suspected and age ranges in which positive rates are higher.

P601 A 3-year descriptive study of intestinal parasite infections in outpatients in Madrid, Spain (2000–02)

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Madrid, E

Background: Intestinal parasite infections have increased in our country mainly because of easier access to international travel and immigration.
Objective: To know the prevalence of intestinal parasite infections in outpatients attending to a health area (Area 1, Madrid, Spain).
Patients and methods: 11 016 faecal samples (10 346 stools, 604 perianal swabs and 66 adhesive tapes for diagnosis of pinworms) were processed from 5757 patients between January 2000 and December 2002. Origin of samples and seasonal incidence of the parasites found together with epidemiological data as gender, age and geographical origin of infected subjects were analysed. Stools were concentrated using a disposable parasite concentrator with formalin-ethyl acetate (Biosepar, Germany). Coccidian oocysts were screened on direct and concentrated faecal smears stained by a modified Kinyoun acid-fast staining.
Results: 53.3% of parasitised patients were from foreign origin, mainly from South America (91.7%; of which 79.8% were Ecuado-

rian subjects). The overall prevalence of intestinal parasitisms was 15.6% (1723 faecal specimens belonging to 882 patients with 2353 parasites identified). The prevalence of the parasites found was: *Blastocystis hominis* (34.1%), *Giardia intestinalis* (17%), *Entamoeba coli* (15.6%), *Endolimax nana* (14.7%), *Enterobius vermicularis* (4.6%), *Trichuris trichiura* (3.9%), *Cryptosporidium parvum* (2.6%), *Entamoeba histolytica/dispar* (1.8%), *Strongyloides stercoralis* (1.3%), *Ascaris lumbricoides* (1.2%), *Hymenolepis nana* (1.2%), *Lodamoeba butschlii* (0.6%), *Taenia saginata* (0.5%), hookworms (0.4%) and *Chilomastix mesnili* (0.2%). Considering only the pathogen species, *Giardia intestinalis* was the most prevalent (48.8%) followed by *Enterobius vermicularis* (13.2%), *Trichuris trichiura* (11.4%), *Cryptosporidium parvum* (7.6%), *Entamoeba histolytica/dispar* (5.3%), *Strongyloides stercoralis* (3.8%), *Ascaris lumbricoides* (3.5%), *Hymenolepis nana* (3.5%), *Taenia saginata* (1.6%) and hookworms (1.2%). The frequency of double, triple, quadruple and quintuple infections was 19.7, 5.9, 1.8 and 0.4%, respectively. Samples processed and parasite detected in 2002 have increased by 82.7 and 166.7%, respectively with regard to 2000.
Conclusions: Our findings suggest that parasitic infections are still a public health problem with a high prevalence. The knowledge of the situation in each area facilitates its management and control.

P602 Enteropathogenic bacteria isolated from patients with diarrhoea: frequency of isolation and susceptibility testing to commonly used antibiotics in a Tehran hospital: a 1-year study

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Objectives: The aim of this study was to determine bacterial aetiology of the diarrhoea, frequency of isolated various enteric pathogens and susceptibility to commonly used antibiotics.
Methods: During 1 year study from November 2001 to November 2003, in total 2291 stool specimens were examined microscopically in microbiology laboratory. All specimens inoculated to routine microbiological cultures media including: XLD, Selenit F MacKonky agar Hekton Enteric and SS agar. All isolated bacteria identified by biochemical tests and stereotyped by relevant antisera (Bahar Afshan Company) Susceptibility testing performed by disk diffusion method as recommended by NCCLS.
Results: Of 2991 stool sample 123 enteropathogenic bacteria isolated. The frequency of isolated bacteria was: *Shigella* spp. 58 (47.8%) strains, enteropathogenic *Esherchia coli* (EPCE) 28 (22.8%) and *Salmonella* spp. 28 (22.8%). *Shigella sonnei* was the most prevalent serotype with 43 (74.5%) isolates followed by *Shigella flexneri* 11 (12%) and *Shigella dysenteriae* and *Shigella boydi* each two strains. *Salmonella group* D was the predominant serotype with eight isolates followed by *Salmonella* group B and *Salmonella typhi*. Susceptibility of *Shigella* isolates to ciprofloxacin, ceftrixone, amikacin, nalidix acid co-trimoxazol, ampicillin and tetracycline was 98.2, 91, 88.3, 20.4, 19.3 and 15.1% respectively. All *Salmonella* isolates were susceptible to cefotaxim, followed by gentamycin 99.6% chloramphenicol 89.6% co-trmoxazole 80% and ampicillin 19.34% and teracyclin 15.1%. About 60% of all EPCE were susceptible to ciprofloxacin, gentamycin, chloramphenicol co-trimaxazol and cefotaxim. All isolates were resistant to ampicillin.
Conclusion: This study reveals that *Shigella sonnei* was the predominant serotype among *Shigella* isolates. The majority of *Shigella* spp. and EPCE were resistant to ampicillin and co-trmaxazole. The rate of resistance among *Salmonella* spp. isolates was not high except for ampicillin.

P603 Intestinal parasites found in native and foreign workers during a 7-year period in Greece

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Infections with intestinal parasites constitute both a medical and a public health problem. The improvement of sanitation since 1960 led to a significant reduction of parasitosis in Greece. However, the high numbers of foreign workers entering the country have contributed to the increased numbers of faecal examination and isolation of intestinal parasites in our laboratory. The objective of this study was to monitor the number of people carrying intestinal parasites and to compare them with those of earlier years.
Material and methods: During a 7-year period (1997–2003) samples from 1879 individuals (natives and foreigners) who were examined as outpatients at the hospital, in order to obtain a heath clearance certificate, were included in the study. Single stool samples obtained without purgatives were subjected both to macroscopic and microscopic examination: (1) direct unstained smears, (2) iodine stained direct smears, (3) unstained wet films after concentration (sedimentation method: Ritchie).
Results: Out of 1879 individuals who were examined, 1061 were natives and 818 foreigners. Parasites were found in 17 natives (1.6%) and in 97 foreigners (11.8%). The species of isolated parasites were: (a) Protozoa [*Giardia lamblia* (34), *Blastocystis hominis* (9), *Entamoeba histolytica/E. dispar* (6), nonpathogenic Amoeba (77)] (b) Intestinal Nematodes [*Ascaris lumbricoides* (5) and *Enterobius vermicularis* (3)]. In 23 individuals a mixed infection with two species of parasites was found while in one, three species were present.
Conclusion: The alertness of public health services is critical in order to constrain the spreading of parasitic infections to indigenous populations by locating and treating, if possible, foreign carriers.

P604 Prevalence of intestinal parasites in women 15–45 years old (in pregnancy age) in a healthy village (Kargan), in Ardabil, Iran

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Objectives: Intestinal parasites are one of the major health problems in developing countries and cause malnutrition, diarrhoea and anaemia in human, especially in women. The aim of this study was to determine the prevalence of intestinal parasites among women 15–45 years old.
Methods: A cross-sectional study was performed on 90 women (15–45 years old) of this village. A questionnaire was designed to include relevant information. Faeces samples of them were collected and examined with wet mount smear and formalin-ether concentration methods. Data was analysed by Chi-square test.
Results: The present study indicates that 51.1% of women infected at least one type of intestinal parasites as follow: *Giardia lamblia* 10%, *Entamoeba coli* 43.3%, *Blastocystis hominis* 28.8% and *Hymenolepis nana* 3.3%. More than 27.7% of the subjects were infected with more than one parasite: 17.7, 7.8 and 2.2% had 2, 3 and 4 parasites, respectively.
Conclusion: Because of the high prevalence rate of intestinal parasites among women 15–45, contact them with children and role of them in preparing food, preventive measures and treatment seems to be necessary.

P605 Cryptosporidiosis associated with child care settings in Madrid, Spain

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Madrid, E

Background: *Cryptosporidium parvum* is a protozoan parasite transmitted by ingestion of oocysts excreted in the faeces of infected humans or animals. *Cryptosporidium* infection usually causes a self-limited diarrhoeal illness but it can be life-threatening in immunocompromised individuals. Groups at particular risk of cryptosporidiosis include immunocompromised persons, especially those with HIV infection, family and sexual partners of infected patients, children and caretakers in day-care centres, animal handlers and travellers.

Objective: To assess the prevalence of *Cryptosporidium parvum* in outpatients attending to an urban health area (Area 1, Madrid, Spain).
Patients and methods: A total of 1250 faecal samples from 929 outpatients were processed between September 2000 and December 2002. All faecal samples were studied by direct iodine wet mounts visualization followed by a concentration technique. Stools were concentrated using a disposable parasite concentrator with formalin-ethyl acetate (Biosepar, Germany). Coccidian oocysts were screened on direct and concentrated faecal smears stained by a modified Kinyoun acid-fast staining.
Results: During the 28-month period, *Cryptosporidium parvum* oocysts were detected in 62 faecal samples from 42 patients (25 males and 17 females). All were children between 4 months and 9 years; 35 of them (83.3%) were aged <4 years, of which 27 (77%) attended to day-care centres. Of the 42 cases of cryptosporidiosis, 20 (47.6%) exhibited an elevated excretion rate of oocysts and 27 (64.3%) showed faeces with pasty consistency and a characteristic yellow colour in the moment of diagnosis. The highest incidence of *Cryptosporidium parvum* was observed in autumn and spring (31 and 16 isolations, respectively).
Conclusions: Our data revealed that *Cryptosporidium parvum* should routinely be sought in children with diarrhoea. A correct aetiological diagnosis may permit to detect outbreaks and to avoid spreading. A modified acid-fast stain should be performed as part of a routine examination. Epidemiological studies are necessary to better quantify the public health impact of cryptosporidiosis.

P606 Cryptosporidiosis in children

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Objectives: During an outbreak of acute watery diarrhoea among the residents children of Cadiz (Spain) occurred from August 12 through November 18 2003, *Cryptosporidium parvum* oocysts were identified on 22 stool specimens from these patients.
Methods: Stool specimens were collected from patients with diarrhoea, and were preserved in sodium acetate-acetic acid-formaldehyde. Stool were sedimented by centrifugation. The microscopic examination of a direct smear and the cold acid-fast Kinyoun stain were realisated. An extensive questionnaire with demographic, clinical and epidemiologic characteristics was defined.
Results: Of the 22 patients with cryptosporidiosis, a 59% was male, a 27% required hospitalization, 21 were immunocompetents and one was HIV positive. Their mean age was 4 years. The clinical manifestation included watery diarrhoea (100%), anorexia (63%), abdominal cramps (54%), vomiting (35%), and fever(13%). The median duration of diarrhoea was 13 days. *Cryptosporidium parvum* was identified as the unique pathogen in 86% of the cases.
Conclusion: *Cryptosporidium parvum* was not a common cause of gastroenteritis in immunocompetent children resident in Cadiz. However, we recommend that clinicians and laboratories consider performing routine stool test for *Cryptosporidium* in people with watery diarrhoea. This outbreak highlights the importance of surveillance for cryptoporidiosis and the need for guide for the prevention of infections among HIV infected persons. Further studies are needed to determine the prevalence and spectrum of the clinical patterns of this parasitic disease.

P607 Human and animal trichinellosis in Belgrade

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Objectives: To determine the number of humans infected with *Trichinella spiralis* in the area of Belgrade and the kind and source of meat contaminated with the parasite.
Methods: Epidemiologic data on the number of human trichinellosis in Belgrade in the period from 1996 to 2000 were collected from the Institute for Infectious and Tropical Diseases and Institute of Public Health of Belgrade and the data on pig trichinellosis from the Ministry of Agriculture and Forestry. We collected data on the connection between the infected humans and source of meat from the Food and Drinks Centre.
Results: In the period of investigation on the territory of Belgrade 399 individuals were infected with *T. spiralis* what makes 12.3% of the infected individuals in Serbia. The greatest number of infected people was in 1997 and 2000 (morbidity: 6.0/10 000 and 5.1/10 000 respectively). The disease had seasonal character and appeared in epidemics (42 epidemics with 306 patients). In this period 77 pigs were found to harbour the parasite. Fifteen of them were the source of infections, while the others were eliminated thanks to the timely action of veterinary inspection. It makes 30.61% of the total of 49 pigs infected with *T. spiralis* that were the source of infection for inhabitants of Belgrade. The other pigs (69.39%) originated from the other parts of the country. The smallest number of infected individuals was observed in central parts of the town and the greatest number of them was in three suburbs, where was also found the greatest number of pigs infected with *T. spiralis*.
Conclusion: 30.61% of all pigs that were sources of epidemics of inhabitants of Belgrade originated from the territory of Belgrade. There was topographic correlation between trichinellosis of pigs and humans. The greatest number of infected humans and pigs was observed on the territory of three suburbs, that implicates the foci of infections in these areas.

P608 Study of rodents and their parasitic infections in Bandar Abbas City, Southern Iran

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Objectives: This investigation is conducted in Bandar Abbas City, Southern Iran, to determine the fauna, abundance, distribution map, ectoparasites and blood parasites of rodents, during the basic study for their control programme.
Methods: Rodents were captured with live traps from different parts of the city, during spring and summer of 2003 and transferred to the parasitology laboratory of Bandar Abbas training and public health research centre. After anaesthetization six thin and thick blood films were prepared from the heart of each animal. Smears were prepared from the ears of rodents to study the leishmaniasis infection. After recording the morphometric characters, ectoparasites were removed on water surface by combing their hairs. Finally the head and skin of each rodent was removed and ectoparasites were conserved in ethanol 70%. The obtained slides were stained with Giemsa and observed with light microscope.
Results: A total of 105 rodents demonstrated four species: *Rattus norvegicus, R. rattus, Tatera indica* and *Mus musculus* were caught and investigated from 20 areas of Bandar Abbas city. The major species was *R. norvegicus* (78%), followed by *T. indica* (16%), *R. rattus* (3%) and *Mus musculus* (3%). The most frequency was observed in Ayatollah Ghaffari quarter, 17 rodents per 24 traps. Ectoparasites were identified as *Xenopsylla cheopis, Polyplax* sp., *Ixodes* sp. and *Ornithonyssus* sp. We found 35 rodents infected with blood parasites. The observed parasites were *Trypanosoma* sp., *Babesia* sp., *Borrelia* sp. and one unknown bacteria. The most infection rate was due to *Trypanosoma* followed by *Babesia* (13.3%) and *Borrelia* (0.95%). There was no leptomonad infection in the studied rodents. *Mus musculus* (0%) and *R. norvegicus* (88.6%) had the lowest and highest parasitic infection, respectively. *Borrelia* sp. was only observed in the blood films of *T. indica*.
Conclusion: The reason for high frequency of rodents in some quarters of Bandar Abbas is the waste food materials, sweepings gathered in some parts of town, illegal storing and transporting of some food materials such as wheat, corn and so on. Removed ectoparasites are important because of their role in transmission of some dangerous disease agents such as plague, typhus,

relapsing fever and also haemorrhagic fevers. The observed blood parasites in the studied rodents show that these animals can be potential reservoir hosts for some zoonoses in Bandar Abbas, especially in quarters with low sanitation.

P609 Epidemiology of *Blastocystis hominis* and other intestinal parasites in female marriage emigrants in Taiwan

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Background: There were 139 735 foreign marriage immigrants (exclude the Mainland China) until the end of 2002 in Taiwan and 58.2% of them came from Vietnamese. There were several studies mentioned about the prevalence of parasitic infection among foreign workers but none did concern with these marriages immigrants. This is the first study about the prevalence of intestinal parasitic infections in the female marriage immigrants from Vietnam in Taiwan.
Methods: The female marriage immigrants from Vietnam who were required to take a complete physical examination for the residence approval July 1998 to June 2001 in southern Taiwan were included in this study. Examination for intestinal parasites used the merthiolate-iodine formaldehyde concentration method. Student's *t*-tests and multiple regressions were used to test for significance and for statistical adjustment.
Results: The prevalence of intestinal parasite infection of 1434 female Vietnamese marriage immigrants was 37.3% and there was a significant increase from 1999 to 2001 in statistically ($P < 0.0001$) but decreasing trend in the prevalence by age in statistically ($P < 0.0001$). There were 20 species of intestinal parasites were found in the study. 30.5% for 12 species transmitted via faecal–oral route, 11.8% for three species from soil-mediated route, and 0.7% for five species by food-bone infection. The prevalence of blastocystosis (20.4%) and hookworm infection (9.7%) was remained high in the protozoa and helminthes infection in the immigrants. The results of the prevalence of intestinal parasite examinations were aged adjusted by using multiple regression analysis. This also showed significant differences in the prevalence of intestinal parasite infection in different annuals in statistically (adjusted with age, $P < 0.001$).
Conclusion: The results provide systematic information on intestinal parasitic infection among female marriage immigrants in Taiwan and advised appropriate health care after parasite infection confirmed in these migration communities.

P610 Characterisation of casein kinase 1 in *Trichomonas vaginalis*

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Casien kinases (CK) are important regulators of many cellular processes in higher eukaryotes; however, casein kinase related genes has never been identified in *Trichomonas vaginalis*. The full length cDNA of two casein kinase 1 (CK1) partial cDNA clones isolated from a *T. vaginalis* expressed sequence tags (EST) library were obtained by 5′ rapid amplification cDNA ends (5′-RACE). The complete open reading frames of TvCK1.1 and TvCK1.2 are 1467 bp and 1572 bp. The Trichomonads CK1-like gene encodes a putative protein of 489- and 524-amino acid residues with a predicted molecular weight of 60 and 63 kDa, respectively. The amino acid sequences of the trichomonal TvCK1.1 and TVCK1.2 showed only 40 and 26% identity with human CK1-delta. However, 3D structure modelling simulated by ExPASy Molecular Biology Server showed the structure of the Trichomonad CK1 are highly conserved. The expression of TvCK1s were determined in synchronised cell division cycle. Results from quantitative real-time PCR showed that TvCK1.1 are highly expressed during the cell division cycle but not TvCK1.2.

P611 Comparison of direct microscopy and *in vitro* cultures in detection of *Trichomonas vaginalis*

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Objectives: The parasitic protozoan *Trichomonas vaginalis* is a common pathogen that causes trichomoniasis and has been linked to preterm birth, acquisition of human immunodeficiency virus, infertility and nongonococcal urethritis. Diagnosis is made by identifying motile unicellular flagellates on a vaginal saline wet mount, by using different culture media and serological and molecular methods. This clinical study performed to evaluate wet mount microscopy and two broth culture methods for the detection of *T. vaginalis* in swab specimens obtained from female patients.
Methods: A total of 128 women, ages between 18 and 48 years with abnormal vaginal discharge who applied to Obstetrics and Gynecology Department were enrolled to this study. The samples of vaginal secretions from the posterior fornix collected on a sterile cotton tipped swab. The smears were examined using wet-mount preparations and culturing on cystein pepton maltose (CPLM) medium and tripticase yeast extract maltose (TYM) medium in 1 h after specimen collection. We determined the optimal days on which to read culture tubes by inoculating aliquots of secretions in to each medium and reading the tubes 1, 2, 3, 4 and 7 days later and evaluated the test performance criteria of three methods.
Results: Of the 128 patients 12 (9.37%) had positive results for *T. vaginalis*. All the 12 positive cases were detected in TYM medium. TYM medium is accepted as gold standard. CPLM medium detected only nine of these 12 positive cases. Sensitivity, specificity, positive predictive values and negative predictive values were 75, 100, 100 and 97% respectively. Wet mount examination detected only seven of the 12 positive cases. Sensitivity, specificity, positive predictive values and negative predictive values were 58, 100, 100 and 96% respectively. One CPLM negative case was positive in wet mount examination. Optimal growth observed in 2 days for CPLM and in 4 days for TYM medium.
Conclusions: Culturing on TYM medium was the most sensitive technique which we used in *T. vaginalis* diagnosis. Although vaginal saline wet mount is an easy and low-cost technique, culture is more sensitive than the direct examination and TYM medium is superior to CPLM medium for growth of *T. vaginalis*. We project to expend this study with large numbers of clinical specimens.

P612 Seroprevalance of *Toxoplasma gondii* infection among some risk groups in Erzurum, Turkey

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Objectives: Toxoplasmosis is an infection caused by a single-celled parasite called *Toxoplasma gondii*. The parasite is found throughout the world. Toxoplasmosis can be transmitted to humans by ingestion of tissue cysts in raw or inadequately cooked infected meat or in uncooked foods that have come in contact with contaminated meat, by inadvertent ingestion of oocysts and sporozoites in cat faeces, or transplacentally. Our objective was to determine the seroprevalence of *T. gondii* antibody among some risk groups to ascertain whether they have an increased risk through occupational exposure.
Methods: The blood samples collected from three different risk groups including 30 veterinarians, 43 butchers, 43 slaughterhouses worker and, 100 healthy people as control groups, and obtained sera were stored at −20°C until used. Anti-toxoplasma IgG and IgM antibodies were determined by using ELISA (Trinity Biotech USA). Data and results were analysed by software Microsta (hypothesis test for two proportional from independent groups).
Results: The percentage of Toxoplasma Ig G seropositivity were 61.2% in the risk groups and 38% in control group. The differences between risk and control groups was found to be significant ($P < 0.001$). Toxoplasma Ig G seropositivity rates were 53.5% in veterinarians, 60.5% in butchers, 67.4% in slaughterhouses workers. Toxoplasma Ig M was negative in all groups.

Conclusion: Given the risks to health because of *T. gondii* infection, prophylactic measures such as wearing gloves and mask when handling meat and animals and washing hands after risk of contamination from soil, raw meat etc. are warranted.

P613 Comparison of DNA extraction methods and PCR assays for detection of *Toxoplasma gondii*

B. Edvinsson, S. Jalal, B. Evengård on behalf of the ESGT

Objectives: The use of PCR for detection of *Toxoplasma gondii* is sensitive and more relevant to use than serological techniques as a diagnostic tool in immunocompromised hosts. There are different DNA extraction methods and PCR assays available. We compare two different DNA extraction methods and three different PCR assays for detection of *T. gondii*.
Methods: DNA from *T. gondii* tachyzoites extracted either with QIAamp DNA mini Kit or MagNa pure DNA extraction methods was analysed with LightCycler SYBR green 1. Sensitivity of a real-time PCR TaqMan assay was determined using dilution series of extracted DNA. Also, DNA from 2 mL blood samples spiked with 104 to 10 tachyzoites per sample was extracted using the two extraction methods and analysed with conventional PCR, PCR in combination with oligochromatography or real-time PCR SYBR green 1 or TaqMan. All assays targets the B1 gene.
Results: The two DNA extraction methods showed no difference in extracting DNA from *T. gondii* tachyzoites. Analysis of spiked blood samples revealed no difference in sensitivity between the two DNA extraction methods when followed by PCR oligochromatography or real-time PCR TaqMan. Conventional PCR was more sensitive when DNA was extracted using QIAamp DNA mini Kit. Detection limit of the TaqMan assay was one parasitic genome in a run using dilution series of pure parasitic DNA. When analysing DNA extracted from 2 mL spiked blood samples a less sensitive detection limit was observed. Real-time PCR SYBR-green 1 was unable to detect parasitic DNA in all spiked blood samples.
Conclusions: The two DNA extraction methods are equally efficient in extraction of DNA from *T. gondii* tachyzoites. LightCycler PCR SYBR green 1 yielded a high background signal when analysing blood samples making the detection signal undistinguishable. Presence of blood cell DNA also altered the detection limit of the TaqMan assay. Conventional PCR and PCR oligochromatography were more sensitive than real-time PCR TaqMan for spiked blood samples. Our results also show that conventional PCR was more sensitive in the spiked blood samples using QIAamp DNA mini Kit, suggesting that the choice of extraction method may affect different PCR assays differently.

P614 Evaluation of the ELISA IgE test for diagnosis of acute toxoplasmosis

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Objectives: Usefulness of the IgE ELISA test (TEST-LINE Brno, Czech Republic) for the detection of acute toxoplasmosis was evaluated by comparing the course of quantitative and qualitative results after infection, and also the sensitivity, specificity, positive and negative predictive value with parameters of other tests.
Methods: 545 sera samples taken from Toxoplasma-infected patients with known clinical status and duration of the infection were, besides the IgE test, tested also with ELISA IgA, IgM (TEST-LINE, BIO-RAD), the complement-fixation test (CFT-Sevapharma, Prague) and IgG avidity test (in-house, NRL TOXO). As a criterion of acute toxoplasmosis, the presence of clinical symptoms was considered.
Results: Clinical signs persist for longer than 4 months in only 30% of patients. While low avidity of IgG prevails in samples taken up to 4 months after onset of symptoms, IgE ELISA is predominantly positive up to month 6, IgA ELISA up to month 8, and IgM ELISA up to month 12, like CFT (titres up to 256). Sensitivity of the IgE test (94.3%) is lower than that of IgM ELISA (98.1%), but the specificity and predictive value of a positive test for IgE (91.7%; 75.6%, respectively) are superior when compared with the same parameters of IgM (65%; 43.3%). No relation of IgE positivity with an allergy in patients was found. The IgE test showed the lowest rate (7.7%) of false positive plus false negative results evidencing the best correlation with clinical features.
Conclusions: Similarly like the IgG avidity test, the IgE ELISA TEST-LINE is a highly specific test which can be very useful in combination with some sensitive test (IgM, CFT). As the increase in IgG avidity is strictly related to the time since infection, this method is preferred when the duration of the Toxoplasma infection is needed to be known. However, in some patients, high avidity after 4 months can be accompanied by persisting clinical symptoms of toxoplasmosis. If data reflecting the clinical state of the patients are preferred, the IgE test is the method of choice.

P615 Effect of testing for IgG avidity for serodiagnosis of toxoplasmosis in pregnant women

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Objects: Measurement of *Toxoplasma gondii* immunglobulin G (IgG) avidity (binding strength) is a powerful tool for distinguishing recent Toxoplasma infection from past. This study was planned to determine Toxo IgM and IgG specific antibodies against *T. gondii* and IgG toxo avidity in pregnant women.
Materials and methods: Blood samples were taken from all 1363 female patients attending the gynecology and obstetric department of Selcuk University Meram Faculty of Education's Research and Practice Hospital during the period 2001–03. The presence of specific *T. gondii* antibodies was determined using VIDAS system; screening test, test for screening IgM and IgG specific antibodies and IgG toxo avidity test (bio-Merieux, France).
Results: Out of 1363 pregnant women 410 (30.08%) were found IgG toxo antibodies positive. Thirty-two (7.8%) pregnant women have both IgM and IgG positive tests. Four (1%) have equivocal IgM toxo antibodies tests. Low IgG avidity (<0.200, may be seen in acute primary infection with *T. gondii*) was found in 10 (2.4%) and high IgG avidity (>0.300 excludes primary infection within last 16 weeks) was found in 398 (97.1%) of the samples (toxo IgG positive). In two (0.5%) sera the avidity of IgG antibodies was borderline (0.200 to <0.300) indicating possible primary infection during the last 6 months. In 87.5% of IgG positive and IgM positive pregnant women, we determined high IgG avidity (showing low risk pregnancy) reducing unnecessary pregnancy terminations. Besides of this; in 1.1% of IgG positive and IgM negative women we determined low IgG (showing high risk pregnancies that were not defined before).
Conclusion: Toxo IgG avidity test is more precise test in showing high risk and low risk pregnancies.

P616 Can free-living amoebae be an environmental niche for *Vibrio cholerae*?

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Background: *Vibrio cholerae* is a highly infectious bacterium responsible for large outbreaks of cholera among humans. A seasonal distribution of epidemics is known but the role of naturally occurring habitats are rather unknown. Plankton has been suggested to play a role, as bacteria can be attached to this type of organisms forming a bio-film. The water born free-living amoebae, *Acanthamoebea castellanii*, is an amoeba that has been shown to be able to ingest and promote growth of several bacteria of different origin. The aim of the present study was to disclose whether or not an intra-amoebic behaviour of *V. cholerae* exists.

Methods: *V. cholerae* strains (*V. cholerae* O139 from the outbreak in Bangladesh 1993, the seventh-pandemic *V. cholerae* O1 El Tor-Inaba strain N16961, the sixth-pandemic *V. cholerae* O1 Classical-Ogawa strain 395, and the nonepidemic strain *V. cholerae* O54) were co-cultivated with *A. castellanii* for 16 days to examine whether an interaction could be established. Each day the number of live bacteria as well as amoebae was estimated. Intracellularly growing bacteria were distinguished from extracellularly bacteria by gentamycin treatment to show the intra-amoebic growth of bacteria. Bacteria were located to different compartments of amoebae, which could be mirrored by microscopy.
Results: When *V. cholerae* strains and *A. castellanii* were co-cultured it was found that *V. cholerae* O139, *V. cholerae* O1 El Tor-Inaba, and *V. cholerae* O54 multiplied intracellularly from 0 to 105 cfu/mL, while *V. cholerae* O1 Classical-Ogawa strain 395 could not grow in *Acanthamoeba* cells. All *V. cholerae* strains tested stimulated growth of amoebae during the co-culturing period, as the number of amoebae increased. Approximately 2 weeks after infection of amoebae, bacteria could be found in the inter-space between walls of the amoeba cysts. These data show a role of free-living amoebae as hosts for growth of *V. cholerae* and that this interaction can play an important role in the ecological niche of the bacterium.
Conclusions: Besides showing a new ecological niche of *V. cholerae*, this study redirects the bacterium from being pure extracellular parasite to the constellation of facultative intracellular bacterium that, thus, opens new medical aspects of the disease. This will have implications on the cholera epidemiology, immunology, and pathogenicity.

Tropical and parasitic diseases

P617 Characteristics of 117 malaria cases in Turkey

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Objective: To evaluate the epidemiologic and clinical characteristics of 117 adult malaria patients.
Methods: The charts of the patients, who had been hospitalized between 1985 and 2001 in the Infectious Diseases and Clinical Microbiology Department of Ankara Numune Education and Research Hospital, were reviewed. Diagnosis was established by thin and thick smears of blood preparations obtained in the febrile period. Logistic regression was modelled to predict the factors that could determine the presence of the classical cyclic pattern of the malaria based on the follow up notes of the nurses.
Results All the patients were >14 years of age, 78% were male. The mean age was 28. Travel to malaria endemic region was common (55%). Forty per cent of the patients acquired the infection in Southeastern Anatolia, while they were performing their military duty. Prehospital antibiotic use for any other diagnosis was common (50%) among the patients. The most common complaints were fever (100%), rigor (93%), sweating (90%), headache (76%), nausea (45%) and fatigue (38%). The most common physical examination findings were splenomegaly (86%), hepatomegaly (62%). Fifty-one per cent of the patients were diagnosed without demonstration of the classical cyclic pattern of fever. Herpetic lesions were detected in 10% of the cases. Four cases needed transfusion. Anaemia was detected in 23% of the patients, leukopenia in 47%, thrombocytopenia in 73%, twofold increase in ALT or AST level in 32% of the patients. The plasmodium was detected in 100% of the thick smears, and 83% of the thin smears. *Plasmodium vivax* was detected in 115 patients, whereas *P. falciparum* was detected in two patients. Chloroquine and primaquine were given to all patients with *P. vivax*. One of the *P. falciparum* detected case was given mefloquin, and the *P. falciparum* case was given quinin sulfate and tetracycline. Mean length of stay was 5 days. All the patients had been discharged with cure. The previous use of antibiotics, and gender had no effect on presence of classical cyclic pattern of fever, however the detection of the classical pattern declines by the increase in age (odds ratio; 0.8, confidence interval; 0.79–0.99, $P = 0.039$).
Conclusion: Malaria is still a public health problem. The febrile patients with a history of travel to the endemic regions should raise the suspicion of malaria.

P618 Relapse rate of vivax malaria with shorter duration of antirelapse treatment in Iran

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Objectives: Every year thousand of malaria cases mostly the vivax type Occur in Iran. The recommendation of WHO for antirelapse therapy of vivax malaria is the usage of primaquine (15 mg daily) for 14 days but in area where G6PD deficiency rate is high 8 weeks therapy (each week 45 mg of primaquine) is recommended. Considering the fact that in Iran drug therapy of malaria is implemented by direct observation of health care workers, 8 weeks regimen of antirelapse therapy of vivax is very expensive and in some situations impractical. In this study shorter duration of antirelapse therapy was compared with that of 8 weeks regimen.
Material and method: In 1996 and 1997 antirelapse therapy of vivax in Sistan & Baluchistan province in southeast of Iran was done with different regimens according to manpower and executive facilities. This is a cohort study that compare clinically and parasitologically relapse of vivax malaria in 200 patients with one week therapy, 200 patients with 2 weeks, 800 patients with 4 weeks and 500 patients with 8 weeks therapy by primaquine .
Results: Relapse rate of vivax malaria by each regimen were 23.8, 13.2, 5.4 and 4.6% respectively. Relative risk of relapse in comparison with 8 weeks regimen were 5.2, 2.9 and 1.2 respectively. Number need to therapy (NNT) were 5, 12 and 125. Interval of first relapse varied between 6 and 52 weeks with mean of 28.8 weeks. The relapse rate was not different by sex.
Discussion: The relapse rate by 4 and 8 weeks regimens were low and there was not significant difference of relapse rate in 4 and 8 weeks regimens. So in special situations we can use 4 weeks therapy instead of 8 weeks therapy.

P619 Persistent HRP2 and pan-malarial antigen reactivity after successful treatment of *P. falciparum* infection

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Introduction/objectives: Malaria infection is still prevalent in most of the tropical and subtropical countries of the World. More than 1.5 million people die from malaria each year. In Kuwait more

than 700 malaria cases are detected each year by microscopy, which is an insensitive technique.
Materials/subject & methods: In this study, 229 patients with the microscopic diagnosis of *P. falciparum* infection were monitored with microscopy, ICT Malaria Pf/Pv test and OptiMAL assay to detect persistent antigenaemia in patients negative for asexual-stage parasitaemia following antimalarial treatment.
Results: Following successful antimalarial chemotherapy, the ICT Malaria Pf/Pv test detected persistent antigenaemia in 160 of the 229 (70%) patients on day 7. 144 (63%) of the patients reacted with histidine rich protein 2 (HRP2) antigen and 110 (48%) reacted with panmalarial antigens (PMAs). However, the reactivity to HRP2 antigen and PMAs dropped to 35 and 23% respectively on day 14. A higher proportion of patients were positive for HRP2 antigen than for PMAs. The majority of the patients were positive for both HRP2 antigen and PMAs however, 16 (7%) patients were HRP2– but PMAs+. Compared with ICT Malaria Pf/Pv test, the OptiMAL assay detected significantly less number of post-treatment persistent reactions. Levels of parasite-specific lactate dehydrogenas (pLDH) and panmalarial pLDH, as detected by the OptiMAL assay, were shown to decline in parallel with clearance of asexual-stage parasitemia. On day 7, persistent pLDH reactivity was detected in 89 (39%) and panmalarial pLDH in 34 (15%) cases that dropped significantly to 21 (9%) and 14 (6%) respectively on day 14.
Conclusion: Thus OptiMAL may be superior to ICT Malaria Pf/Pv in monitoring therapeutic responses. However, a further improvement in quantification of current antigens is required to further enhance the sensitivity and specificity these assays.
Acknowledgements: Financially supported was granted by Kuwait University Grant MI 109.

P620 Drug resistance to malaria in Orissa

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Objective: To find out the resistance status of malaria parasite to 4-aminoquinolin in Orissa.
Methods: The place of study is selected on the basis of predominance of *P. falciparum* cases and deaths because of malaria. The team follow the method of 28 days *in vivo* study prescribe by the World Health Organization (WHO).
Results: The team carried out total 14 studies from 1998 to 2002 over a span of 5 years (see Table).

Table 1. The total number of test case were 2033

S	1722	34.60%
RI	147	7.20%
RII	121	5.90%
RIII	43	2.10%

Conclusion: We get RII & RIII resistance in all most all studies. The summary findings for last 3 years shows there is increasing of RII & RIII in persistent areas of transmission. As per the drug policy on malaria in the country, change of drug in resistance areas only required when there is 25% increase of RII & RIII put together. So these areas do not warrant change of cloroquine to some second line drug in the region.

P621 *In vitro* recrudescence of *P. falciparum* parasites suppressed to dormant state by atovaquone alone and in combination with proguanil

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Objective: The primary objective was to study the viability of *Plasmodium falciparum* parasites reappearing during long-term follow up cultures after repetitive exposures to various concentrations of atovaquone and proguanil.
Methods: Two *P. falciparum* parasite strains were used for the *in vitro* experiments, i.e. F32 and FCR3 originating from Tanzania and Thailand respectively. The parasite strains were kept in continuous culture according to known method of Trager and Jensen, 1976.
Results: Parasites (F 32 and FCR3) exposed to 100–5000 nM atovaquone for 96 h were reduced to <5% of initial parasitaemia but recrudesced after 16–21 days. Similarly, parasites exposed to 1000 nM atovaquone for 48, 72, 96 and 144 h recrudesced after 9, 14, 21 and 23 days respectively. After the removal of drug exposure, only one to three parasites and only schizonts were consistently found per 10 000 RBCs, apparently unable to produce trophozoites and thus possibly adopting a 'dormant state'. Parasites (F32 and FCR3) exposed to 500 nM atovaquone for 72 h, reappeared after 14 days. These recrudescing parasites were then similarly re-exposed and suppressed by atovaquone in three consecutive follow up experiments. They then reappeared after 10, 9 and 6 days respectively. No known point mutations in cytochrome b gene (cytb), associated with atovaquone resistance, were however detected in any recrudescing parasites. Finally, parasites (F32) exposed to various concentrations of atovaquone and proguanil in combination for 72 h reappeared after 9–17 days. Relatively low concentrations of proguanil (0.2 times EC90) were required in combination with atovaquone to kill more than 95% of the parasites. The baseline susceptibilities of the parasites to both individual drugs were similar before and after recrudescence in all experiments.
Conclusions: *In vivo*, the 'dormant state' parasites may represent a small fraction of cytostatic parasites, unable to grow and unsusceptible to further treatment with the drug. Return from 'dormant state' to normal growth within few days after removal of the drug pressure may represent the failure of drug action.

P622 Typhoid and paratyphoid fever in the Czech Republic, 1993–2003

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Objectives: Typhoid fever is endemic in many developing countries. Number of the cases is estimated between 12 and 33 million/year in the world. In the Czech republic these diseases occur very rarely. Since the World War II, the occurrence has decreased. While in 1951 the incidence rate was more than 14/100 000 inhabitants, in 2002 was only 0.01/100 000. The incidence rate of paratyphoid fever B decreased from 0.75/100 000 in 1951 to 0/100 000 in 2002. Almost all cases are imported. Between the years 1993 and 2003 50 cases of enteric fever were reported in the Czech republic. In our department we admitted 20 patients with imported typhoid or paratyphoid fever during this time. Seventeen patients had typhoid fever and three paratyphoid fever A. Seventeen patients were from the Czech republic, three were foreigners. Men prevailed women (16:4). Majority of cases were from the age group between 20 and 30 years. Eighty-five per cent of all patients were infected in Asia, the rest in Africa and Europe. The highest risk of infection was in India, where 13 patients travelled.
Methods: All patients were physically examined and diagnosis was verified by haemoculture, stool culture and Widal's test. In all patients blood count, liver function test and other biochemical examination were examined.
Results: Clinical picture was usually mild or moderate, only one patient had severe course with confusion. Fever occurred in 100%, hepatosplenomegaly in 75%, diarrhoea in 70% and headache in 68.4% of all patients. The other signs (hypotension, abdominal pain, bradycardia) were less frequent. Roseola was found only in 10% of all patients. 94.5% of patients had hepatopathy, 40% leucopenia. Complications occurred very rarely, relapse was seen only once. Four patients had dual infection. Diagnosis was usually made by haemoculture (90% positive), stool culture was positive only in 35%. Fifty per cent of all strains were resistant to antibiotics. Multiresistant strain occurred only

once from India. Patients were treated by fluoroquinolones in 75%, sometimes by antibiotic combination. Corticosteroids were used only once.
Conclusions: Typhoid and paratyphoid fever are very rare in the Czech republic, usually are imported. In our department were treated 41% of all cases occurring in the Czech republic between the years 1993 and 2003. The highest risk of infection was in Asia (India) followed by Africa and Europe. Fifty per cent of all strains were resistant to antibiotics. Fluoroquinolones were the drug of choice and treatment was successful.

P623 Imported dengue fever in the Czech Republic

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Two institutions in the Czech Republic possess the facilities for the diagnosis of dengue fever (DF): the National Reference Laboratory for Arboviruses, Ostrava, and the Department of Virology, Teaching Hospital Na Bulovce, Prague. During 1997–2002, 417 patients were examined for suspected DF. Serological evidence, i.e. the presence of anti-dengue IgM and IgG antibodies, was based on ELISA using PANBIO kits. Epidemiological data on DF have been collected by the National Reference Center for Epidemiological Data Analysis in Prague since 1997. Up till now, 15 DF cases (diagnostic code A90) and one case of dengue haemorrhagic fever (DHF, diagnostic code A91) have been registered.

P624 Contribution of PCR in laboratory diagnosis of visceral Leishmaniasis

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Background: Visceral leishmaniasis (VL) is a severe zoonotic and often life-threatening disease caused by the protozoan *Leishmania* spp. The disease is widespread in the Mediterranean region and also endemic in many parts of the world. The aim of this study was the evaluation and the contribution of PCR in the early diagnosis of VL and also the comparison of PCR to the serological tests and the direct detection of parasite in bone-marrow aspirate (BMA).
Materials and methods: A total of 24 patients (36 whole blood and sera samples) with confirmed VL were assessed in this study. The patients were divided in to two groups: In group 1, 11 patients (16 whole blood and sera samples) with definite VL confirmed by the demonstration of the parasite in BMA were included. In group 2, 13 patients (20 whole blood and sera samples) with clinical manifestations of VL (fever, enlargement of spleen, pancytopenia), positive results in serological tests (indirect immunofluorescent antibody test, IFA, indirect haemagglutination test, IHA) and negative direct examination of BMA were included. Twenty patients with fever of other aetiology in whom Leishmaniasis was evaluated as part of the differential diagnosis and 20 healthy persons were also included in the study, as a control group. PCR was performed in all samples. In PCR amplification two different pairs of primers and the highly repetitive kinetoplast DNA as target were used. PCR amplification revealed products of 120- and 145-bp. IFA and IHA methods were used for the detection of the specific IgG and total antibodies of *Leishmania* spp.
Results: The two PCR methods produced positive results in all 24 patients (group 1, 2). Both serological tests gave positive result in 17 of 24 patients, while only one of the two serological tests was positive in six patients (group 1, 2). There was also one patient with positive direct examination of BMA and low serological reactivity (group 1). All healthy persons and patients of other infections were found negative for Leishmaniasis by serological methods and PCR.
Conclusions: PCR methods contribute in the early and definitive diagnosis of VL in areas where the disease is endemic, such as southern Europe. The demonstration of the parasite in the direct examination of BMA is essential for establishing the diagnosis, but presents low sensitivity. The use and the combination of serological tests are useful in the diagnosis of VL.

P625 Haemophagocytic syndrome associated with visceral leishmaniasis

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Introduction: Haemophagocytic syndrome, more properly referred to as haemophagocytic lymphohistiocytosis (HLH), is a rare distinct clinical entity of infancy and early childhood. HLH is commonly associated with various systemic infections and to a broad spectrum of malignancies and genetic disorders. Although the association of HLH and visceral leishmaniasis (VL) has been rarely described, the diagnosis of the disease may be difficult when VL and HLH together present in adults. We report a case of haemophagocytosis in a 34-year-old man to draw attention to the difficulties in diagnosing VL when accompanied by HLH.
Case: A 34-year-old man presented with a 4-week history of fever, malaise, poor feeding and abdominal pain. Past medical history was unremarkable. Physical examination revealed high fever (39.2°C) with pallor, hepatomegaly and splenomegaly. Lymph nodes were not enlarged and vital signs were normal. The laboratory findings showed pancytopenia and elevated erythrocyte sedimentation rate; serum triglycerides were increased to 267 mg/dL with normal cholesterol. Transaminase activity and total bilirubin were high, whereas serum fibrinogen was low with elevated circulating fibrin degradation products. There was no serological evidence for infection with cytomegalovirus, Epstein–Barr virus and toxoplasmosis. Blood cultures were negative. The patient symptoms deteriorated within 24 h and he was referred to intensive care unit where intestinal bleeding with disseminated intravascular coagulation occurred. The patient died 48 h after admission in the ICU. Bone marrow and liver biopsy performed after death were compatible with diagnosis of HLH, whereas peripheral blood culture for parasites yielded *Leishmania infantum*, identified as zymodeme MON1 which is predominant in our country.
Conclusion: Leishmaniasis should be considered when discussing the cause of haemophagocytosis in countries where the disease is endemic, such as Tunisia. When revealed by haemophagocytosis, diagnosis may be difficult particularly in adults.

P626 Detection of Leishmania major in visceral infection by a nested-PCR assay

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Leishmania infantum is the typical agent of visceral leishmaniasis in Iran and middle east, but a few reports suggested that *L. tropica* could cause this type of leishmaniasis too. In this study, we detected *L. major* in bone marrow and lymph node samples of a village dweller from booshehr, who had kala-azar signs.A 30-year-old man with kala-azar symptoms was bedridden in Namazi hospital. Four bone marrow smears and two paraffin-embedded blocks of him were tested. At first, DNA extraction from smears performed by proteinase k and from blocks DNA extracted by boiling and proteinase k. Then variable regions of leishmania KDNA minicircles amplified by CSB1XR and CSB2XF primers in step 1, 13Z and LiR primers in step 2 of nested-PCR assay. We determined the species of parasite by electrophoresis of PCR product on agarose gel and comparing between their bands with marker and standards. *Leishmania major* has a 560 bp variable region. PCR was performed several times rigorously, and in all of samples only

L. major has be detected. All clinical signs suggested to kala-azar and leishman bodies were seen in lymph nodes and skin lesions of patient. The skin lesion have been appeared on the left foreleg of this man 10 years ago and did not be cured by plastic surgery, but began to cure contemporary with kala-azar therapy. According to increasingly spreading of *L. major* in Iran, if this parasite can cause visceral symptoms, it could be reckoned to a major common health problem in this country. Therefore, it is necessary to carry out widespread molecular studies in typing of various agents of leishmaniasis.

P627 Study of glucantime resistance in cutaneous leishmaniasis by PCR-RFLP method in Shiraz, Iran

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Glucantime is an effective drug for cutaneous leishmaniasis (CL) therapy in endemic regions of CL including Iran. In recent years, there are several reports of resistance to it. In this study, PCR-restriction fragments length polymorphism (RFLP) was used for determination of any relationship between the genome and drug resistance of causative agents of CL in Shiraz in southern Iran. For this purpose, Giemsa-stained positive slides of 102 patients with different degrees of amastigote density were used. Nineteen slides belonged to healed patients who had been treated by glucantime and 28 slides have been prepared from unhealed patients. The surface materials of these slides scraped and DNA extraction was carried out by Proteinase K and amplified by CSB1XR and CSB2XF primers in step 1, 13Z and LiR primers in step 2 of nested-PCR amplification method. The products were characterised by agarose gel electrophoresis in comparison with reference strains. The amplified fragments digested by restriction enzymes were electrophoresed and produced schizodeme patterns were analysed. Among 19 samples from healed patients, six were *Leishmania tropica* and 13 were *L. major*. Among 28 samples from unhealed patients, 14 were *L. tropica* and 14 were L. major. Analysis of these results showed that patients infected with *L. tropica* have more drug resistance. Those infected with *L. major* have more drug sensitivity to glucantime in this region. Schizodeme analysis showed high genomic diversity in *L. tropica* and *L. major* in Shiraz. The genomic diversity of *L. tropica* was considered higher than *L. major*. The results of this study determine a relationship between genomic diversity and incidence of drug resistance in patients infected with *L. tropica* in Shiraz.

P628 Subconjunctival *Dirofilaria repens* infection confirmed by PCR-based approach

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Objectives: *Dirofilaria repens*, a mosquito-born filaria of dogs and cats causes subcutaneous and submucosal lesions in human beings. More than 50% of nodules are located on the head with the most common site being the conjunctiva and orbit. We report a case of infection in man from Belgrade, Serbia and Montenegro who visited Spain 6 months before.
Methods: The clearly visible, motile parasite was localised under the right bulbar conjunctiva. The parasite was extracted intact from the lesion and preserved in 10% formalin. One part of the worm was examined in Mason trichrome and Movat stained sections. For detection of parasites in blood concentration tests were used. DNA extraction and PCR for *D. repens* and *D. immitis* using specific primers was performed according to procedure for formalin preserved tissues.
Results: Macroscopically the parasite was cylindrical, whitish measuring 90 mm in length and 0.70 mm in width. The distance from the anterior end to vulva was 1.40 mm and the distance from the caudal end to anus was approximately 0.08 mm. Examination of cross-sections of the parasite revealed an immature female filaria. The microscopic features included a pseudocoelom with intestine and two empty uteri, muscle cell layer and multilayered cuticle with longitudinal ridges. The distance between ridges was wider than the width of the ridge itself. Previous macroscopic and microscopic characteristics indicated that the worm was *D. repens*. A PCR product of 246 bp was amplified when specific primers for *D. repens* were used which confirmed morphological means in accurate identification. No microfilariae were detected in patients blood sample.
Conclusons: According to recent reports the worm appears as an emerging pathogen in Europe, especially in Mediterranean area. We assume the infection was contracted locally, as autocthonous cases of human superficial and visceral *D. repens* infections were described from urban and suburban areas in the last few years. Molecular identification of *D. repens* is of great value in detection of parasite in removed tissue particularly when regressive phenomena render the morphological characteristics of the parasite but according to some reports, duration of tissue/parasite preservation in formalin (more than 20 days) could interferes with DNA amplification. Although in this case the worm was in fixative for several months, it did not appear to influence the outcome of our PCR-based protocol.

P629 First cases of *Acanthamoeba keratitis* in Slovakia

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Objectives: Two dominant risk factors are responsible for initiating the amebic keratitis: wearing of the contact lenses and corneal trauma. Both these factors are associated with the first isolations of the *Acanthamoeba* species as the causative agents of human keratitis in the Slovak Republic. Three cases report are presented here.
Results: The first case of amebic keratitis manifested in the right eye of a 53-year-old man after the eye injury. Amoebae were identified as *Acanthamoeba* sp. of group III. The course of the disease was influenced mainly by seeing a physician at a late stage and discovering the aetiology of disease no sooner than 10 months after the eye injury. The disease progress has not been staunched either by itraconazole (Sporanox), or by corneal transplantation and the patient had to undergo enucleation. *Acanthamoeba lugdunensis* was identified as a causative agent of amebic keratitis in the second case. A 39-year-old man wearing contact lenses visited thermal swimming pool. A month later, the first disease symptoms occurred. The disease has manifested as herpetic keratitis of the left eye with cloudy cornea, circular infiltrate and deterioration of vision. The cultivation of the eye swab has revealed polyresistant strain of *Pseudomonas aeruginosa* and the cultivation of corneal scraping has revealed amoebae. Due to immediate clinical and laboratory diagnosis the propamidine-isethionate gtt. (Brolene) therapy has significantly improved the eye condition. Wearing contact lenses is probably connected also with the third case. A 15-year-old woman worn the contact lenses during bathing in various swimming pools and in the sea. She even cleaned the contact lens case under tap water regularly. In the used contact lens disinfecting solution, there were found cysts of *Acanthamoeba* sp. of group II and we assume that it was the way of the eye infection. The Brolene therapy was successful.
Conclusion: Presented cases suggest probably the glacier phenomenon of the occurrence of amebic keratitis in the Slovakia. Thanks to the first record of this disease, the amebic keratitis has attrac-

ted attention of the ophthalmologists and resulted in successful therapies of the following cases as well as in increased requirements for acanthamoebae examination in patients with eye diseases.

P630 First report of human myiasis caused by *Chrysomya bezziana* Villeneuve (Diptera; Calliphoridae) in Iran, May 2002

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A 45-year-old farmer from Espand village (located in west of Iranshahr county, Sistan and Baluchistan Province, south-east Iran) with sever headache, vertigo, nosebleed, oedema in face and agitation symptoms, referred to the Khatam-Al-Anbia Hospital in Iranshahr. CT scan showed an extensive oedema in nasal and paranasal sinuses. Endoscopy consideration revealed the presence of numerous larvae lodged inside the nasal cavity. In the operating room, on 6 May 2002 about 60 larvae of third instar fly larvae were pulled out under the general anaesthesia condition. Some of the larvae were preserved in 70% methanol and the rest were reared on ground beef placed on the top of moist soil in a mosquito net cage under insectry condition (RH % 70 ± 5, temperature 30 ± 2°C). The full-grown larvae crawled into the soil and developed to adult flies later on. The larvae and adult flies were identified according to James (1947), Zumpt (1965) and Spradbery (1991) keys. Our precise identification indicated that the flies were the Old World Screw-worm (*Chrysomya bezziana*). Six adult flies and six full grown larvae have been deposited in the collection of Entomology Museum, School of Public Health, Tehran University of Medical Sciences. The patient's life history implied that he usually was resting near the goats in his farm at mid-days. On the contrary, most of the goats usually being attacked with myasis larvae.

P631 Imported case of brucellosis complicated by liver abscess

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Hepatic involvement is a common feature of brucella infection, but liver abscess is a less frequent complication. We report a case of brucellar hepatic abscess in a 42-year-old man with a 4 week complaint of fever, abdominal pain, anorexia and weight loss. Forty days earlier, he was back from a 2 years staying in Albania. A detailed epidemiological history showed that he consumed, about 2 weeks before the onset of symptoms, fresh unpasteurised cheese. At admission the patient was febrile (39°C), and tachycardic (100 bpm). Abdomen was diffusely painful, especially in the right upper quadrant. Liver was 2 cm below the costal margin. Blood tests showed mild neutrophilia (6680 cells × mm^3), anaemia (haemoglobin 10.6 g/dL) and hypoalbuminaemia (3034 mg/dL), ESR 94 mm/h and C-reactive protein 15.1 mg/dL. Abdominal ultrasound scan showed an abscess within the VI hepatic segment of about 55 mm in diameter which was promptly drained. Blood cultures were positive for *Streptococcus anginosus*, but Wright test for *Brucella* was also positive at high titre (>1600). Clinical course was complicated by right sided pleuric effusion that needed evacuation. Ciprofloxacin i.v., doxycycline p.o., and metronidazole p.o. were initially administered. Antibiotic regimen was then switched to doxicicline p.o. and gentamicin i.v. for 6 weeks, accomplishing a progressive improvement of general conditions. *Escherichia coli* and *S. anginosus* were isolated from the abscess drainage. Any attempt of isolating *Brucella* spp., including biomolecular approaches, failed. Wright serology was repeated at discharge, showing a decrement in titres. A review of published cases was done, finding a brucellar hepatic abscess incidence of some 1%. As the isolated strains in this patient did not clearly indicate a brucellar aetiology, some question could be raised on the real involvement of *Brucella* spp. in this case. The possibility are that normal intestinal flora could reach, by contiguity, the liver. Otherwise, the initial brucellar colonisation could have been replaced, during abscess evolution, by superinfecting strains that made the specific diagnosis impossible. The latter mechanism could also explain the low incidence of confirmed brucellar liver abscesses in other series.

P632 Neurobrucellosis: report of five cases

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Brucellosis is a zoonotic disease which is still a problem in Turkey. Neurobrucellosis is a severe complication of systemic brucella infection and it can be seen 0.5–25% of the patients. The clinical symptoms and findings are variable as follows: meningoencephalitis, transient ischaemic attact, serebral infarct, subarachnoid haemorrhagia, myelitis, myelopathy, cranial or peripheric neuritis, radiculopathy, serebral abcess and progressive myelopathy. There are some problems in early diagnosis of neurobrucellosis. Systemic signs and symptoms can be indistinct and the results of serological and bacteriological tests can make clinicians mislead. Positive CSF cultures are difficult to obtain. We aimed to report five cases of neurobrucellosis followed in our clinic. The mean age of our patients was 32.4 (range 11–65) years. Of our patients, three were male and two were female. All of the patients had neurological symptoms and findings when they submitted to hospital. Three of the patients had sensorineural hearing loss. Except one patient who has a history of symptoms lasting 1–2 months, their symptoms began more than 2 years ago. Radiological findings were all normal except two patients; one had ventriculer shrink and indistinct brain sulcus at the beginning of the course of the disease and the other had hiperintence lesions in white matter of frontal and temporal lobes in cranial BT scanning. The biochemical results of CSF were: the pressure and protein concentration were increased, pandy reactions were all positive, glucose levels were decreased in all cases, the mean cell count was 105/mm^3 (mostly mononuclear cells). Rose Bengal test in serum and CSF were all positive. The Wright agglutination test (SF) in serum ranged between 1/80–1/640, in CSF 1/40–1/160. In two of the patients, CSF cultures revealed *Brucella melitensis*. The other three cases' results for blood and CSF cultures were negative. The hearing loss did not improved but the neurological signs and symptoms improved in all of the patients except one patient who had organic brain syndrome 1 year after the therapy. In conclusion; there are still problems in diagnosis of neurobrucellosis which can cause important neurological sequelae. In the patients who has neurological symptoms, neurobrucellosis have to be suspected by clinicians. The clinicians must carefully evaluate clinical symptoms and laboratory results when neurobrucellosis is suspected.

P633 Evaluation of available stains for detection of *Acanthamoeba* sp. from brain specimen: an assessment

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Objectives: *Acanthamoeba* sp. and others free-living amoebae are rare cause of opportunistic cerebral and mucose disease above all

in AIDS and usually are diagnosed post-mortem. The aim of this study is to determine the better and more fast stain for detection of granulomatous amoebic encephalitis (GAE) and their recuperation of strain from culture.
Methods: A patient, 33-year-old was admitted to the hospital in late January 1998 with a 2 days history of headache, confusion and a left field visual defect. A cranial computed tomography scan revealed a mass in the right occipital lobe. Toxoplasmosis was suspected. A biopsy specimen was obtained which multiple double walled cyst-like structures and granulomatous reaction were seen. We never has seen visualised this image but we think in *Acanthamoeba* sp. or *Balamuthia mandrillaris*. Six weeks after, a right parieto-occipital craniotomy was performed and microscopical examination, wet and stained preparations was carried out, Kop Color (Innogenetics), periodic acid-Schiff (PAS), trichrome, haematoxylin-eosin, Gomori methenamine silver and others.
Results: An indirect immunofluorescence and culture in non-nutrient agar plates precoated with *Escherichia coli* culture and incubate at 37°C about 8 days was identified as *Acanthamoeba castellanii*. This is the first case of GAE identified from Spain.
Conclusions: We think as others authors that GAE is not diagnostic sometimes probably, special cultures are required for the identification. As all but a few isolations from human tissue have yield it is generally believed that most human infections have been due to free-living amoebae. The tissue can have portion with amoebae and others without it. Fast stains including wet mounting can do more easy the recuperation of strain. Then a positive culture can facilitate the identification, *Balamuthia* not grow on non-nutrient agar but can be grown on mammalian cell lines.

P634 Leptomeningeal form of neurocysticercosis with chronic meningitis

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Objectives: During 2002 and 2003, we treated 46 patients with neurocysticercosis. Neurocysticercosis was the most frequent parasitosis of the central nerve system. Parenchymal form was seen in 60–90% of patients; Leptomeningeal and spinal form are rare. We treated four patients with this form of disease. The objective of our work was to present clinical course and therapeutic outcome in patients with chronic meningitis during neurocysticercosis.
Methods: We present clinical, laboratory and neuroradiological findings and therapeutical outcome in these patients.
Results: Our patients had pleocitosis in cerebrospinal fluid (CSF), average 74 ± 20 cells, CSF proteins was elevated, more then 6.4 g/L, and CSF glucose levels were 0–1.5 mmol/L. The pathological findings were present in prolong time period (6 months to 2 years). In neurological findings, all of them have a meningeal symptoms, with neurological deficiencies (paresis, ataxia, etc.). Duration of which was not correlated with CSF findings. Pathological changes in CSF were present longer then neurological problems. Serological conformation of cysticercosis was performing by ELISA test from blood and CSF. Intrathecal synthesis was positive. Tests for echinococcosis and toxoplasmosis were negative. Magnetic resonance imaging (MRI) was reviled in two cases parenchimal and leptomeningeal form of neurocysticercosis. One patient has only leptomeningeal form, and one patient has parenchimal, leptomeningeal and spinal form of neurocysticercosis.
Conclusions: Leptomeningeal form of neurocysticercosis was rare (8%). All of our patients have chronic meningitis during 6 months to 2 years. Neurological and laboratory findings are correlating with MRI. Repeated treatment (3–5) was necessary for successful cure. We used albendasol with corticosteroids. Side effects were mild and well tolerated.

P635 Distribution of soft and hard ticks and their association with *Borrelia* and CCHF in Isfahan province, Iran

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Objectives: A faunistic study was carried out to estimate the frequency and distribution of different species of soft ticks and Ornithodoros association with intermittent relapsing fever and have ticks infestation with CCHF.
Methods: Ten per cent of villages in 18 county of Isfahan province were selected randomly and ticks were collected from their habitats from June 2002 to June 2003. All of ticks were detected alive and studied for infection rate of *Borrelia* and CCHF by RT-PCR.
Results: Overall 6600 ticks were collected in this survey. The identified adult ticks were *Argas persicus*, *A. reflexus*, *Ornithodorous lahorensis*, *O. canestrinii*, *O. erraticus*, *O. tholozani*, *Boophilus annulatus*, *Rhipicephalus bursa*, *R. sanguineus*, *Dermacentor niveus*, *D. marginatus*, *Haemaphysalis inermis*, *Hae. Sulcata*, *Hae. conncina*, *Hyalomma anatolicum*, *Hae. punctata*, *H. dromedarii*, *H. asiaticum*, *H. deritum*, *H. marinatum*, *H. schulzei*, *H. Aegyptium* and *Ixodes ricinus*. The soft ticks were infested to *Borellia* in Isfahan, Fereidan, Fereidonshahr, Semirum and Aran-Bidgol county. No infestation by CCHF were observed.
Conclusions: This study confirm infestation by *Borrelia–Persica* and *Microti* in Isfahan province that take advantage of them for control planning of arthropod diseases, and no building in and over rodent colonies, and so physicians must pay attention to this disease in contaminated regions.

P636 The occurrence of *Entamoeba gingivalis* in patients with periodontal diseases

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Objectives: The aim of this study was to assess the frequency of occurrence of *Entamoeba gingivalis* in patients with periodontal diseases and the correlation between the prevalence of these protozoans in relation to clinical symptoms.
Methods: Diagnostic material in the form of swabs collected from dental plaques, pockets and periodontium were used. The diagnostic material was collected from 20 patients with periodontal diseases The way of detecting *E. gingivalis* was based on making microscopic preparations using a special liquid, which extends the vitality of protozoa. Next these specimens were examined in a contrast phase. *Entamoeba gingivalis* was identified by characteristic movement of pseudopodia.
Results: The presence of *E. gingivalis* was found in 60.5% of examined patients. Among this group of patients the characteristic indicators of periodontal diseases such as: oral hygiene index (PI, PI%, API%), gingival index (GI, GI%), bleeding index (PBI,PDJ) and deepness of gingival pockets (GK2, GK4, L>5) were analysed. The statistical analysis of these results indicated the correlation between the occurrence of *E. gingivalis* and PI, PI% and $L > 5$.
Conclusions: Our clinical and microscopic observations led us to consider that infection with oral protozoans should be regarded as an important factor associated with the pathological changes occurring in patients with periodontal diseases.

Meningitis

P637 Demographic, clinical and laboratory data in 140 meningococcal disease cases

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Introduction and Objective: Meningococcal diseases occur in a worldwide distribution as endemic or in epidemics. The overall mortality is 8–10% with deaths occurring mainly in patients presenting with signs and symptoms of meningococcaemia. Several investigators have devised scoring systems using clinical and laboratory parameters available at the time of presentation to prognosticate the outcome of the infection. We conducted this survey to determine the prevalence of demographic, clinical, laboratory data in our patients.
Methods: This was a prospective descriptive study performed upon patients with definite diagnosis of meningococcal infection (blood or cerbrospinal fluid positve culture) in St Zahra Hospital, Isfahan (centre of Iran) during October 1996 till September 2001. The subjects were comprised 140 patients [99 (70.7%) males and 41 (29.3%) females] aged 1–50 years. The data were achieved by checklist and analysed by using SSPS software.
Results: Upon this study, 99 (70.7%) were males and 41 (29.3%) females, and among them 75 (53.6%) were aged between 11 and 20 years. In the study population, 57 (40.7%) were students, 39 (27.8%) workers, 20 (14.3%) infant and children and 24 (17.2%) other jobs. Seasonal frequency: winter 49 (35%), spring 37 (26.4%), summer 30 (21.4%), fall 24 (17.2%). Iranian 92 (65.7%) and Afghan refuges 48 (34.3%). Clinical presentation: pure meningitis 40 (28.6%), meningitis with meningococcaemia 62 (44.3%), meningococcaemia alone 33 (23.5%) and meningoencephalitis five (3.6%). The five most clinical symptoms and signs were: fever 131 (93.6%), vomiting 107 (76.4%), neck stiffness 105 (75%), headache 96 (68.5%), skin rash 92 (65.7%). Paraclinical data: leucocytosis 99 (70.7%), normal range 25(17.8%) and leucopenia 16 (11.5%). The predominant white blood cell was polymorphonuclear 134 (95.7). Thrombocytopaenia 27 (19.3%). Abnormal prothrombin time 46 (32.85%). The mortality rate was 15 (10.7%). All of our patients had the first episode of meningococcal infection. Fifteen (10.7%) had deficiencies of terminal complement components C5–C9. Lumbar puncture was carried out in 121 (86.4%) patients, and the abnormal results were observed in 107 (76.4%).
Conclusion: Meningococci is still a killer, it affects males more than females, the highest age-related attack rate occurs in teenagers and young adults. Afghan refugees were a source of infection in our survey that may be due to crowded and low socioeconomic state of them. Central nervous system is a target organ in meningococcal infection. Our mortality was higher than what to be suggested.

P638 Laboratory surveillance of meningococcal disease in Portugal

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Objectives: In 2002, the control of meningococal disease (MD) was considered a priority in Portugal and a new surveillance system was introduced. Since October 2002, a laboratory-based surveillance system for MD (VigLab MD) started based on a laboratory network, which includes all laboratories from hospitals with MD inpatients. Laboratories notify to the National Institute of Health (INSA) all suspected cases received for laboratory confirmation, make *Neisseria meningitides* isolation and send the strains for further characterisation (antigenic and molecular). Besides, they also should notify all culture negative cases of CSF having previously established criteria, and sent them to INSA where the confirmation is made by non-cultural methods (PCR). The aim of this study was to determine the number of clinical or laboratory suspected cases of MD in which *N. meningitis* was detected.
Methods: *Neisseria meningitides* strains were isolated in hospital laboratories according to their own protocols. Direct DNA detection in clinical samples with negative cultures was performed by real time PCR (Light Cycler System, Roche, Germany) amplifying a 111-nucleotide sequence of gene *ctrA*. In a screening test, we used the fluorochrome SYBR Green and, in a confirmatory test, a fluorescent dye-labelled-specific probes. The melting temperature was used for identification of amplicons. Strain group determination was performed by direct agglutination (Difco) or, when nonreactive, by PCR using primers specific of *siaD* gene for group B, C, Y and W135, and *orf-2* gene for group A. Group determination of noncultural strains present in clinical samples, was also performed by PCR. For serotyping and subtyping, we used monoclonal antibodies from National Institute for Biological Standards and Control in an ELISA technique.
Results: In a 14-month period, we received 181 clinical samples and 118 strains. In 155 cases, we had confirmed MD and characterised serogroups as follows:
- 144 – culture negative + PCR negative;
- 37 – culture negative + PCR positive;
- 118 – culture positive.Seventy-nine strains were in group B, 57 strains group C and seven strains group W135

Conclusion: PCR is a very important tool for detection and characterisation of *N. meningitides*. This network has greatly contributed for clarifying the Portuguese situation in what concerns the MD.

P639 Multidisciplinary research of meningococcal invasive disease in the Czech Republic

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Objectives: Changing severity of MID in the Czech Republic (CR) being related to the high incidence of invasive strain of *Neisseria meningitidis* (NM), belonging to ET-15/37 complex, constrains to elaboration of diagnostic, therapeutic and severity assessment algorithms. High incidence of sepsis initiated our intensive studying of prognostic factors of MID on admitting to the hospital, properties of NM and other factors of MID.
Methods: A new grant project being further to our previous grant researches started in 2002, concentrates complex of MID data from eight departments in CR to the database including haematological and genetic research results. The research of NM in NIPH – identification of electrophoresis genotype ET by multilocus electrophoresis (MLEE) and identification of sequence type (ST) by multilocus sequence typing (MLST) is carried out in all patients as well. NM strains were isolated from patients and/or from PCR products in patients with negative cultivation. Belonging to hyper-virulent complexes (HC) is assessed together with prognostic factors and contextualised with the genetic familiar predisposition to the pathological pathway of fibrinolysis (due to mutation of promoter gene for PAI-1), and polymorphism G 308A in the promoter gene for tumour necrosis factor (TNF). Within 15 months, we have got data from 78 patients (53.4% of MID in the CR). Group characteristics: median of age 17.5 years, male:female = 42:36, mortality = 10.3%.
Results: Data analyses show 6.4% incidence of benign meningococcaemia, increasing incidence of sepsis (33.3%) with mortality 30.7%, decreasing incidence of sero-group B (42.5%) with 53% of HC (20.6% without), increasing incidence of serogroup C (34.6%) with HC in 92.5%. Serogroup was not identified in 16.6%. HC could not be proved in two cases with group C and in seven cases with group B due to the PCR detection only. Relationship among three above-mentioned areas of investigation is evaluated.

Conclusions: High incidence of hyper-virulent strains proved almost in all cases with NM group C and in majority of cases with group B does not correspond with only 33.3% incidence of sepsis. High incidence of proved benign meningococcaemia indicates good awareness of MID in the CR. Complete multidisciplinary approach to the MID research can contribute to get explanation for not yet answered question, why MID sometimes kills and sometimes has a benign course.
Acknowledgements: This study was supported by grant IGA Ministry of Health NI-7109-3 (http://www.neisseria.mlst.net).

P640 Community-acquired enterococcal meningitis caused by *Enterococcus casseliflavus*: first case report

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Background: Enterococcal meningitis accounts for only 0.3–4.0% of cases of bacterial meningitis. *Enterococcus faecalis* and *E. faecium* are the most frequent meningeal isolates accounting for 76–90 and 9–22%, respectively. *Enterococcus casseliflavus* is a motile enterococcus that produces a yellow pigment in agar and has a VanC phenotype (intrinsic low level resistance to vancomycin and susceptibility to teicoplanin). It has been implicated in a wide variety of infections in human beings, especially immunocompromised persons, but never in meningitis.
Case Report: A 77-year-old female presented for evaluation of fever, stupor, diarrhoea and vomiting of 3 days duration. There was no history or head injury or any surgical procedures. She had been suffering from rheumatoid arthritis for 30 years for which she was in treatment with steroids and methotrexate, diabetes in treatment with insulin and moderate renal failure. On admission she had a temperature of 38.0°C. She was alert but not oriented to time and place. Her neck was stiff, and she had a positive Kernig's sign. WBC 15 100/mm^3, 70% neutrophils and 23% lymphocytes. CSF was opalescent, glucose: 14 mg/dL, protein: 472 mg/dL, white cell counts: 200/mm^3. CSF was processed using BACTEC 9120. *Enterococcus casseliflavus* was identified using the VITEK-2 system and on the basis of motility and yellow pigmentation testing. The isolate was (i) susceptible to penicillin, ampicillin, ampicillin-sulbactam, imipenem, teicoplanin, tetracyclines and linezolid; (ii) low level resistant to vancomycin (MIC > 8 mg/L), trimethoprim-sulfamethoxazole, levofloxacin, ciprofloxacin and quinupristin-dalfopristin; and (iii) high level resistant to gentamicin, streptomycin and kanamycin and clindamycin. Echocardiogram revealed no isolates. Colonoscopic examination revealed two ulcerative lesions covered by fibrin in the rectal mucosa and multiple punctuate erosions in the sigma mucosa. She was successfully treated with meropenem and ampicillin–sulbactam.

P641 Factors predicting fatal outcome of purulent meningitis

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Objectives: To define the most accurate factors predicting in-hospital mortality of patients with purulent meningitis.
Methods: Retrospective study of patients hospitalised between 1997 and 2001 in an infectious diseases department of a tertiary care hospital. Records of 149 consecutive patients, older than 15 years with community-acquired purulent meningitis were reviewed. The following data were selected and further analysed: age, sex, duration of symptoms to diagnosis, underlying debilitating conditions, GCS score, APACHE II score, cerebrospinal fluid (CSF) leucocyte count, CSF protein level, CSF glucose level, CSF/blood glucose ratio and aetiology. Each parameter was tested in univariate logistic regression analysis and furthermore all significant variables were tested in multivariate analysis.
Results: There were 30 fatal cases with the overall mortality rate 20.1%. *Neisseria meningitidis* meningitis possessed the lowest mortality rate (2/37 = 5.4%), compared with *Listeria monocytogenes* (2/10 = 20%) and *Streptococcus pneumoniae* (12/39 = 30.8%). The risk of death was significantly higher in older age ($P = 0.002$), presence of underlying condition ($P = 0.005$), lower GCS score ($P < 0.001$), higher APACHE II score ($P < 0.001$), lower CSF/blood glucose ratio ($P = 0.001$).
Conclusion: Significant prognostic factors indicated by univariate logistic regression analysis were age, underlying debilitating condition, GCS score, APACHE II score and CSF/blood glucose ratio. Sex, duration of symptoms to diagnosis, CSF leucocyte count, CSF protein level and CSF glucose level had no significant prognostic value. In multivariate analysis only age and APACHE II score were significant predictors of fatal outcome. These results are in agreement with previously published studies.

P642 Dexamethasone therapy for bacterial meningitis in south-eastern Anatolia region of Turkey

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Objectives: Routine use of steroids as adjunctive treatment of bacterial meningitis remains controversial. We have carried out a retrospective, placebo-controlled, double-blind study of dexamethasone in 145 adult with acute bacterial meningitis in Dicle University of Turkey.
Methods: The patients were randomly assigned to receive either ceftriaxone ($n = 72$) or dexamethasone ($n = 73$) in addition to optimum antibiotic treatment (4 g every day ceftriaxone). Dexamethasone therapy (16 mg every day) was started 10 min before the first dose of ceftriaxone and has given every 6 h for 3 days.
Results: Baseline dermographic, clinical and laboratory features of the two groups were similar. The mean age of the patients was 30.2 ± 15.3 years. The sex of the patients was 91 males and 54 females. We have growth in 23 patients (15.9%) *Streptococcus pneumoniae*, in 12 (8.3%) *Neisseria meningitis*, in three (2.1%) *S. aureus* and 107 (73.7%) patients had no growth in cerebrospinal fluid cultures. CSF glucose concentration significantly increased in dexamethasone therapy than the other group after 24 h treatment ($P = 0.01$). However, other indices of inflammation showed similar changes in both groups. Addition of dexamethasone did not affect the rate at which CSF became sterile. When we compared two group the fatality rate was observed in patients with acute bacterial meningitis which were receiving dexamethasone; only seven of 73 patients died, 12 of 72 patient died which were not receiving dexamethasone.
Conclusion: Acute bacterial meningitis still remains a serious infection. Early diagnosis and treatment may reduce fatal outcome and improve the course of the disease. We conclude that dexamethasone is beneficial in the treatment of adults with bacterial menengitis, particularly in preventing deafness.

P643 Serologic evidence of *Borrelia burgdorferi* infection among patients hospitalised with tick-borne encephalitis

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Objective: The purpose of this work was to evaluate serum and cerebrospinal fluid presence of antibodies against *Borrelia burgdorferi* of patients with diagnosed tick-borne encephalitis.
Methods: Sera and cerebrospinal fluid of 90 patients with diagnosed TBE were analysed. The diagnosis of TBE was based on anamnesis (reported tick bites or endemic area dwelling) clinical symptoms (fever, headache and neurological signs) and results of laboratory tests (inflammatory changes of CSF parameters, serologically confirmed serum/CSF presence of IgM, IgG antibodies against TBE virus). Patients were divided into two groups: group I: 28 patients with severe clinical course of TBE with paresis and

unconsciousness; group II: 62 patients with mild course of TBE. Serological diagnosis of IgM, IgG antibodies against *B. burgdorferi* was performed with use of ELISA recombinant kit. CSF and sera were collected during first days of hospitalisation when diagnosis was established.
Results: In group I, IgM antibodies against *B. burgdorferi* in CSF were detected in two (7.14%) patients and IgG in six (21.42%). One patient showed presence of IgM and IgG simultaneously. In group II, IgM in CSF was not present in any patient but IgG was found in CSF of eight (12.9%) patients. Serum IgM antibodies among patients from group I were detected in two (7.14%) patients and IgG in seven (25%) patients. Patients with CSF antibodies against *B. burgdorferi* showed their presence in serum as well. In sera of group II patients, presence of IgM antibodies was detected in four (6.45%) patients and IgG in 12 (19.35%).
Conclusion: The diagnosis of tick-borne encephalitis should not exclude infection with *B. burgdorferi*. Patients with severe course of TBE showed more frequent presence of antibodies against *B. burgdorferi* in CSF and serum.

P644 Spinal epidural abscess in the MRI age

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Objectives: To evaluate the clinical, microbiological and prognostic characteristics of patients who were seen in our hospital with spinal epidural abscess (SEA).
Methods: Retrospective and descriptive study of patients who were diagnosed of SEA at our institution between January 1998 and November 2003 was used in this study.
Results: During the period of study 11 patients (nine males, mean age 58 ± 14 years) were diagnosed of SEA. The most common symptoms at presentation were fever (100%) and radicular pain (82%). There were signs of cord compression in 55%. In seven patients, the origin was community acquired. Seven patients had comorbid conditions (three alcoholism, two diabetes mellitus and two cancer); two cases presented a potential source of infection (vascular catheter); and in three cases, there was a direct spread from contiguous sources (epidural catheter, previous laminectomy and thoracic empyema). The isolated microorganisms were *Staphylococcus aureus* (six patients), *Streptococci* (two patients) and polymicrobial (one patient). The aetiological agent was not identified in two patients. Bacteraemia was documented in six patients. The lumbar spine was most commonly involved (seven patients), followed by thoracic (five patients) and cervical (two patients) regions. In three patients, there were multiple segments affected. In five patients, the abscess was circumferential, and in four cases was posterior. Spondylodiscitis was associated in five patients. The treatment was laminectomy and antibiotics in eight cases, and three patients were treated only with antibiotic therapy. Two patients died. In the univariated analysis the advanced age was the only variable associated with worse outcome.
Conclusions: In our experience the SEA is essentially produced by *S. aureus*. A high index of clinical suspicion should be kept in mind in patients with fever and spinal pain to avoid the progression and development of cord compression. Less than half of the cases have spondylodiscitis associated. In selected patients, the antibiotics alone can be effective.

HIV

P645 Genotype-guided treatment change in previously heavily antiretroviral treatment HIV patients

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Objective: To study HIV resistance in Hispanic antiretroviral therapy (ART)-experienced patients, and response after genotype (GT)-guided ART changes.
Methods: A retrospective analysis of ART-experienced patients seen in an outpatient clinic in Puerto Rico, assessment of resistance by GT, and evaluation of virological response after GT-guided ART change.
Results: A total of 84 patients, all ART-experienced Hispanic men, had 92 GTs performed for ART failure. Seventy-three per cent had >1 prior change in their ART, 89% were using >3 ART drugs. Prior exposure to all three drug classes was seen in 35% patients, to NRTI + PI in 38%, to NRTI + NNRTI in 18% and to only NRTI in 9%. Mean ART length was 38 (range 3–60) months. Mean VL was 40 000 (811–750 000). Resistance by GT to all three drug classes was seen in 26% of patients, to two classes in 48% and to one class in 14%. Twelve per cent of patients had wild-type virus; 86% had resistance to NRTI, 55% to PI, and 46% to NNRTI. Fifty-eight patients had a GT-guided ART change. VL decrease >0.5 log was seen in 57% patients within 16 weeks. Thirty-four per cent reached VL <400. New ART included two sensitive drugs in 69% patients, only 26% had >2 sensitive drugs. Success in patients having used <3 drugs in the past was 100%, those having used 3–7 drugs 59%, and those having used >7 drugs 20% ($P = 0.05$). Patients with resistance to <2 drug classes were more likely to have virological success vs. those with higher resistance (71% vs. 57%), but not significantly so. Success did not depend on the number of sensitive drugs or on the number of new drugs added.
Conclusions: Genotype-guided ART change was associated with 59% virological success in this clinic, despite having a heavily ART-experienced and heavily resistant population. Success was predicted by the number of drugs the patient had previously used. There was a trend to better success in those with <2 ART drug class resistance. Success was not predicted by the number of sensitive drugs nor the number of new drugs in the patient's ART.

P646 Lipodystrophy as paradoxically marker of efficacy of antiretroviral therapy in AIDS patients: our experience

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Background: After antiretroviral therapy are described lipidic alterations of metabolism and lipodystrophy with peripheral loss of fat and central accumulation.
Objective: To evaluate prevalence of lipodystrophy and its correlations with lipidic alterations.
Patients and Methods: Between 1998 and 2002, 203 AIDS patients, 125 males (62%), 78 females (38%), age 23–71 (mean 47 ± 24) years were hospitalised.
Results: Seventy-three patients (46%) had lipidic alterations: 35 patients (22%) had hypercholesterolemia, 29 patients (18%) hypertrigliceridemia and nine patients (6%) both. Fifty patients (68%) had lipodystrophy. Hypercholesteraemia was associated at age, lamivudine ($P = 0.033$), ritonavir ($P = 0.002$), whereas hyper-

triglyceridaemia at age, sex, time of diagnosis, lamivudine, stavudine, ritonavir and saquinavir.
Conclusions: In our study, lipidic alterations were associated with lipodystrophy especially but there was a better immune-virological answer to antiretrovirals. Lipodystrophy paradoxically can be considered marker of efficacy of therapy.

P647 Head-to-head comparison between first-choice HAART in antiretroviral-naïve patients with HIV infection: lopinavir/ritonavir-versus efavirenz-based therapy

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The updated international guidelines of antiretroviral therapy pose lopinavir/ritonavir (L)- or efavirenz (E)-based HAART as the first-line choice in naïve patients. Aim of our study is to review retrospectively the efficacy and tolerability of L- vs. E-based HAART in 67 naïve patients who started HAART since 2002; 36 consecutive patients treated with L plus two nucleoside analogues (NA) were compared with 31 consecutive patients who received E and two NA. At baseline, the two study groups were matched as to demographic and epidemiological features, as well as mean viral load (4.5 ± 1.3 vs. 4.3 ± 1.7 Log 10 HIV-RNA copies per millilitre, for L and E). However, the L group included a greater number of patients with prior-concurrent AIDS ($P < 0.03$), and showed a lower mean $CD4^+$ count at baseline ($P < 0.004$). The number of early (first month) interruptions due to poor tolerability proved similar: five cases in the L group vs. four among E-treated patients, although untoward events involved the gastrointestinal tract and the CNS, respectively, for L and E. During the subsequent follow-up (9–21 months), laboratory examinations were performed at least quarterly, and showed a comparable virological response (as to mode of decay, and time and rate of viral suppression), in the presence of only one case of virological failure in the E group. Conversely, a more rapid immune recovery occurred in L-treated patients, regardless of the more compromised mean initial $CD4^+$ count of this last patients group. Mid-term toxicity was significantly different, with L-treated patients who experienced an altered serum lipid profile in over 40% of cases vs. <10% recognised in the E group. The overall need of change of antiretroviral regimen due to toxicity, poor adherence, patient's request, or failure, was comparable in the two examined patient groups. When considering our experience on 67 antiretroviral-naïve patients treated with either L- or E-based HAART, more potent and rapid immunological effects were achieved in the L group, which started from a deeper immunodeficiency, while E-treated patients experienced more rare adverse events and had less compliance problems with pill burden and subjective tolerability. Virological efficacy did not prove remarkably different between L- and E-treated patients. Pending the approval of other agents useful for first-line HIV infection therapy, the selection of L- vs. E-based regimens has to take into account of the initial immunological and disease status, while more data are needed on long-term outcome, role of emerging resistance and toxicity, as well as targeted pharmacoeconomic evaluation.

P648 Polyunsaturated fatty acid ethyl ester as therapy of hypertriglyceridaemia related to HIV infection treated with antiretrovirals

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Aim of our study is to assess prospectively the efficacy and safety profile of polyunsaturated fatty acids ethyl ester (PFAEE) in the control of hypertriglyceridaemia complicating antiretroviral-treated HIV disease. Forty-three patients aged 38–61 years (29 males) with a diet- and exercise-resistant hyperlipidaemia and a mean triglyceridaemia of 298.3 ± 42.2 mg/dL, received PFAEE at 1 g twice daily, and were followed quarterly for at least 1 year. A hypercholesterolaemia (mean value 245.0 ± 29.6 mg/dL), concurred in 11 cases only. The dylipidaemia was prompted by a combined highly active antiretroviral therapy (HAART) lasting from 16 to 119 months (mean 47.2 ± 21.7 months), and based on protease inhibitors in 30 cases, and non-nucleoside reverse transcriptase inhibitors in 11 patients. Continued PFAEE administration led to a significant decrease of triglyceridaemia of 24.1, 34.3, 36.8 and 39.2%, after 3, 6, 9 and 12 months, respectively ($P < 0.001$ vs. baseline levels), while negligible changes occurred in serum cholesterol levels. Normal serum triglyceride levels (<172 mg/dL) were reached by 14 patients (34.1%), who continued PFAEE at 500 mg/day after 6–9 months, while five patients had persistently elevated triglyceridaemia (<300 mg/dL), and after 6 months significantly benefited from increased PFAEE dosage up to 1.5 g/day (reaching a mean 31.2% reduction of serum triglyceride levels vs. baseline). Mild and transient gastrointestinal disturbances (possibly attributable to concurrent medications) were referred by 12 patients, but no treatment discontinuation became necessary. Treatment with PFAEE prevented from changes of antiretroviral regimen and its composition, directly caused by persisting dyslipidaemia. Dyslipidaemia is a mounting problem in long-term management of HIV infection, and cardiovascular damage is of particular concern. In our experience (1–3), all administered fibrates and statins showed a similar efficacy in the therapy of HIV-associated dyslipidemia, but significant side effects and drug–drug interactions may occur, especially when antiretroviral drugs and other underlying pharmacological therapies are of concern. For isolated or predominant hypertriglyceridaemia, PFAEE may represent an effective and safe alternative to fibrates and/or statins, to be confirmed in enlarged, randomised, dose-finding comparative trials.
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P649 Pharmacokinetics of non-nucleoside inhibitors of HIV-1 reverse transcriptase of the DATA/DAPY classes of compounds

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Objectives: Diaryl-triazine (DATA) and diaryl-pyrimidine analogs (DAPY) are potent nonnucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) in cell-based assays on wild-type virus and also in a large panel of clinically relevant single and double mutants. Most NNRTIs assume a butterfly conformation within the lipophilic binding pocket of reverse transcriptase (RT). In the case of DATA/DAPY compounds, the two wings of the butterfly conformation are composed of aromatic rings, while the central part contains hydrogen bridge forming fragments. Hence, it is not surprising that these compounds are highly lipophilic with log *P*-values often in excess of 5. In spite of their lipophilicity, these compounds show moderate to good bioavailability in animal species and man. As can be expected, they are also absorbed to some extent through the lymphatic pathway.
Methods: We have investigated the possible causes of this seemingly paradoxical relationship between antiviral activity, lipophilicity and bioavailability. This comparative pharmacokinetic study of DATA/DAPY compounds involves various cell-based assays and physicochemical as well as computed parameters.
Results: A surprising finding is that highly potent and bioavailable DATA/DAPY analogues also form aggregates with radii between 34 and 100 nm. This has been confirmed by means of

dynamic light scattering, electron microscopy and atomic force microscopy.
Conclusion: We propose that aggregate formation can be an important factor in absorption and cell-mediated activity of lipophilic drugs.

P650 Restoration of complement-mediated phagocytosis in HIV-1 infected macrophages by 2′,5′-dideoxyadenosin

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Background: Using cAMP analogues, evidence has been obtained that complement-mediated phagocytosis (CMP) by human monocyte-derived macrophages (MDM) is impaired by cAMP. During HIV-1 infection, increased intracellular concentrations of the nucleotide cAMP may occur which could have negative effects on CMP in macrophages.
Objective: To examine the effect of an activator of adenyl cyclase on CMP and the effect of inhibition of adenyl cyclase on CMP by MDM infected with HIV-1Ba-L.
Materials and Methods: Using a colorimetric assay, sheep-erythrocytes (E) opsonised with human serum as a source of complement components were used as targets to quantify CMP by MDM infected with a laboratory adapted, M-tropic strain of HIV-1 for 7–10 days. Complement receptors were activated using 200 nM phorbol myristyl actetate for 15 min, and E uptake measured in the presence of varying concentrations of forskolin, a potent stimulator of adenylate cyclase, or dideoxyadenosin (ddAD), an inhibitor of adenylate cyclase. HIV-1 infection was assessed by retroviral reverse transcriptase activity, whereas immunolabelling of p24 viral antigen and the cell surface marker CD64 allowed quantification actively infected macrophages by microscopy and digital imaging.
Results: Consistent with results obtained using cAMP analogues, forskolin (100 μM) strongly inhibited CMP by 80% ($P < 0.0016$) in uninfected MDM. A significant increase in phagocytic activity was observed when erythrocytes were incubated with incrementing levels of 2′,5′-ddAD (up to 10 μg/mL) in both HIV-1 infected cells and controls ($57 \pm 20\%$, and $30 \pm 6\%$, respectively). In the presence of 10 MIC/mL 2′,5′-ddAD, particle ingestion by HIV-1 infected macrophages reached levels (97%) of complement-mediated phagocytosis of untreated, HIV-negative controls.
Conclusions: In HIV-1 infected MDM, integrin-dependent phagocytosis can be restored by 2′,5′-ddAD to levels of uninfected macrophages suggesting that elevated cAMP levels in these cells may contribute to decreased complement receptor function.

P651 $CD4^+$ T-lymphocytes counts at AIDS among patients treated by highly active antiretroviral therapy or other antiretroviral regimens

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Objectives: Opportunistic infections (OI) seem to occur at higher CD4 count, as the use of HAART. We reported and compared the CD4 count at four AIDS-defining events for patients receiving HAART or other regimens. The risk to present OI at higher CD4 count was calculated according to HAART or other regimens.
Methods: Data of 405 patients enrolled prospectively from July 1992 to December 2002 in the Lyon section of the French Hospital database on HIV were analysed. The following OI were studied: pneumocystis jiroveci pneumonia (PCP), oesophageal candidiasis (EC), cytomegalovirus disease (CMV) and Kaposi's sarcoma (KS). Patients were stratified by treatment at OI onset. The CD4 count 100 days before to 15 days after the diagnosis was compared by treatment group at diagnosis (HAART vs. bi or mono, B + M, therapy) using Mann–Whitney test (MW). Linear mixed model (LMM) was used to estimate mean CD4 at OI by treatment group, taking into account confounders and CD4 counts within 2 years before the diagnosis. The risk to present OI at higher CD4 count by treatment group at diagnosis was calculated using multiple logistic regression by OI and was quantified by odds ratio (OR) with their 95% confidence interval (95% CI).

Table 1. Median and mean CD4 counts at OI onset by treatment groups.

Treatment at OI onset		HAART (n=114)		B+M (n=291)		p-value	
OI		Median CD4	Mean CD4	Median CD4	Mean CD4	MW[1]	LMM[2]
PCP	($n = 84$)	18	57	29	49	0.62	0.61
EC	($n = 173$)	59	96	21	48	$<10^{-3}$	$<10^{-1}$
CMV	($n = 206$)	48	73	13	36	$<10^{-1}$	$<10^{-4}$
KS	($n = 145$)	155	161	28	58	$<10^{-1}$	$<10^{-3}$

[1]Median comparison
[2]Mean comparison

Results: A total of 84 PCP, 173 EC, 206 CMV and 145 KS were analysed. The follow-up duration, CD4 count at baseline and at treatment initiation was similar for each treatment group. Median CD4 counts were significantly higher among patients on HAART at onset of EC, CMV infection, KS but not for PCP (see table). Mean of CD4 estimated with LMM confirmed these results (see table). The risk of OI at higher CD4 count was increased for all OI, except PCP, in patients receiving HAART compared with those on B + M: at PCP with CD4 > 100, OR 5.8 (95% CI 0.9–33.9); at EC with CD4 > 100, OR 2.4 (95% CI 1.1–5.3); at CMV with CD4 > 100, OR 8.4 (3.1–23.0) and at KS with CD4 > 200, OR 7.7 (95% CI 2.9–20.1).
Conclusions: The risk of OI at higher CD4 count was increased for patients receiving HAART compared with those on B + M. That could be explained by an incomplete restoration of CD4 function in spite of an increase of the CD4 count. OI occurring at higher CD4 count since the use of HAART emphasises the need of appropriate follow-up to detect and prevent OI. Results from other cohorts would be helpful to conclude.

P652 Gender differences in HIV RNA and CD4 counts, but not in AIDS-defining illnesses and CD4-based HAART initiation threshold, in new entrants to HIV care in Cleveland, Ohio

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Objectives: To examine possible differences between HIV-infected men and women new to HIV care at University Hospitals of Cleveland between 1995 and 2002.
Methods: Through database extraction, we identified 1066 patients who sought treatment for the first time at our Special Immunology Unit between 1995 and 2002. Of these, 806 (80.1%) with no history of AIDS-defining illnesses more than 3 months prior to presentation and no prior antiretroviral exposure are included in this analysis. Data were analysed using t-test for independent groups and chi-square (SPSS version 11.0).
Results: Six hundred and thirty-two (78%) of our patients were men. Women had higher CD4 counts and lower HIV RNA than men at baseline (mean: 388 ± 282 vs. 310 ± 242 cells/μL, $P = 0.003$, and $89\,396 \pm 168\,223$ vs. $126\,537 \pm 200\,540$ copies/mL, $P = 0.048$, respectively). The proportion of entrants with CD4 cell counts <200/μL did not differ significantly between sexes (37.8% vs. 30.4%, $P = 0.11$). There was no difference in the proportion of patients presenting with category C AIDS-defining illnesses, category B symptomatic conditions and STD history between men and women (15.8% vs. 12.0%, $P = 0.23$; 27.8% vs. 29.8%, $P = 0.63$; and 17.7% vs. 14.9%, $P = 0.42$, respectively). Seroprevalence of hepatitis B (HbsAg) was higher in men (8.7% vs. 0.6%,

$P < 0.001$), but there was no difference in hepatitis C Ab (18.8% vs. 15.6%, $P = 0.40$).
Conclusions: In Cleveland, female new entrants to HIV care had higher CD4 cell counts, lower HIV RNA levels and were less frequently seropositive for hepatitis B infection. Whether women here seek HIV care earlier in the course of disease or whether early markers of HIV disease differ between sexes in this population remains to be determined.

P653 Incidence and predictors of opportunistic infections and mortality in HIV/AIDS patients admitted to a university hospital: a 16-year follow-up

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The study aimed to determine the incidence and significant risk factors of opportunistic infections in HIV/AIDS patients followed in the Department of Internal Medicine between 1986 and 2002, and to investigate predictors of mortality in this group. The charts of all HIV-AIDS patients ($n = 110$) were retrospectively examined. Data were collected on patients' sociodemographic characteristics, morbidity features at admission, presence and type of opportunistic infections, antiretroviral treatment modalities used and outcomes as of 31 December 2002. The mean (±SD) age was 37.1 ± 12 years and 68% were men. Heterosexual contact was the major route of transmission (68.2%). According to CDC-93 classification (modified in 1997), 36, 38 and 33 patients were at categories A, B and C, respectively, and 42 patients had $CD4^+$ cell count of $<200/mm^3$ at the first visit. Forty-two patients experienced at least one opportunistic infection. The most common AIDS indicator condition was pneumocystis carinii pneumonia ($n = 17$, 15.5%), followed by tuberculosis (12.7%), CMV infection (9.1%), toxoplasmosis (8.2%), cryptococcal meningitis (2.7%) and PML (1.8%). Ninty-one patients (82.7%) received at least one antiretroviral drug. Twenty-four (21.8%) patients died during therapy. Cox proportional hazards modelling was conducted to identify statistically significant predictors of opportunistic infections: adjusting for initial $CD4^+$ cell count, antiretroviral therapy and route of transmission, patient's age at admission (HR = 1.03, 95% CI 1.01–1.06) was the only significant predictor of opportunistic infection. In the study group, mortality was significantly associated with age at admission (HR = 1.06, 95% CI 1.02–1.10) and presence of at least one opportunistic infection (HR = 2.58, 95% CI 1.01–6.58). Study findings confirmed that opportunistic infections are significant predictors of mortality in HIV/AIDS patients. In the study, age at admission was indicated as a significant predictor of both occurrence of opportunistic infections, and subsequent mortality.

P654 Venezuelan HIV patients and prevalence of HPV infection in anal samples

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Objective: The aim of the study was to characterise HPV infection in a group of HIV positive male patients to determine the association between HIV infection, anal squamous intraephitelia lesions (ASILs) and HPV genotypes.
Methods: Twenty HIV positive and 9 HIV negative men were enrolled after informed consent and complete physical examination. Anal exfoliated cells were collected for PCR assay. The HPV typing were performance by using FRLP (HPV fast, Pharmagen, Spain).
Results: In the group of HPV positive patients, 10% had ASCUS lesions, 35% low grade anal squamous inthraepitelial lesion (LgASIL), 15% high grade ASIL (HgASIL) and 5% presented HgASLI/invasive anal cancer. In the HIV negative patients 67% had normal cytology, 11% with ASCUS and 22% with LgASIL. The overall prevalence of HPV infection was higher in HIV positive group (95%) the negative HIV patients (78%) ($P < 0.001$). The HPV type 16 was the most frequent genotype in HIV positive male (55%) associated with ASCUS, LgASIL and in all cases of HgASIL/anal cancer; only 33% of HIV negative patients presented this HPV type. A high spectrum of HPV genotypes in the HIV positive group: 6, 11, CP8304, 70, 58, 31, 33, 61 and 83. A high prevalence of oncogenic HPV types (30%) was found in the group of HIV positive patients with normal anal cytology.
Conclusions: The results in this study shown both high rate anal HPV infection and anal disease in the HIV positive patients (95%). The HPV 16 is the most frequent genotype in our HIV positive men population and another common feature of HPV infection in this group was the mix viral genotypes observed in the anal samples. In the HIV positive patients with normal anal cytology, we found a high prevalence of high-risk oncogenic types of HPV. This group represents a high-risk population for development anal diseases.
Acknowledgements: This study was supported by grant FONACIT-S12000000643.

P655 HCV viraemia and the degree of hepatic affectation in HCV-HIV co-infected patients

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Objective: We study the relationship between HCV viraemia (determined by quantitative-PCR) and the degree of hepatic fibrosis in patients with HCV-HIV co-infection prior to treatment.
Materials and Methods: Twenty-three sera from 23 patients with chronic hepatitis C were studied. The level of RNA HCV was determinate by PCR, previous reverse transcription RNA-DNA complementary (Cobas Amplicor; Roche Diagnostics). The degree of hepatic fibrosis was studied by histological activity rate (HAR-Metavir) in hepatic biopsy.
Results: Nine (39.2%) patients have RNA levels lower than 500 000 IU/mL; five of these have a severe hepatic affectation; three have a severe HAR-Metavir (3) and two have cirrhosis HAR-Metavir (2). In the remaining four patients, three have a moderate HAR-Metavir (2) and one has a slight HAR-Metavir (1). Eight (34.8%) patients have RNA levels from 500 000 to 1 000 000 IU/mL: one patient has a slight HAR-Metavir (1), four patients have a moderate HAR-Metavir (2) and three patients have a severe HAR-Metavir (3). Six (26%) patients have RNA levels higher than 1 000 000 IU/mL: two patients have a slight HAR-Metavir (1), and four patients have a moderate HAR-Metavir (2).
Conclusion: In patients HCV-HIV co-infected there is an association between viraemia and degree of hepatic affectation (HAR-Metavir).

Table 1. Distribution of patients by viremia level and degree of hepatic affection

	HAR-Metavir			
Q-PCR (IU/mL)	Slight (1) No.	Moderate (2) No.	Severe (3) No.	Cirrhosis (4) No.
0–500 000	1	3	3	2
500 001–1 000 000	1	4	3	0
>1 000 000	2	4	0	0

Spearman's Rho: −0.522

P656 Prevalence of GBV-c/HGV in HIV-infected patients and potential influence of co-infection on the course of the disease

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Objectives: Assessment of prevalence of GBV-c/HGV infection in HIV-infected patients and evaluating of a possible influence of GBV-c/HGV on the course of HIV infection by assessment of immunological and virological markers of progression of HIV infection.
Methods: We have investigated sera of 273 HIV-infected patients from AIDS Center of the University Hospital Na Bulovce Prague during 2002–2003. Our target was to assess the presence of markers of GBV-c/HGV infection by semiquantitative HGV PCR evaluation and anti-E2 antibodies by ELISA testing. 271 of serum samples were tested for HGV PCR and 269 samples were tested for anti-E2 antibodies. HIV viral load and CD4 count were tested concurrently. We used Spearman's test to rule out the dependency of CD4 count and HIV viral load on HGV infection.
Results: Eighty-nine (33.3%) of patients were positive in PCR HGV test and 101 (38.5%) of patients were positive in anti-E2-ELISA. No statistically significant effect of GBV-c/HGV infection was observed on CD4 count and HIV viral load in our cohort of patients.
Conclusion: The effect of GBV-c/HGV infection on predictive laboratory markers of HIV infection was not confirmed in our study. Further investigations regarding this subject seem to be necessary.
Acknowledgements: Our study is constituent of the grant project no.7509-3/2003 IGA MH CR.

P657 Cardiovascular neurovegetative dysfunction in HIV-infected patients

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Objectives: Spectral analysis of heart rate variability (HRV) is a sensitive technique for measurement of cardiovascular neurovegetative function, which also allows differential assessment of parasympathetic and sympathetic components. Moreover, spontaneous baroreflex sensitivity (BRS) is a simple and non-invasive method for the study of the baroreflex function. The objective of this study was to evaluate the presence, nature and degree of cardiovascular neurovegetative dysfunction in HIV-infected patients compared with HIV-negative controls, and to correlate the dysfunction with stage of HIV infection and antiretroviral treatment.
Methods: A total of 57 HIV-infected patients (29 men), aged 34–58 (median 38) years, were enrolled in the study. Twenty-two patients were at stage A, 22 patients at stage B and 13 patients at stage C, according to the CDC classification system. Forty-seven patients were receiving highly active antiretroviral therapy (HAART), and half of them were on treatment with protease inhibitors. Cardiovascular neurovegetative function was assessed by spectral analysis of HRV and by BRS method. Blood pressure and consecutive R–R intervals were recorded continuously for 20 min on supine resting, and for 20 min during a passive tilt-test. Data obtained from each CDC stage group were compared with those obtained from 14 HIV-negative controls recruited from patients and medical staff at the hospital.
Results: HRV parameters were not statistically different among patients at CDC stages A and B in comparison with controls; when compared with controls, patients at stage C had: (i) a reduced BRS in baseline condition (8.5 ± 1.7 ms/mmHg vs. 21.8 ± 4.1 ms/mmHg; $P < 0.015$); (ii) a reduced increase of systolic arterial pressure during tilt-test (8.2 ± 2.0 mmHg vs. 17.0 ± 3.2 mmHg; $P < 0.03$); (iii) a significant difference in the low frequency (LF)/high frequency (HF) ratio (3.9 ± 1.3 vs. 13.0 ± 5.4; $P < 0.019$) during tilt-test, related to a different LF component (44.8 ± 6.3 vs. 75.6 ± 4.7 normalised units; $P < 0.001$). No differences were observed between HIV-infected patients with respect to HAART regimen.
Conclusions: Cardiovascular dysfunction is common in association with HIV infection and occurs more frequently and with greater severity in patients with AIDS. However, it may be present in the early stages of HIV infection and progress during the illness.
Acknowledgements: This work was funded by Ricerca Corrente IRCCS.

P658 Pancreatic abnormalities during HIV infection. What about epidemiology, management and outcome?

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HIV-infected patients on HAART are exposed to direct and indirect (hyperlipidaemia-mediated) pancreotoxicity and multiple predisposing conditions (biliary-liver disorders, alcoholism). In order to assess frequency and significance of pancreatic anomalies, a case–control study involved 976 HIV-infected patients followed for 1.5 years. Octreotide ± gabexate administration was carried out in patients with severe, prolonged or symptomatic abnormalities. In our single-centre cohort, 349 of 976 (35.7%) patients experienced altered amylase and/or lipase at least once. Compared with the remaining 627 patients without pancreatic alterations a multivariate analysis detected a relationship with duration of HIV infection, AIDS, a $CD4^+$ count <200 cells/μL, duration of a protease inhibitor-based HAART, hypertriglyceridaemia and underlying acute-chronic hepato-biliary diseases ($P < 0.01$–0001), while no association was found with nucleoside analogues. Only 36 of 349 (10.3%) patients had signs and symptoms of pancreatic involvement and a frank pancreatitis occurred in only nine (2.6%) patients. A specific treatment with gabexate (46 patients), octreotide (17 patients), or both (35 patients) was performed when pancreatic enzymes were greater than threefold normal values for >6 months, and when signs-symptoms of pancreatitis became evident. A drop of pancreatic enzymes >50% vs. baseline, combined with improvement-cure of clinical-instrumental picture, was achieved in 68 of 98 (69.4%) patients treated for 13–35 days, in absence of untoward events. A more rapid and effective response was obtained in the 35 patients treated with gabexate + octreotide vs. 63 patients who received a single drug ($P < 0.05$). A further case–control study compared the 98 treated patients with 76 HIV controls with matched pancreatic abnormalities, but followed with dietary-conservative measures: a significantly better short- and long-term evolution was seen in patients treated pharmacologically as found by a more frequent-rapid drop of pancreatic enzymes, improvement of clinical-instrumental alterations, reduced relapse rate, and better HAART tolerability. Limited literature data are available about pancreatic abnormalities and their management during HIV disease and HAART. However, a crude 35.7% rate of patients in our cohort had laboratory alterations, while pathogenetic pathways are broadening, owing to emerging dysmetabolism and mitochondriopathy. Further studies are needed to give reliable estimates of both frequency and evolution of HIV-associated pancreatic abnormalities, their consequences on HAART administration and specific treatment.

P659 Laboratory and clinical pancreatic abnormalities during HIV infection treated with antiretroviral therapy

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The epidemiological and clinical features of HIV-associated pancreatic abnormalities are changing in the HAART era. Their frequency, risk factors and clinical and therapeutic features were assessed in an observational case–control study involving 986

patients, assessed for pancreatic anomalies in a case–control comparison including the whole follow-up period of each patients; 149 patients with high and prolonged laboratory abnormalities underwent further assessment. Compared with controls, the 352 patients (35.7%) who experienced greater than one episode of confirmed pancreatic laboratory abnormality had a longer duration of seropositivity, exposure to protease inhibitors, a more frequent immunodeficiency, AIDS diagnosis, liver-biliary disease, and hypertriglyceridaemia ($P < 0.04$–0.001), while no relation was found with type and duration of antiretroviral combinations (as well as specific nucleoside analogue use). Among these 352 patients, high and prolonged laboratory alterations eventually associated with signs of organ involvement occurred in 149 cases, and seemed related to the administration of didanosine, stavudine, lamivudine, pentamidine, cotrimoxazole, or anti-tubercular therapy, substance or alcohol abuse, opportunistic infections, liver-biliary disease, a protease inhibitor-based HAART, and hypertriglyceridaemia ($P < 0.02$–0.001). However, no difference was noticed between the 39 patients with clinical and/or imaging evidence of pancreatic involvement and the remaining 110 asymptomatic patients, as to the same risk factors. Although recurrences of enzyme alterations involved >70% of patients, in only 33.8% a change of antiretroviral or antimicrobial therapy became necessary. An acute but uncomplicated pancreatitis occurred in eight of 29 overall symptomatic patients (27.6%). A 2–4 weeks gabexate and/or octreotide administration (performed in 68 patients of 149), attained a significant laboratory, clinical and instrumental cure or improvement in 73.5% of patients, with a better success rate of combined versus single therapy, a reduced tendency to disease relapses in the subsequent 6–42-month follow-up, and a better tolerability of antiretrovirals ($P < 0.05$–0.004). Epidemiological and pathogenetic studies are warranted to re-evaluate pancreatic abnormalities in the HAART era, and their consequences on continued anti-HIV and antimicrobial therapy. The management of antiretroviral therapy and the indication to gabexate and/or octreotide administration in the different clinical and laboratory settings, deserve controlled investigation.

P660 Antiretroviral treatment and gynecomastia: which correlations?

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Aim of our study was to recognise all episodes of gynecomastia occurring during anti-HIV therapy in our patient (p) cohort, and to search for all correlations with several demographic–epidemiologic variables, clinical-laboratory markers, prior-underlying therapy, metabolic anomalies, and subsequent evolution. A cross-sectional survey of 988 p treated with antiretrovirals for >1 year (661 males:66.9% of p), allowed us to retrieve all p with ultrasonography-confirmed, true gynecomastia, considered after exclusion of all potentially involved conditions. Particular attention was deserved to metabolic alterations, including the lipodystrophy syndrome, dysmetabolism, and administered antiretrovirals. A complete hormonal workout failed in detecting significant abnormalities (i.e. hypogonadism) in all p but one, and hyperprolactinemia was not found. Fifteen p of the 513 evaluable males (2.92%), developed gynecomastia when aged 12–58 years. The duration of HIV-infection, antiretroviral therapy, and HAART, varied significantly in our p group, and no correlation was found with clinical-laboratory markers of HIV disease, but five of 15 p (33.3%) never received protease inhibitors (PI), while an efavirenz-based therapy apparently prompted gynecomastia in four p who were naïve for PI, and worsened this sign in three more p who switched from a PI-based HAART towards efavirenz. One p developed gynecomastia while on prolonged, isolated dual nucleoside analogue (NA) therapy, without administration of PI and non-NA reverse transcriptase inhibitors. A concurrent lipodystrophy was present in all p who developed gynecomastia, while hypertriglyceridemia, -cholesterolemia, and -glycaemia were found in 11, six, and three p, respectively. Among NA, stavudine represented the more frequently used drug, administered during a more prolonged time in all p with gynecomastia. During the subsequent follow-up (7–23 months), no significant clinical amelioramentof gynecomastia was observed, despite therapeutic changes (determined by regimen failure and/or toxicity), but surgery never proved necessary. Gynecomastia, as an emerging untoward event of HIV infection treated with antiretrovirals, warrants investigation, from an epidemiologic, clinical, and pathogenetic point of view. The apparently frequent association with other metabolic anomalies suggests some common pathway with other HIV- and antiretroviral-associated disturbances, so that special attention should be deserved to anti-HIV therapy, and the role of single and associated antiretroviral compounds.

P661 Lipomatosis and HIV disease during antiretroviral therapy

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Objective: The fat redistribution syndrome and its variably associated metabolic abnormalities, emerged as a result of the administration of potent anti-HIV combinations. Local fat accumulation may present as central adipositiy, increased breast dimension, gynecomastia, lipomastia and the so-called buffalo hump. Lipomas and other benign tumours of fatty tissue have not been yet reported during HIV disease, and the HAART era.
Methods and Results: Eight of approximately 1000 HIV-infected patients (p) experienced multiple lipomas since the year 2000. All p suffered from ultrasonography-confirmed multiple lipomatous lesions (3 to >20), predominantly involving limbs, thorax, and anterior abdomen, associated with limited local discomfort. Six p were male and two were females, with age ranging from 36 to 58 years, and duration of seropositivity between 38 and 116 months. Risk factors for HIV disease included iv drug use and heterosexual contacts in three p each, and homo–bisexual transmission in two p. At the time of onset of lipomas, all p were on a protease inhibitor (PI)-based HAART regimen since 17-56 (mean 24 ± 14) months. Our p experienced four to nine different anti-HIV therapeutic lines: almost all available PI and nucleoside analogues (NA) had been used, while these p never took non-NA reverse transcriptase inhibitors. Laboratory markers of HIV disease tested satisfactory:mean viral load 3.1 ± 0.6 log10 HIV-RNA copies/mL, and mean CD4+ count 432 ± 146 cells/μL. Lipoatrophy was present in six of eight p, associated with central adiposity in four p, while no localised fat accumulation was present (i.e. breast enlargement, buffalo hump).Increased triglyceridemia, cholesterolemia, and glycaemia were detected in five, three, and one p, respectively, with some correlation with the onset of signs of the fat redistribution syndrome. The 15–31-month follow-up allowed us to identify the appearance of further lesions in four p, and a substantially stable disease in the remaining four p, while spontaneous regression never occurred,as well as resort to surgery.
Conclusion: The relationship between lipomas, HIV infection, and HAART is a novel entity, but the frequent association with other clinical and metabolic disturbances possibly related to antiretroviral therapy should prompt further epidemiological, pathogenetic, and clinical studies. Malignant degeneration should be rare, but careful surveillance seems recommendable. The possible pathogenetic role carried out by antiretrovirals, and the concomitant occurrence of lipodistrophy and dysmetabolism, warrant investigation.

P662 Drug-induced alopecia after Lopinavir/ritonavir (Kaletra) treatment

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The most commonly reported side effects related to Kaletra are: diarrhoea, vomiting, headache, nausea, increases in triglycerides

and cholesterol. About 4% stopped taking Kaletra because of side effects. Alopecia is a well-recognised adverse effect of chemotherapy, but is uncommon with antiretroviral therapy. Alopecia, generally involving the scalp, has been reported in patients with HIV infection treated with Indinavir but not with Kaletra. Hair loss has been linked to certain other drugs.We present a 62-year-old man with HIV infection, stage B2, CD4 count 432 and plasma viral load <50 copies/mL experienced alopecia totalis of his scalp, eyebrows and eyelashes beginning 18 months after initiating antiretroviral treatment including Kaletra. No hair loss of arms, legs and pubic area was observed. Our patient's drug regimen consisted of Lopinavir/Ritonavir (four caps bid), Efavirenz (600 mg qd) and Stavudine (40 mg bid); in addition, the patient was receiving treatment for diabetes with Glivenclamide and Metformin for the last 3 years. These drugs have not been shown to cause alopecia. The alopecia reversed completely 2 months after Kaletra substitution by Nelfinavir without any other change of treatment and his eyelashes and eyebrows grew back as well.To our knowledge, alopecia totalis has not been reported in patients with HIV infection treated with Kaletra. In conclusion, the course of alopecia related to Kaletra seems to be reversible.

P663 Modification of lipid parameters during structured interruptions of treatment in HIV chronically infected patients

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Objective: Analysis of the modifications of anthropometric, biochemical and lipid parameters as well as those molecules implied in the lipid metabolism (leptin, tumor necrosis factor alpha – TNF-α) in patients with chronic infection by human immunodeficiency virus (HIV) during structured interruptions of antiretroviral treatment (STI).
Patients and Methods: Forty chronically HIV infected patients were evaluated (CD4 cell count, nadir 374; at the beginning of STI 968; viral load, VIH nadir 41521; at the beginning of the STI < 50 copies; the median duration of antiretroviral treatment at entry of the study was 60 months). The STI consisted of HAART 2 months, nontreatment 1 month. Patients were evaluated at the end of each period and after four STI, with particular attention to the following parameters: CD4 cell count, viral load, waist and hip circumference, tricipital fold, arm circumference and serum concentrations of cholesterol, triglycerides, leptin levels, TNF and its soluble receptors (TNFR1 and TNFR2).
Results: There were no significant modifications in lymphocyte T CD4 counts or in the viral load at the end of 4 STI. Viral load persisted undetectable in the patients who completed the study. Likewise, significant differences were not detected between values observed at the beginning of STI and at the end of the intervention with reference to the anthropometric parameters (waist circumference 84 vs. 87; hip circumference 92 vs. 100 cm; tricipital fold 14 vs. 21 cm; arm circunference 31 vs. 31 cm, $P > 0.05$ in each case), serum cholesterol (173 vs. 226 mg/dL) or triglycerides levels (124 vs. 144 mg/dL). Nevertheless, a significant increase in the concentration of leptin (15 vs. 66 pg/mL, P 0.043) and a diminution of the TNFR1 (100 vs. 80 vs. pg/ml, P 0.0017) and TNFRII (253 vs. 185 vs. pg/mL, P 0.093) levels were detected.
Conclusions: The STI is associated to significant modifications in lipid markers receptors (TNF and leptin), suggestive of an increase in lipogenesis without significant changes in the anthropometric parameters during the 12 months of follow-up.

P664 Metabolic changes in HIV-infected patients on HAART

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Objective: The use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV infected patients, but it has been associated with various adverse effects. A number of metabolic disorders have been reported among patients on HAART, such as high serum lipids and elevated liver enzymes. However, results are still limited and controversial. To assess the impact of NNRTI on serum lipids and liver enzymes in HIV infected patients.
Methods: The study was conducted during the year 2001 at a large outpatient clinic, in Lisbon (Portugal). Clinical charts from 695 consecutive HIV infected patients were analysed. Total and high-density lipoprotein (HDL) cholesterol, triglyceride levels, and AST/ALT were determined. Values represent the average of at least four time-points through the 12 months of the study. Data from patients who were either taking nevirapine or efavirenz-based regimens was compared with patients treated with other combinations, as well as with nontreated HIV infected patients.
Results: Data from 25 patients was excluded from analysis, because of the poor quality of the data. Complete records were available from 670 patients: 175 patients (26.1%) were on a combination of NNRTI and NRTI: 68 patients were taking efavirenz (10.1%, Group EFV), and 107 patients were taking nevirapine (16.0%, group NVP); 418 patients (62.4%) were on other drug combinations (group O), and 77 HIV patients (11.5%) were not taking antiretroviral treatment (group X) (Table 1). No age or gender differences between the groups were noted. Group NVP had lower triglyceride levels and higher HDL cholesterol when compared with group O (triglyceride: 1.63 vs. 1.70, $P < 0.001$; HDL cholesterol: 1.36 vs. 1.07, $P < 0.001$), and higher HDL cholesterol when compared with group X (1.36 vs. 1.03, $P < 0.001$). Group EFV had lower HDL cholesterol than group X and group O (HDL cholesterol: 1.24 vs. 1.03, $P = 0.01$; 1.24 vs. 1.10, $P < 0.001$). No significant differences were found in other variables.
Conclusion: Our data show that there is an association between the use of NNRTI and better lipid profiles (higher HDL cholesterol and lower triglycerides). No increase in liver enzymes was found on association to NNRTI. The use of nevirapine improved the lipid prole both by reducing the triglycerides and increasing the protective HDL fraction. These changes are expected to contribute to the reduction of cardiovascular disease in HIV-1-infected patients on HAART.

P665 Nephrolitiasis induced by indinavir plus boosting dose of ritonavir in a Belgrade study population

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Objectives: Efficacy of Indinavir (IDV) has been known for more than 6 years. Its major disadvantages are limited absorption, a short elimination half-life and adverse events such as nephrolithiasis. IDV in combination with ritonavir (RTV) is believed to ameliorate these disadvantages. We investigate if the use of IDV singly or with boosting dose RTV in combination with nucleoside reverse transcriptase inhibitors (NRTIs) was associated with a higher risk of nephrolithiasis.
Methods: We performed prospective, open-label study and continuously monitored drug naïve patients for incidence cases of nephrolithiasis. The patients received IDV alone (400 mg bid) or IDV plus RTV (400 mg + 100 mg bid) in combination with NRTIs. The prevalence of nephrolithiasis between groups of patients on IDV alone (IDV arm) and on IDV with boosting dose RTV (IDV+RTV arm) was compared using the chi-square test. The

probability of developing nephrolithiasis was estimated by Univariate and stepwise Multivariate logistic regression.
Results: There were 189 patients; 99 patients were included in the IDV arm and 90 patients in the IDV + RTV arm. Nephrolithiasis developed in 38 (26.57%) patients in total. The prevalence of nephrolithiasis was 26.97% on IDV arm and 25.93% on IDV + RTV arm, respectively ($P < 0.001$; d.f. = 1). Multivariate logistic regression shown that the relative risk of developing nephrolithiasis is 1.9-fold greater (RR = 1.9; 95% CI 0.88–2.48) in IDV arm and 6.6-fold greater in IDV + RTV arm (RR = 6.2; 95% CI 1.68–17.12).
Conclusion: We demonstrate that boosting IDV with RTV increased the risk of developing nephrolithiasis by 6.2-fold. We support the need for therapeutic drug monitoring in patients using IDV with or without RTV in order to monitor the number of patients who discontinued IDV due to toxicity.

P666 Prevalence of resistance mutations in subtype F strains isolated from Romanian naïve children

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With the advent of highly active antiretroviral therapy (HAART) the life expectancy of HIV infected patients have been substantially prolonged. However, it is now clear that mutations accumulate in treated patients, limiting the efficacy of the therapy and requiring a change in the existing medication. Resistance genotyping has become a SOC in HIV infection management. Furthermore, resistance mutations are more and more encountered in recently diagnosed/newly infected individuals. Information has accumulated so far mainly on type M clade B strains which are dominantly circulating in Western Europe and North America. Sporadic communications have suggested that resistance mutations can occur spontaneously in the genome of viruses of other subtypes isolated from untreated patients.In this study we present data coming from strains from Romanian children obtained before the onset of the treatment. Most of the samples had viral loads over 10 000 copies/mL. The genotyping has been performed with the ViroseqTM (Applied Biosystems) kit according to the manufacturer's recommendations. Previous anecdotal information suggested that subtype F strains are rather common in Romania. Our findings have confirmed these observations (all strains tested so far belonged to the clade F), although the strains displayed several dissimilarities with subtype F strains available from databases. In order to show the relatedness of the Romanian and international strains phylogenetic trees are being displayed. Bearing in mind that resistance mutations are more readily selected in subtypes other than B, we evaluated several genomic positions belonging to the RT and protease genes. Our results reveal that while the reverse transcriptase gene are relatively stable in respect to the resistance mutations, the frequency of the resistance mutations is significantly higher in the protease gene. Some of the genomic positions seem more prone to evolve towards a resistant genotype. These findings suggest that some resistance calculation algorithms of clinical interest might need to be revised for other subtypes than B – taking in consideration that the virological response of these patients was good.

P667 Phylogenetic analysis of subtype F HIV 1 strains isolated in Romania

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One of the particularities of the HIV epidemics in Romania is the high prevalence of subtype F strains. Virtually all of the strains that have been tested so far have the closest relationship to reference and field clade F strains. Several epidemiological explanations have been proposed so far which took in account local factors: the relatively closed status of the country in the late 1980s, the explosion of the epidemics in closed institutionalised children communities, etc., were supposed to allow a cuasi clonal expansion of a limited number of original HIV strains. Few – if any – of the approaches used so far have relied on molecular epidemiology tools. Phylogenetic trees are a powerful tool that allows to evaluate the relatedness of field strains. The purpose of our study was to use this tool in order to analyse the strains originating from children infected in orphanages between 1987 and 1990, and currently circulating subtype F strains from Romania as well as from neighbouring countries (Moldavia). Sequence information was generated for the protease and partially for the reverse transcriptase gene by using the commercial Viroseq^{TM} kit (Applied Biosystems), following the manufacturer's protocol. Every new sequence was analysed against the available sequences in the major databases. The relationship of the sequences was then analysed using some of the available phylogenetic software (PHYLIP, PUZZLE, etc.). As possible recombinant strains were encountered we performed separately for the reverse transcriptase and protease comparison as well as the analyses of the entire amplified segment. Subtype F strains have been reported to circulate in different parts of the world as well; such regions are South America (Brazil, Argentina), Portugal, Central Africa. Our analyses assess the genetic relatedness of the Romanian subtype F strains with such other strains isolated worldwide.

P668 Comparison of line probe assay vs. sequence analysis for detection of human immunodeficiency virus type 1 (HIV-1) mutations conferring resistance to antiretroviral drugs

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Objective: To compare the line probe assay (LiPA) vs. sequence analysis for detection of mutations conferring resistance to nucleoside (NRTI) and non-nucleoside inhibitors (NNRTI) of HIV-1 reverse transcriptase and protease inhibitors (PI).
Material and Methods: In a retrospective study, 54 plasma samples from 54 HIV-1 infected patients were analysed for drug resistance. All the patients were followed in our hospital. Baseline characteristics of the patients were studied. Forty-nine patients received antiretroviral therapy. The more frequently treatment was two NRTI plus a PI in 42.6%, and two NRTI plus a NNRTI in 33.3% of the patients. Sequencing of the protease (PR)gene and the reverse transcriptase (RT) gene was performed by the TruGene HIV-1 assay (Visible Genetics, Canada). The new version of the LiPA test, LiPA HIV-1 RT and Protease assay v 2.0 (Innogenetics, Belgium) was compared with the reference method: the sequence analysis. The LiPA assay allows the study of wild-type and mutant sequences at codons 41, 69, 70, 74, 75, 184, 215, 103, 106 and 181 of the RT gene (LiPA RT) and at codons 30, 46, 48, 50, 54, 82, 84 and 90 of the PR gene (LiPA P). A descriptive study was carried out with the aid of the statistical program SPSS for Windows version 9.0. Each codon was scored as wild type, mutant, a mixture of both or uninterpretable results. Concordance was defined as the same interpretable results obtained by the two methods. Discordances were defined as minor or major.
Results: LiPA gave uninterpretable results for 36 (5,95%) of 605 analysed codons in the RT gene and for 13 (3.77%) of 344 analysed codons in the PR gene. The concordance between LiPA and sequence analysis was 97.6% for codons of the RT gene and 96.7% in the PR gene. Minor discordances was 1.2% in the RT gene and 2.4% in the PR gene.
Conclusions: (1) The LiPA HIV-1RT and Protease assay v 2.0 give a high rate of codon hybridisation failures and it's do not improve the previous version. It is unacceptable in the clinical practice. (2) LiPA to detect more minority mutant-wild-type mixtures than DNA sequencing. It is useful for the screening of primary HIV-1 resistance.

P669 Evaluation of the Roche COBAS TaqMan 48 HIV-1 test

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Objectives: Analytical and clinical performance of the real-time based Roche COBAS TaqMan 48 HIV-1 Test was evaluated and compared with the Roche COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test, v1.5.
Methods: HIV-RNA purification for the COBAS TaqMan HIV-1 test was conducted by using glass fibre-based High Pure System Viral Nucleic Acid Kit. Plasma panels for intra-assay, linearity and analytical performance studies were obtained from AcroMetrix Corporation. Genotype panels (A–H) were obtained from Boston Biomedica Inc. Plasma specimens for specificity, linearity and competitive sensitivity for low viral titres and high titre viral quantitation of clinical performance were obtained from our previously well-characterised HIV-1 positive plasma panel depository. For analytical performance the specimens were tested by both methods either in duplicate or triplicate. Clinical performance samples for specificity and linearity were tested in singlicate, for competitive sensitivity in duplicate and for high titer quantitation in quadruplicate.
Results: Intra-assay results showed good correlation ($R = 0.996$) between the two methods and CV%s were similar, 2–44% within detection range of the assay. Similar correlation and CV%s were obtained in linearity studies. However, COBAS TaqMan HIV-1 test proved to give slightly higher copy numbers, especially within low copy number range. This was observed also when studying competitive sensitivity for low viral titres in clinical performance. Testing quantitation equality across different genotypes COBAS TaqMan HIV-1 Test showed slightly better sensitivity in all genotypes compared with the COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test, v1.5. The spcificity of the COBAS TaqMan HIV-1 Test, when testing negative sample panel, was 100%. In quantitation of high titer specimens the CV%s varied between 6 and 19%. When using COBAS TaqMan HIV-1 Test there was no need for reruns.
Conclusion: The analytical and clinical performance of the Roche COBAS TaqMan HIV-1 Test is comparable with the Roche COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test, v1.5 depicting better sensitivity especially with low copy numbers. Wider detection range of 40–10 $\times$ 10^7 copies/mL and shorter assay run time of the COBAS TaqMan HIV-1 Test are considerable improvements in HIV RNA-quantitation.

P670 Comparison of two commercial assays for the quantification of HIV-RNA

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Objectives: In this study we compared two of the commercially available assays for the measurement of HIV-1 RNA, the QUANTIPLEX HIV RNA 3.0 (Bayer) which uses the branched DNA signal amplification technique (v3bDNA) and the AMPLICOR HIV-1 MONITOR TEST 1.5 (Roche) which uses reverse transcription (RT-PCR) to assess their quantitative relationship. It is useful to find a quantitative relationship between these two assays because patients may be monitored with more than one assay over the course of their treatment.
Methods: Total of 44 plasma samples from 44 antiVIH-1 infected patients monitored at the specialised unit of HIV at our hospital, were tested with v3bDNA and RT-PCR. Both are ultrasensitive assays with lower detection limits of 50 copies/mL.
Statistical analysis: for the correlation analyses, the intraclass correlation coefficient was calculated. Wilcoxon test was used to determine the statistical significance. Assays values were transformed to common (log10) logarithms and expressed as log 10 v3bDNA or log10 RT-PCR. These assays were also expressed by groups. Three groups (A, B and C) were classified based on numbers of copies/mL.
Results: The data are shown in the following table:

	bDNA			
	A < 1000	B 1000-40000	C > 40000	Totals
ROCHE				
A < 1000	27			27
B 1000–40000	2	7		9
C > 40000		4	4	8
Totals	29	11	4	44

The undetectable samples were 19 (43,18%) tested by RT-PCR and 24 (54, 5%) tested by v3bDNA. The two assays were found to be significantly correlated ($P = 0.002$) coeff. = 0.976. Statistical significant differences were observed among the A group ($P < 0.035$) and B group ($P < 0.021$). No statistical significant differences were observed in C group ($P > 0.263$).
Conclusions: (1) Both test are highly correlated and are used to monitor patients. (2) In patients with low viral load it is not recommended to mix both techniques. (3) These results indicate that variability between assays increases as values approach the lower detection limit.

P671 Evaluation of a simplified protocol of the Versant bDNA HIV 3.0 for HIV-1 viral load quantitation

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Objectives: In this study, we evaluate the validity of a new protocol that simplifies and shortens the standard procedure of the Versant bDNA HIV-1 RNA 3.0 for viral load (VL) quantitation using the System 340 instrument (S340).
Methods: A total of 252 samples were studies in parallel by the standard (SP) and alternate (AP) procedures. Frozen aliquots stored at −70°C were used for performing the AP. SP implies manual vortexing of RNA extracts (x96), incubation for 2 h in thermal block, and then run the S340 HIV program; in contrast, only direct incubation of the nucleic acid extracts in the S340 was required for AP, but running the HCV program (the same. Samples were allocated in groups A–F according to their respective VLs determined by SP: A ($n = 50$) <50 RNA copies/mL (c/mL); B ($n = 50$) 50–100 c/mL; C ($n = 47$) 100–1000 c/mL; D ($n = 45$) 1000–10 000 c/mL; E ($n = 30$) 10 000–100 000 c/mL; F ($n = 30$) >100 000 c/mL.
Results: Mean log-transformed values between SP and AP differed in 0.05 log units. As for groups B–F, these differences were −0.14, 0.01, 0.07, 0.06 and 0.04, respectively. Eight samples in group A (below the limit of quantitation of the SP) gave detectable VL with the AP (range 51–143 c/mL); conversely, seven samples from group B (range 53–75 c/mL) were undetectable by AP. An excellent correlation between SP and AP values were observed, either considering the numeric VLs ($R = 0.936$; $P < 0.001$) or log-transformed values ($R = 0.987$; $P < 0.001$). The AP numeric values of the VL appear to be an overall 1.5% lower than the corresponding SP quantitations.
Conclusion: (1) Both procedures gave comparable VL values, but the alternate protocol is simpler and less time-consuming than the standard procedure. (2) A trend towards lower values was observed for the alternate procedure. (3) Overall differences were far below the 0.2 log units accepted as the normal technical variability.

P672 HIV-1 viral load by NucliSens EasyQ HIV-1 v1.1 in combination with the NucliSens mini MAG instrument

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Objectives: bioMérieux has developed a new nucleic acid isolation method (NucliSens Magnetic Extraction Reagents) that uses Boom chemistry in combination with magnetic silica particles. The NucliSens mini MAG instrument is used to wash and collect the silica particles in a user friendly and efficient way. In principle, the extraction method is generic and can be applied for a broad range of different sample types. The objective of this study was to verify this new extraction platform for the isolation of HIV-1 RNA from plasma samples in combination with the NucliSens HIV-1 v1.1 assay.
Methods: Spiked plasma samples were used to measure the analytical sensitivity of NucliSens EasyQ HIV-1 v1.1 in combination with the new extraction method. The extraction performance on clinical samples was measured by using 123 EDTA plasma samples obtained from HIV-1 infected individuals. As a reference extraction method the NucliSens Extractor was used. Testing was performed on two different sites. In this study also carry over was addressed by processing high positive samples (spiked with >1 000 000 HIV-1 IU/mL) and negative samples obtained from healthy blood donors in the same run.
Results: The analytical sensitivity of NucliSens EasyQ HIV-1 was 53 IU/mL and 92 IU/mL for the new extraction method and the reference method, respectively. In the clinical samples, HIV-1 RNA was detected in 121 of 123 (98%) and 115 of 123 (93%) of the clinical samples for new extraction and the reference method, respectively. In addition, for both extraction methods, excellent correlation was found in the quantification results, the overall *R*-value was 0.94. No case of sample-to-sample carry over during extraction was observed. With the new extraction method 24 samples were processed within 90 min.
Conclusion: The NucliSens mini MAG instrument was successfully used to isolated HIV-1 RNA from plasma samples for determination of viral load. Compared with the reference extraction method (NucliSens Extractor) an improved analytical sensitivity was obtained in combination with NucliSens EasyQ HIV-1 v1.1, and a good clinical reactivity was measured. The sample throughput time is <90 min for 24 samples. Moreover, not a single case of sample-to-sample carry over was detected.

P673 Comparative study of two techniques to measure HIV-1 viral load in a French commercial laboratory

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Objectives: The aim of the study was to compare the performance of the NucliSens EasyQ HIV-1 V1.1 assay (bioMérieux, Boxtel, The Netherlands), a real time NASBA amplification assay for the quantitative determination of HIV-1 RNA in clinical specimen with the Versant HIV-1 RNA 3.0 (bDNA, Bayer Diagnostics) technique routinely used in our laboratory.
Methods: A total of 221 plasma samples were collected from HIV positive/AIDS patients. For each sample, HIV-1 viral load was measured using the NucliSens EasyQ HIV-1 V1.1 assay and the assay we use for routine i.e. Versant HIV-1 RNA 3. An eight-member calibration panel from Versant was used to check for proper set-up and functioning of both systems.
Results: Concordant results were obtained in 202 of 221 samples (91.40%). Seventeen results were found to be discordant. Among them, six were negative with Versant HIV-1 RNA 3 and quantified with EasyQ HIV-1 V1.1 and 11 were negative with EasyQ HIV-1 V1.1 giving a quantification result with Versant HIV-1 RNA 3. These discordant results showed very low values of viral load, always inferior to 2.89 log, corresponding to <776 copies/mL. Two EasyQ HIV-1 V1.1 results were considered as invalid. The results obtained demonstrate a good correlation between the two techniques for both low and high viral loads.
Conclusion: Analysis of a set of 221 clinical routine samples revealed that EasyQ HIV-1 V1.1 has a comparable performance to the Versant HIV-1 RNA 3 assay used in our laboratory. No difference in quantitation was observed between NucliSens EasyQ HIV-1 and Versant HIV-1 RNA 3.These performance results would enable us to switch from our routine method to NucliSens EasyQ HIV-1 without any impact on patient follow-up.

P674 Evolution of hypervariable region 1 of hepatitis C virus in HIV–HCV co-infected patients after treatment with highly active antiretroviral therapy

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Objectives: HCV variability is mainly attributed to virus ability to respond to host immune pressure. This study was aimed at studying the effects of highly active antiretroviral therapy (HAART) on heterogeneity HCV quasispecies in HIV/HCV co-infected patients.
Methods: Sixteen HIV/HCV co-infected patients were selected from I.Co.N.A. Cohort. The selected patients harboured HCV genotype 1, were not co-infected with HBV, and had been on HAART for at least 1 year. With respect to HAART, eight patients showed stable or increasing CD4 counts (immunological responders, I-R) after 1 year of therapy, and eight showed decreasing CD4 counts (I-NR); 11 patients showed HIV viral load <400 cp/mL after therapy (virological responders, V-R), and five showed HIV viral load >10 000 cp/mL (virological nonresponders, V-NR). For quasispecies investigation, plasma samples at baseline (T_0) and after 1 year HAART (T_1) underwent clonal analysis of HVR-1 region of HCV (10–16 clones per patient per time point). Complexity (Shannon entropy) diversity (mean genetic distance), ratio synonymous to nonsynonymous substitutions (K_a/K_s) was considered, as parameters representative of quasispecies herterogeneity at T_0 and T_1.
Results: All parameters of quasispecied heterogeneity showed significant correlation, but did not correlate with HCV viral load, as already shown for patients mono-infected with genotype 1 HCV. Mean values of HVR-1 complexity and diversity were comparable at T_0 and T_1, in patients classified on the basis of either virological or immunological response. However, when analysing the individual variations of complexity, diversity and K_a/K_s, a general tendency was observed, with increasing values in V-R and decreasing values observed in V-NR. This behaviour reached high statistical significance for complexity ($P = 0.005$), was significant for K_a/K_s ($P = 0.045$), and approached statistical significance for diversity ($P = 0.082$). No correlation was observed for the variation of heterogeneity parameters according to immunological response.
Discussion: In HIV-infected patients undergoing HAART, control of HIV replication and not CD4 cell count restoration is predictor of increased HVR-1 quasispecies heterogeneity after 1 year of therapy. This is rather surprising, and warrants further investigation on the relationships between HIV and HCV viral replication dynamics.

Antimicrobial resistance mechanisms - I

P675 Development of endogenous resistance by staphylococci to BAL9141 and comparators

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Introduction: BAL9141 is a novel broad-spectrum cephalosporin strongly cidal towards homogeneous MRSA and vancomycin-resistant staphylococci. As emergence of resistance to antibiotics inevitably follows their clinical use, we examined the proclivity of MR staphylococci to develop high-level endogenous resistance to BAL9141 and comparators.
Materials and Methods: MICs (mcg/mL) towards linezolid, moxifloxacin, and BAL9141 of three staphylococcal strains were as follows: MRSA strain 745: 4, 0.06, 4; MRSA strain P8(+)-Hom: 1, 0.06, 4; MRSE strain CNS 184: 1, 2, 1. Each strain was serially passaged (10^8 CFU/passage, 48-h intervals, 35°C) on Mueller–Hinton agar (MHA) containing linezolid, moxifloxacin, or BAL9141 for 50 passages or until the MIC stabilised at ≥64 mcg/mL. Cells harvested from the last BAL9141 passage were subsequently passaged on MHA without antibiotic, and their MICs towards the cephalosporin checked periodically by agar dilution. BAL9141-passaged cells were also examined by DNA sequencing of the mec operon and by QRT-PCR to assess changes in structure and expression of genes involved in beta-lactam resistance.
Results: Cells transferred in the presence of linezolid achieved MICs of ≥64 mcg/mL after 27 (MRSA strain 745), 28 [MRSA strain P8(+)-Hom], and 28 (MRSE strain CNS 184) serial passages. Cells transferred with moxifloxacin achieved MICs of ≥64 mcg/mL after eight (745), 22 [P8(+)-Hom], and four (CNS 184) serial passages. In contrast, the MICs of cells transferred in the presence of BAL9141 reached a plateau level of 32 mcg/mL after, respectively, 37 (745), 32 [P8(+)-Hom], and 15 (CNS 184) serial passages. The MICs towards BAL9141 remained at this value through the 50th passage. When isolates of BAL9141-acclimated MR staphylococci were passaged subsequently on antibiotic-free MHA, their MICs towards BAL9141 fell from 32 to 8 mcg/mL within 1–2 transfers and remained at 8 mcg/mL through 30 serial passages in the absence of antibiotic pressure.
Conclusions: The frequency of chromosomal mutations conferring resistance in MR staphylococci follows the order moxifloxacin >> linezolid > BAL9141. Emergence of endogenous resistance during a course of treatment with BAL9141 is considered exceedingly unlikely.

P676 Emergence and dissemination of glycopeptide- and high-level quinupristin-dalfopristin resistant *Enterococcus faecium* clones in various wards of a tertiary hospital

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Objectives: To investigate the dissemination of quinupristin/dalfopristin- and glycopeptide-resistant *Enterococcus faecium* isolates in a tertiary Greek hospital prior to the clinical use of quinupristin/dalfopristin and their resistance mechanisms.
Materials and Methods: Twenty quinupristin/dalfopristin resistant vanA gene carrying *Enterococcus faecium* (glycopeptide-resistant *E. faecium*, GREF) isolates were recovered during a 10-month period from clinical infections of separate patients in various departments of Sismanoglion General hospital (Athens, Greece). The isolates were tested phenotypically for their MICs to multiple antimicrobials used against enterococci, by PCR for genes encoding glycopeptide and streptogramin resistance, by pulsed-field gel electrophoresis (PFGE) and by plasmid analysis.
Results: The MICs of the isolates for quinupristin/dalfopristin ranged from 4 to >32 mg/L. All isolates carried the gene erm(B) but not genes vat(D), vat(E) or other known genes encoding streptogramin A acetyltransferases, efflux pumps or rRNA methylases [vat(D), vat(E), vat(A), vat(B), vat(C), vga(A), vga(B), vgb(A), vgb(B), and erm(B)]. Macrorestriction analysis of the isolates showed that the highly quinupristin/dalfopristin GREF isolates fitted to two genetic lineages, while the remaining isolates belonged to various unrelated clones. Plasmid analysis of the highly streptogramins resistant isolates showed that some isolates did not carry any visible plasmid, possibly suggesting a chromosomal location of the resistance mechanism(s).
Conclusions: The absence of detectable genes encoding streptogramin resistance indicates that further unknown mechanisms confer the high level resistance to quinupristin/dalfopristin. The emergence of clonally unrelated GREF isolates resistant to quinupristin/dalfopristin prior to its clinical use in Greece suggests the circulation of such isolates in the community and represents a potential threat for the usefulness of quinupristin/dalfopristin in the clinical practice.

P677 Evidence of reserpine-affected mechanism of resistance to tetracycline in *Neisseria gonorrhoeae* clinical isolates

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Objective: To analyse the effect of reserpine in the MIC of tetracycline in *N. gonorrhoeae*.
Methods: Seventeen clinical isolates of *N. gonorrhoeae* and three strains with a characterised mutation in the mtrCDE operon were analysed. The MIC of tetracycline both in the absence or the presence of reserpine was performed by *E*-test and read both at 24 and 48 h. Moreover, the MIC of erythromycin was performed following the same methodology in the three control isolates. Presence of tetA and tetM were established by PCR. To discharge a direct effect of reserpine the MIC of this compound was calculate.
Results: Twelve intermediate and five tetracycline-resistant isolates were studied. All tetracycline-resistant strain carried the tetM gene (Dutch variant) whereas none presented the tetA gene. Nor tetA neither tetM genes were present among the intermediate isolates. In all strains the MIC of tetracycline was affected by reserpine. Thus, among intermediate isolates the MIC in presence of reserpine decreased from 4 to >62-fold (from 0.5 to 1 mg/L to <0.016 to 0.25 mg/L), without differences when the plates were read at 24 or 48 h, while those strains carrying tetM gene presented a MIC ranged from 12 to 16 mg/L and 1–1.5 mg/L at 24 h which was determined in the absence and the presence of reserpine, respectively, and of 32 mg/L, and 6–8 μg/ml when read in absence and presence of reserpine at 48 h. The isolates were able to grow on concentrations of reserpine higher than 100 mg/L. The effect of reserpine on mtrCDE system was discharged due to its null effect inhibiting the MIC of erythromycin.
Conclusion: Our results suggest an intrinsic mechanism of resistance to tetracycline in *Neisseria gonorrhoeae* clinical isolates probably associated with the basal expression of some reserpine inhibitable efflux pump different from mtrCDE.

P678 Distribution of ermA, ermC, msrA and linA genes in methicillin-resistant staphylococci in the Czech Republic

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Objectives: The aim of this study was to investigate an incidence of different resistance mechanisms to macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic.
Methods: Phenotypes were determined by triple-disc diffusion method. The presence of the genes ermA, ermC (resistance by target site alteration) msrA (efflux resistance) and linA (resistance by antibiotic inactivation) was tested by Southern blot analysis. A bioassay for the detection of lincomycin inactivation mechanism was performed in lincosamide-resistant strains, where none of the resistance genes was detected.
Results: In 99 clinical isolates in vitro resistant to one of erythromycin, lincomycin or clindamycin, triple-disc diffusion method reveals seven different phenotypes corresponding with resistance mechanisms. The resistance was mainly because of the presence of msrA gene, which was detected in 53 strains. Genes ermC and ermA were detected in 42 strains and the linA was detected in 28 strains. In 15 lincosamides-resistant strains no resistance gene was detected. Lincosamides were not inactivated in those strains indicating a new type of resistance different from inactivation.
Conclusion: The dissemination of resistance types differs strongly from the published data. While in other countries cross-resistance to macrolides and lincosamides conferred by ermA and ermC predominates, in the Czech Republic the gene msrA is the most frequent genetic determinant conferring resistance to macrolides only. It follows that one third of macrolide-resistant staphylococci remains lincosamide sensitive. A group of lincosamide-resistant strains with unknown resistance mechanism was newly defined.

P679 Fluoroquinolone-selected resistance among *Pseudomonas aeruginosa*: impact on susceptibility to imipenem, ertapenem and meropenem

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Objectives: Fluoroquinolone-selected mutants of *Pseudomonas aeruginosa* can exhibit significant changes in carbapenem susceptibility. However, little is known about which fluoroquinolones are more likely to select for carbapenem resistance. This study assessed the occurrence and mechanism(s) of altered carbapenem susceptibility among *P. aeruginosa* mutants selected with levofloxacin and ciprofloxacin.
Methods: *Pseudomonas aeruginosa* PAO1 and a clinical isolate *P. aeruginosa* 164 were the parent strains, and single-step mutants were selected in-agar with 1X–4X MIC of ciprofloxacin and levofloxacin. Confirmed mutants were evaluated for changes in susceptibility to imipenem, ertapenem and meropenem. Mutants with significant changes in carbapenem susceptibility were evaluated for changes in transcriptional expression of four efflux pumps and oprD.
Results: Fifty-six confirmed fluoroquinolone-resistant mutants were selected, with none exhibiting decreases in imipenem susceptibility or changes in expression of mexEF-oprN. In contrast, four mutants demonstrated significantly decreased susceptibility to meropenem and ertapenem. However, these four mutants did not alter their expression of the four efflux pumps or oprD, and they were selected with both fluoroquinolones from both parent strains. The small numbers prevented meaningful comparisons between the fluoroquinolones. Three additional mutants exhibited hypersusceptibility to imipenem and ertapenem, with associated six- to 11-fold overexpression of mexCD-oprJ. One of these mutants was hypersusceptible to all three carbapenems, and meropenem hypersusceptibility was associated with fourfold decreased mexAB-oprM expression.
Conclusions: Ciprofloxacin and levofloxacin exhibited a propensity to select for both dual resistances to fluoroquinolones and meropenem/ertapenem, as well as carbapenem hypersusceptibility. The mechanism(s) of meropenem and ertapenem resistance remain unknown and warrant further investigation. Furthermore, similar studies using *in vitro* pharmacodynamic models should be conducted to determine if pharmacokinetically relevant exposure to levofloxacin and ciprofloxacin provides different selection pressures for mutant selection compared the static conditions used in this study.

P680 High prevalence of macrolide resistance in *Streptococcus mitis* from neutropenic patients

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Background: The purpose of this study is to determine antibiotic susceptibility of *Streptococcus mitis* and to characterise the mechanisms of macrolide resistance.
Material: In 2002, 169 isolates of *S. mitis* were recovered at the Tunisian Bone Marrow Transplant Centre. A total of 120 (70%) strains were nonsusceptible to erythromycin. From this collection, 33 strains were randomly chosen and studied.
Methods: Susceptibility of erythromycin-resistant isolates to antibiotics was tested by agar dilution technique (CA-SFM). Amplification of ermB and mefE genes was carried out by multiplex PCR technique. For strains harbouring ermB genes, amplification of int-Tn genes encoding the integrase of the Tn916–Tn1545 family of conjugative transposons was carried out by PCR technique.
Results and Discussion: The rate of macrolide resistance was impressive and remarkably higher than those in previous studies. In neutropenic cancer patients, exposure to antimicrobial pressure is high and selection of resistant strains may occur. Of the 33 erythromycin-resistant strains studied, 14 were cross resistant to erythromycin, spiramycin and lincomycin, including 10 strains containing ermB genes and four containing a combination of ermB and mefE genes. Fourteen strains displayed M phenotype contained mefE genes. Five strains were apparently susceptible to lincomycin but resistant to spiramycin, four contained ermB genes and one a combination of ermB and mefE genes. In our study and in Canada, ermB and mefE genes are prevalent. However, ermB genes are prevalent in Japan and mefE genes in Spain. The duplicity of genes has not been described in *S. mitis* but already been identified in *S. pneumonia*e, *S. agalactiae*, *S. oralis* and *S. pyogenes*. A correlation was found between lower erythromycin MIC (4–64 mg/L) for isolates carrying mefE and higher level (MIC of 4 to >1024 mg/L) for those carrying ermB genes.The int-Tn gene, was detected in all strains harbouring ermB genes indicating that this gene is disseminated by conjugative transposon to different strains.
Conclusion: This study shows high erythromycin resistance rate among *S. mitis* isolates with prevalence of mefE and ermB genes in our centre, which argue against the clinical usefulness of erythromycin to prevent viridans group streptococcal bacteraemia in neutropenic cancer patients.

P681 Frequency and stability of mutants selected with fluoroquinolones from *Klebsiella pneumoniae* containing the plasmid-mediated resistance determinant QNR

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Objectives: To evaluate the frequency and stability of mutants derived from a porin-deficient *Klebsiella pneumoniae* (Kp) strain KpIMP17 (wild-type strain) containing the Ser83Phe mutation in gyrA and its derived transconjugant KpIMP22 containing plasmid pMG252, which codes for the plasmid-mediated fluoroquinolone

(FQ) resistance determinant qnr. MICs (mg/L) of ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MFX) against KpIMP17 and KpIMP22 were 0.5/0.5/0.25 and 4/4/8, respectively. Moreover, the presence of mutations in the genes gyrA and parC, in these mutants, were studied.
Methods: Mueller–Hinton (MH) agar plates containing 4x MIC of CIP, LEV or MOX were inoculated with bacteria grown *in vivo* (pneumonia model in mouse) or *in vitro* (MH broth), and incubated at 35–37°C for 48 h. Mutants (up to eight from each plate) were subcultured at least twice in MH agar without antibiotics, and MICs of the selecting quinolones were determined by microdilution (NCCLS guidelines). True mutants were defined as those for which the MIC has increased greater than or equal to fourfold with respect to the parental strain. Mutations in the quinolone resistance determining region (QRDR) of the genes gyrA and parC were evaluated by PCR and sequencing.
Results: All mutants selected on agar plates containing FQ showed stable increased resistance to the selecting agent. Frequencies of mutation of KpIMP17 were 5×10^{-7} (*in vitro* grown bacteria) and 5×10^{-4} (*in vivo* grown bacteria), respectively. For KpIMP22, these values were 10^{-6} and (*in vitro* grown bacteria) and 10^{-3} to 10^{-4} (*in vivo* grown bacteria), respectively. MICs of FQ against mutants from KpIMP17 were 16 to 64-folds higher than against KpIMP17. In the case of KpIMP22-derived mutants MICs increased eight- to 16-fold. None of the mutants presented any additional mutation in the QRDR of gyrA or parC.
Conclusions: In KpIMP22, pMG252 coding for qnr increases 10 times the emergence of mutants with increased resistance (eight- to 16-fold) to FQ. This increase is not caused by additional mutations in the QRDR of gyrA or parC. One hundred per cent of the selected mutants presented stable resistance to fluoroquinolones.

P682 Resistance mechanisms of fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae* collected in Belgium, 1999–2003

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Objectives: Study of point mutations in the quinolone resistance determining regions (QRDR) i.e. gyrase and topoisomerase IV, and the role of pmrA efflux or other efflux pumps.
Methods: Different centres collected clinical strains of *S. pneumoniae* during the winters of 1999 (205), 2001 (314) and 2003 (394). These strains were screened for ciprofloxacin (CIP) resistance by the NCCLS microdilution technique. *Streptococcus pneumoniae* R6 was used as a susceptible reference strain. As positive control the strains F4 and J5 from A. Dickens (UK) were used. The QRDR regions of gyrA, parC and parE of each strain were amplified with the corresponding primers by PCR and then sequenced using the ABI PRISM 3100 Avant Genetic Analyzer. To determine the contribution of efflux to the FQ-resistance, the strains were cultured on blood agar, with or without reserpine. E-test for CIP and moxifloxacin (MOX) were used to determine the MIC values on both media. A PCR for the pmrA-gene and its regulator (mta1) was carried out on each strain.
Results: Resistance caused by QRDR mutations: of a total of 913 clinical isolates, 89 (10.2%) were intermediate resistant (I) and 29 (3.2%) resistant (R) to CIP. All the resistant strains, 38 intermediate and four susceptible (S) strains were sequenced (SIR: 1999: 3-15-3; 2001: 1-5-9; 2003: 0-24-17). Of a total of 71 strains, 16.9% had mutations in gyrA, 0% gyrB, 29.6% parC and 74.6% parE. Combined mutations: 1999: 38.9% with two mutations and 5.6% with three. For 2001: 28.6, 7.1 and 14.3% with 2, 3 and 4 mutations, respectively. For 2003: 7.7, 10.2 and 2.6%, respectively. Only 30.4% of the multiple mutations resulted in I or R to CIP and 8.7% to MOX.
Efflux resistance: All strains were positive for pmrA. An efflux activity for CIP was found in 46 strains (64.8%) while only one strain (1.4%) had a low efflux activity for MOX. In eight strains with no mutation, a high efflux activity for CIP was found.
Conclusions: The relative prevalence of mutations did not increase through the years. Multiple mutations did not necessarily result in more resistant strains and only the nature of the mutation is important. Efflux pumps were present in all strains but efflux activity was only detected for CIP and not for MOX.

P683 Inducible metronidazole resistance in nim-positive clinical *Bacteroides fragilis* group strains

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Objectives: To survey the incidence of nitroimidazole resistance (nim) genes among 1502 clinical strains of *Bacteroides fragilis* group species originating from a pan-Europe study. Selected nim-positive metronidazole susceptible strains were tested for inducibility of metronidazole resistance.
Methods: All strains were screened for nim-genes by PCR. Determination of specific nim-genes (A-F) was carried out by RFLP. Presence of insertion sequence elements in the nim-positive strains was detected using PCR. Seven randomly chosen nim-positive clinical strains (*B. fragilis*, *B. thetaiotaomicron* and *B. ovatus*) with low initial MIC values (0.25–8 mg/mL) were assayed for induction using metronidazole *E*-test strips. Micro colonies were picked within the ellipse area of the E-test strips for three passages, followed by testing of the stability of the resistance by inoculation on blood agar plates without exposure to metronidazole over three additional passages. *Bacteroides fragilis* ATCC 25285 *B. thetaiotaomicron* CCGU 29741 and three clinical strains (*B. fragilis*, *B. thetaiotaomicron* and *B. ovatus*) all lacking nim-genes, were used as control strains.
Results: Two per cent ($n = 30$) of the isolates tested harboured nim-genes. These strains belonged to four species, *B. thetaiotaomicron*, *B. vulgatus* and *B. ovatus* and to the highest extent by the clinically most important species *B. fragilis*. The nimA was detected in 16 of the strains two had nimB, two nimC, seven nimD, two nimE and one nimF. In 23 of the 30 nim-positive strains an IS element possibly involved in regulation of the nim-gene expression was identified. All seven nim-positive strains tested for inducibility of metronidazole resistance could be induced to express high levels of resistance (MIC 64 to $\geq$256 mg/mL) after three passages on subinhibitory concentrations of metronidazole. After three subsequent passages without metronidazole, the resistance was maintained at the same induced level only in one strain. The other six strains yielded a lower stability of resistance and had lower MIC-values after the three final passages. However, four of these strains still had elevated MIC-values compared with preinduction. In contrary, the five nim-negative control strains demonstrated no significant increase of resistance.
Conclusion: The fact that nim-positive strains were easily induced to high levels of resistance is of great clinical concern and emphasises the importance of acknowledging metronidazole resistance in the clinical setting.

P684 Diversity of the mechanisms involved in imipenem resistance in *Acinetobacter baumannii*

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Objectives: *Acinetobacter baumannii* (Ab), a nosocomial pathogen, is often resistant to multiple antibiotics now including ip. At this time, ip resistance represents about 5% of the strains, when several mechanisms have been described already. These strains are more often involved in hospital outbreaks. The diversity of the mechanisms involved in ip resistance was studied in nine clinical isolates of Ab.

Methods: The minimal inhibitory concentrations (MICs) of ip (MSD Laboratory) were determined by E-test method (AB Biodisk) and serial dilutions (1–128 mg/L) in Muller–Hinton broth (MHB). Susceptibility testing of seven beta-lactams (amoxicillin, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanate, ceftriaxone, ceftazidime) was performed by the agar diffusion method in accordance with the French committee of antibiogram guidelines. Including cloxacillin in agar medium that is known to inhibit cephalosporinase revealed cephalosporinase activity. The presence of a metallo-enzyme responsible for imipenem resistance was studied, comparing the MICs of ip with and without EDTA. Enzymes probably produced by the strains were characterised by isoelectic-focusing method with precast polyacrylamid gels containing ampholines with pH range 3.5–9.5 (Amersham Bioscience).
Results: MICs of ip, obtained with *E*-test method, were ranged from 4 to 128 mg/L. Discordance was noted between *E*-test method and susceptibility testing in MHB: four strains had ip MICs first considered superior or equal to 32 mg/L because of the presence of mutants when MICs in MHB were 4 mg/L. Other beta-lactams also showed a decreased activity. Cephalosporinase activity was involved in imipenem resistance in two strains for which, inhibition of the cephalosporinase activity by cloxacillin not only restore activity to ureidopénicillin, carboxypenicillin or cephalosporins but also to ip. Imipenemase activity was found in four strains: the MIC of ip plus EDTA was lower than the MIC of only ip. Electro-focusing showed one or two enzymes with alkaline pI, consistent with those described for IMP-1 and IMP-4 (pI 8–9). For three strains no enzyme were produced. A modification of the PLP or an impermeability mechanism was suspected.
Conclusions: In this study, we confirmed that multiple mechanisms should be involved in the resistance of Ab to ip. Nevertheless, this is the first time that some cephalosporinase produced by Ab are suspected to be responsible for this resistance.

P685 Cross-resistance between voriconazole and fluconazole due to the expression of efflux pumps

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Voriconazole (Vor) is a new triazole antifungal agent, structurally related to fluconazole (Flu), with improved potency and spectrum of activity.
Objective: To evaluate the activity of Vor on clinical resistant strains to Flu.
Methods: Thirty-six resistant and 20 susceptible strains to Flu were used. Minimal inhibitory concentration (MIC) to Flu and Vor were determined according the NCCLS protocol M27-A. Flow cytometry analysis of conidia stained with FUN-1 after 1 h incubation with Flu (1) and 2 h with Vor allowed us to determine a staining index (SI), a ratio between the intensity of fluorescence of treated conidia and the control (nontreated conidia). These assays were repeated after incubating the conidia with sodium azide and with four modulators of efflux pumps (verapamil, β-estradiol, progesterone and ibuprofen), at a concentration able to block efflux pumps. High MIC values to Flu, a SI < 1 and a reversion of resistance following the reduction of the energetic pool with sodium azide or with the modulators was suggestive of resistance of efflux pumps (2).
Results: Sixteen resistant strains to Flu showed very high MIC to Vor (>4 μg/mL), and evidence of active efflux. Low MIC values to Vor and no evidence of efflux were found on the remaining resistant strains to Flu, including five *C. krusei* strains (known as intrinsically resistant to Flu). All susceptible strains to Flu showed low MIC to Vor.
Conclusions: Vor is a more active compound on Candida strains than Flu. The presence of efflux pumps seems to be responsible for the cross resistance between the two antifungals.
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P686 Substrate inhibition of the *Pseudomonas aeruginosa* metallo-beta-lactamase, SPM-1, in acidic and alkaline conditions

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Objectives: To date, there is limited information on the mechanism of hydrolysis for metallo-beta-lactamases (MBLs) with therapeutic beta-lactams. Recently, we described a new-type of MBL, SPM-1, which has unusual kinetics. Accordingly, in order to provide an insight into the mechanism of hydrolysis by SPM-1, a detailed kinetic analysis under different pH conditions was undertaken.
Methods: SPM-1 was purified from a recombinant plasmid using a periplasmic preparation followed by a two-stage purification process. SPM-1 was 98% pure, confirmed by SDS-PAGE. Beta-lactamase assays for pH 4.5–5.5 were carried out in acetate buffer, pH 6.0–7.5 in cacodylate buffer and pH 8.0–9.0 in Tris–HCl buffer. The purified enzyme was transferred to the relevant pH by ultrafiltration and enzyme concentration determined for each preparation. Kinetic parameters (k_{cat}/K_m) were determined for penicillin G, cefuroxime, meropenem and nitrocefin at each pH from the initial rates of hydrolysis for different substrate concentrations.
Results: Substrate inhibition is seen for penicillin G and cefuroxime at high substrate concentrations under acidic conditions and is particularly marked for penicillin G. As the pH values increase, substrate inhibition is reduced. No substrate inhibition is observed between pH 6.0 and 7.5 but once again becomes apparent for penicillin G and cefuroxime in the alkaline pH range. No substrate inhibition is seen for cefuroxime or meropenem. The hydrolytic efficiency (k_{cat}/K_m ratio) of SPM-1 for all beta-lactams is optimal between pH 6.0 and 6.5. The hydrolytic efficiency of SPM-1 decreases towards the acidic and alkaline pH ranges. Overall, pH has a limited effect on the hydrolytic activity of SPM-1. The greatest effect being seen for meropenem where a 20-fold order of magnitude between pH 4.5 and 6.5, and pH 9.0 and 6.5 is observed.
Conclusion: SPM-1 shows substrate inhibition for penicillin G and cefuroxime at acidic and alkaline pH only, indicating a mechanistic explanation for these effects. The lower hydrolytic efficiency at acid and alkaline pH's has previously been seen for both IMP-1, with similar orders of magnitude to SPM-1, and BcII where greater orders of magnitude were seen between pH 4.5 and 6.5. In contrast to these enzymes, the effects of pH on the efficiency of SPM-1 are substrate dependant.

P687 Class 1 and class 2 integrons among ESBL and non-ESBL *Escherichia coli* isolates recovered from nosocomial and community environments

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Objectives: To analyse the overall prevalence of integrons among *E. coli* (EC) isolates from different environments, phylogenetic groups and susceptibility profiles.
Methods: We studied 135 isolates: (i) 52 PFGE ESBL(+) clonal types from hospital setting (16 TEM, nine SHV, 22 CTX-M-9, one CTX-M-14 and four CTX-M-10) (1987–2000); (ii) 43 ESBL(−) isolates causing bacteraemia (1987–2000); and (iii) 40 ESBL(−) isolates from faecal samples of healthy volunteers (HV) living in Madrid (2000–2001). EC phylogenetic groups were determined by a multiplex PCR assay. Class 1 and 2 integrons were detected by PCR, typed by RFLP using AluI and HaeIII as restriction enzymes, respectively, and identified by sequencing (one per RFLP type).
Results: Class 1 were most prevalent than class 2 integrons (48% vs. 14%). Class 1 integrons were more commonly found among ESBL than non-ESBL from blood or HV isolates (69, 40 and 30%) due to the high prevalence of EC containing blaCTX-M-9, which

is located in an In6-like class 1 integron. Class 2 integrons were more common among community than nosocomial isolates [18, 7 and 7% for ESBL(−)HV, blood ESBL(−) and ESBL(+), respectively]. A number of integrons did not contain gene cassettes (nine of 65 of class 1 and one of 19 of class 2). Presence of integrons was more frequent among group D than A, B1 or B2 phylogenetic EC groups (39, 25, 11 and 25% for class 1, and 61, 22, 11, and 6% for class 2). Seven class 1 integron types and three class 2 integron types were detected. Among them, class 1 integrons aadA1 (15 of 56), dfrA1aadA1 (12 of /56) and dfrA16aadA2 (13 of 56) were the most frequently found. Class 2 integrons were mainly associated to Tn7 (15 of 18).

Conclusions: Integrons are widely distributed among EC isolates from both community and nosocomial environments, being frequently associated to EC group D. The low diversity of integrons found might indicate a wide dissemination of other elements (plasmids or transposons) in which they are located. The capture of genes as blaCTXM-9 from the metagenome community pool might be facilitated by the potentially adaptive functions encoded in its genetic neighbourhood.

P688 Induction of the *Acinetobacter baumannii* RND efflux pump AdeB with ciprofloxacin and gentamicin

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Objectives: The *Acinetobacter baumannii* RND efflux pump AdeB has been shown to confer resistance to gentamicin (GEN) and is involved in reduced sensitivity to the fluoroquinolones. AdeR is the putative regulator of adeB, however, expression levels of the genes encoding the pump and regulator is at present unknown. We investigated the effect of ciprofloxacin (CIP) and GEN on expression of these genes by growing isolates in subinhibitory concentrations of the agents and looked at mRNA expression levels quantitatively by RT real-time PCR.

Methods: The standard *A. baumannii* laboratory strain ATCC 19606 (CIP/GEN MICs 1 and 4 mg/L, respectivley) and a multidrug resistant clinical isolate SB13 (CIP/GEN MICs 8 and 128 mg/L, respectively) were used for this study. Log-phase cells were challenged with a subinhibitory (1/2) MIC of CIP or GEN and aliquots extracted at time $t = 0$, 1, 3, 5 and 10 min. RNA was stabilised before extraction and treatment with DNase. RNA (1 μg) was reverse transcribed into cDNA template with random hexamers. Quantitative real-time PCR was performed in a LightCycler with primers for internal sequences of adeB and adeR. 16sRNA was used as a housekeeping gene for internal control.

Results: Quantitative real-time PCR against 16sRNA confirmed that samples had equal concentrations of cDNA; 3–4E + 07 transcripts/ng RNA. At $t = 0$, ATCC 19606 expressed a higher number of adeB transcripts than SB13; 1.7E + 04 vs.1E + 03 transcripts/ng RNA. Levels of adeR were lower than those of adeB by 1E + 02. Ten minutes after addition of CIP there was no difference in adeB levels with ATCC 19606. However, strain SB13 showed a 13-fold increase in adeB transcripts over 10 min. ATCC 19606 adeR levels dropped 80% after 3 min and increased back up to prechallenge levels by 10 min. With SB13, levels of adeR decreased by 50%. A similar but weaker response in adeB expression was seen after challenge with GEN; no change with ATCC 19606 and a sixfold increase with SB13. ATCC 19606 adeR levels decreased by 50% but rose twofold with SB13.

Conclusions: The gene encoding AdeB is induced in a multidrug resistant clinical isolate by ciprofloxacin and gentamicin but is not induced in a sensitive strain. Total levels of adeB or adeR transcripts may not be an indicator of reduced sensitivity whereas inducibility is. This suggests that other factors such as stability of transcripts may play a greater role in *A. baumannii* drug resistance.

P689 Detection of carbapenemases in *Pseudomonas aeruginosa* clinical isolates resistant to imipenem

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Objectives: The aim of this study was to detect and identify the presence of carbapenemases in clinical isolates of *Ps. aeruginosa* resistant to imipenem.

Methods: The study included all resistant and intermediate isolates (33 and 15, respectively) obtained at a Hospital from Bilbao (Northern Spain) during 2002. Phenotypic detection was carried out using the Hodge and EDTA tests. Genetic experiments to detect bla-OXA 40, bla-IMP and bla-VIM genes were performed by DNA amplification with the primers P1/OXA-24: 5′-GTACTAATCAAAGTTGTGAA-3′ and P2/OXA-24: 5′-TTCCCCTAACATGAATTTGT-3′), BLAIMPF: 5′-CTACCGCAGCAGAGTCTTTG-3′ and BLAIMPR 5′-AACCAGTTTTGCCTTACCAT-3′) and VIM-DIA/f 5′-CAGATTGCCGATGGTGTTTGG-3′; VIM-DIA/r 5′-AGGTGGGCCATTCAGCCAGA-3′; VIM1-upv 5′-GTCGCAAGTCCGTTAGCCCAT-3′ and VIM 2-upv 5′-GATTCTAGCGGTGAGTATCCG-3′. To detect class 1 integrons, primers 3′CS and 5′CS were used in amplification experiments.

Results: Results with Hodge and EDTA tests were as follows: (a) Hodge+ and EDTA+, nine isolates; (b) Hodge+ and EDTA−, six isolates; and (c) Hodge− and EDTA+, four isolates. The inhibiton of the growth of the control strain and the mucoidity of the clinical isolates tested did not allow the correct interpretation of the results in many experiments.All bla-IMP experiments were negative but some fragments were obtained when the bla-VIM genes were amplificated. OXA-40 gene was detected in two isolates (both were Hodge+ and EDTA−), which also bored integrons of 1500 and 850 bp.

Conclusions: Phenotypic methods did not allow the correct detection of carbapenemases in the majority of the *Ps. aeruginosa* isolates tested. Genetic experiments showed the presence of OXA-40 gene in two isolates resistant to imipenem, enzyme firstly identified in *A. baumannii* isolates from the same hospital.

P690 Nonenzyme-mediated imipenem resistance in a clinical isolate of *Citrobacter freundii* from Trondheim, Norway

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Objectives: *Citrobacter freundii* K2-23 was recovered from a surgical site infection of a 39-year-old kidney-transplanted male during treatment with imipenem and was shown to possess high-level resistance to third and fourth generation cephalosporins as well as imipenem (MIC 8 mg/L). Initial phenotypic screening for extended spectrum beta-lactamases (BL) using Etest (AB biodisk) showed that the resistance was not sensitive to clavulanic acid. Therefore, we molecularly investigated the mechanism of this broad-spectrum beta-lactam resistance.

Methods: Genetically screening for ESBL genes (TEM, SHV, CTX-M and OXA-type) and CMY-type BL genes involved PCR. Degenerate primers used were designed on the conserved regions of the genes and often more than one primer set was required. PCR amplicons of the desired size were sequenced by a AB sequencer. Crude cell extracts for BL hydrolytic activity were prepared by sonication and centrifugation. Assays were carried out in a Perkin Elmer Lamda 35 spectrophotometer with a range of β-lactams. Isoelectric focusing (IEF) was determined using a Novex (Invitrogen, CA, USA) vertical gel system. Outermembrane preparations were carried out on K2-23 and 5 *C. freundii* β-lactam sensitive strains by standard extraction methods and visualised using SDS-PAGE (Invitrogen). N-terminal protein sequencing was performed on those outermembrane differentially expressed in K2-23.

Results: PCR amplicons were obtained for CTX-M-type and CMY-type BL genes but only the sequence of the latter was creditable and that was of the nascent *C. freundii* AmpC. IEF revealed

only a single weak band with a pI of 8.0 and the BL assays demonstrated no significant hydrolysis with cefotaxime, ceftazidime, cefepime or imipenem. SDS-PAGE on the *C. freundii* isolates revealed that only K23 had a band missing at a molecular weight of 43 kDa. Another protein of a similar size also appeared to be weakly expressed. Preliminary sequencing of the N-terminus of this and revealed the amino acid sequence of AEIYNK. These sequencing residues showed similarity with the ompK35 of *Klebsiella pneumoniae* indicating that the protein missing in strain K2-23 is likely to be an OMP-like outermembrane protein.
Conclusion: The data arising from these studies would indicate that key beta-lactam resistant determinant from *C. freundii* K2-23 is not over-production of the nascent AmpC but down-regulation in one or more of its outermembrane proteins.

P691 Extended spectrum beta-lactamase (ESBL) producers among enterobacteriaceae from patients in Bulgarian hospitals

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Objective: To preliminary characterise the main types of ESBLs among Bulgarian Enterobacteriaceae strains and to determine their rate of resistance.
Methods: A total of 427 Enterobacteriaceae (*K. pneumoniae* – 238, *K. oxytoca* – six, *E. coli* – 129, *C. freundii* – five, *E. cloacae* – eight, *E. aerogenes* – seven, *Proteus* – five, *Serratia* – three, *Salmonella* – one) strains identified as ESBL producers were collected from seven medical centres in Sofia, Pleven and Stara Zagora during 1996–2003. They were confirmed by phenotypical tests (DDS, NCCLS method). MICs were determined by an agar dilution technique (NCCLS, 2002). Conjugative plasmid transfer was performed, followed by an isoelectric focusing according to Mathew/Bauernfeind. The hydrolytic activity of the bands was proved by Bioassay (Bauernfeind).
Results: The rate of resistance was: amoxicillin/clavulanate – 86%, ceftazidime – 85%, cefotaxime – 95%, ceftriaxone – 94%, aztreonam – 74%, cefoxitin – 14%, ceftibuten – 19%, cefepime – 37%, imipenem – 0%, tobramycin – 95%, gentamicin – 84%, amikacin – 41%, ciprofloxacin – 56%, tetracycline – 86%, co-trimoxazole – 53% chloramphenicol – 58%. MIC of ceftazidime (CAZ) ranged 1 to >512 mg/L and of cefotaxime (CTX) – 2–512 mg/L. The strains were divided into two main groups: the first one: MICCAZ > MICCTX – 251 strains and the second: MICCAZ < MICCTX – 161. In all strains sulbactam in combination with CAZ and CTX showed an inhibitory effect. CAZ and/or CTX resistance was transferable in 91 from 100 mating experiments. IEF analysis of 91 strains showed the presence of three clusters. One cluster (42 strains *Klebsiella* and 4 E. cloacae) presents CAZ hydrolytic bands with isoelectric points (pI) 8.2 (SHV type). The second cluster (18 strains *Klebsiella*, two *C. freundii*, 1 *E. coli*) demonstrated enzymes focusing at pI 6.3, which suggests TEM type. The strains with MICCTX > MICCAZ – (11 *K. pneumoniae*, 12 *E. coli*, 1 *S. marscescens*) showed CTX hydrolytic bands with pI 8.4 or 8.8 (CTX-M type). The pI data were from transconjugants and wild type strains.
Conclusion: The SHV type was predominant among ESBLs Enterobacteriaceae producers in Bulgarian hospitals. TEM type was proved in one hospital. CTX-M types ESBLs have increased from 2001 after their first detection and become emerging problem in Bulgaria. All strains were highly polyresistant.

P692 Temporal trends of *Streptococcus pneumoniae* isolates with dual resistance to beta-lactam and macrolide antibiotics

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Objectives: *Streptococcus pneumoniae* (SPN) is a leading cause of pneumonia, meningitis, and otitis media. The evolution of antimicrobial resistance and concomitant clinical failures has become a global concern. Recent mathematical modelling predicted that the resistance to both penicillin and erythromycin antibiotics will increase significantly faster than resistance to either agent alone. We examined the longitudinal changes in dual resistance to these agents in clinical isolates collected by an ongoing Canadian surveillance programme.
Method: The Canadian Bacterial Surveillance Network (CBSN) is comprised of private and hospital-affiliated laboratories from across Canada. Laboratories were asked to collect a defined number of consecutive clinical isolates followed by all sterile site isolates of SPN. In vitro susceptibility testing was performed by broth microdilution using NCCLS guidelines.
Results: In our population, penicillin-nonsusceptibility and erythromycin resistance in SPN is presently 16.25 and 16.46%, respectively. The proportion of these isolates dually resistant to these antibiotics has slowly increased at a rate of approximately 1% per year since 1993. Analysis of the subpopulation of penicillin-nonsusceptible isolates revealed that erythromycin resistance has increased dramatically (rate approximately 5.5% per year). Conversely, among erythromycin-resistant isolates, the acquisition of penicillin nonsusceptibility occurred at a much slower rate (rate approximately 1.3% per year).
Conclusion: Ten years of surveillance of clinical isolates in Canada indicates that the increase in penicillin and erythromycin dual resistance in SPN is largely attributed to an increased propensity for penicillin-nonsusceptible isolates to acquire resistance to macrolide antibiotics. At the present rates of increase, all penicillin-nonsusceptible isolates will be erythromycin-resistant within several years, at which point additional increases in dually resistant isolates will be limited by the increase of SPN isolates resistant to penicillin alone.

P693 CdeA of Clostridium difficile, a new multidrug efflux transporter of the MATE family

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Introduction: *Clostridium difficile* is a Gram-positive spore-forming anaerobic bacterium that is the major cause of nosocomial diarrhoea. Its habitat is the human gastrointestinal tract, an environment rich in lipophilic inhibitors such as bile salts and fatty acids. Furthermore, this species is intrinsically less susceptible to antibiotics, notably to beta-lactams, fluoroquinolones, chloramphenicol, and lincosamides, than the other clostridia. It is likely that active efflux may be a crucial mechanism implicated in survival in the gastrointestinal tract and intrinsic antibiotic resistance of this species.
Methods: We tried to clone in *Escherichia coli* and *Clostridium perfringens* an efflux gene from total DNA of *C. difficile* strain 714 responsible for norfloxacin or ethidium bromide resistance. Ethidium bromide accumulation was measured by a whole-cell fluorometric assay in the presence or in the absence of sodium ions.
Results: We cloned a gene, cdeA, that made *E. coli* and *C. perfringens* resistant to ethidium bromide and acriflavin but had no effect on the susceptibility of the hosts to the antibiotics tested: norfloxacin, ciprofloxacin, gentamicin, erythromycin, tetracyclin, and chloramphenicol. It caused ethidium bromide energy-dependent efflux in whole cells of *E. coli*. The deduced protein was homologous to the protein sequences of known efflux pumps from the third cluster (the so-called DinF branch) of the multidrug and toxic compound extrusion (MATE) family. Efflux activity was stimulated by addition of Na+ ions suggesting that CdeA, like other pumps of the MATE family, is a Na+-coupled efflux pump.
Conclusion: CdeA is the first multidrug efflux transporter of the third cluster of the MATE family identified in gram-positive bacteria. It did not cause significant resistance to antibiotics when cloned in *E. coli* and *C. perfringens*. Gene inactivation would be helpful to appreciate its exact role in *C. difficile* but this experiment could not be performed due to incapacity of the transformation in that species.

P694 First appearance of the CfiA metallo-beta-lactamase gene in Norway and its association with a novel insertion element

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Objectives: A *Bacteroides fragilis* blood culture isolate (K2-28) was recovered from a 61-year-old man with severe general atherosclerosis, 7 days after amputation of both lower extremities and during treatment with meropenem. K2-28 was shown to possess high-level resistance (MIC > 128 mg/L) to carbapenems and most other beta-lactam antibiotics. This resistant pattern, indicative of a metallo-beta-lactamase (MBL), is unique in Norway where resistance to antibiotics is extremely low. Accordingly, we molecularly investigated the precise mechanism of this broad-spectrum beta-lactam resistance.
Methods: Production of a MBL was verified using the Etest MBL strips (AB biodisk, Solna, Sweden) plated onto Columbia blood agar with 5% blood. Genetically screening for the *B. fragilis* MBL gene was undertaken using primers based on the cfiA gene using standard PCR techniques. Primers were also designed against upstream insertion elements known to provide a strong promoter for the expression of the cfiA gene. Degenerate primers used were designed on the conserved regions of the genes and often more than one primer set was required. PCR amplicons of the desired size were sequenced. The entire sequence of the gene and the insertion element was obtained by the 'primer-walking' strategy.
Results: *E*-test results confirmed the presence of a functional MBL with a reduction in MIC from 256 to 3 mg/L in the presence of EDTA. Sequence analysis of the cfiA revealed it was 100% identical to previously described sequences, presenting with the principal zinc binding sequence of HWHGDC. Sequencing of the upstream region of cfiA revealed a novel insertion sequence (IS) element, being most similar (94% identity) to IS612 recently described from Japan. These data designates the element within the IS4 family. The element had a typical imperfect terminal inverted repeat sequences at the distal ends of the IS element. This IS614-like element demonstrates regions homologous to the −10 and −35 promoter regions of the nascent cfiA gene. However, the −10 was most similar to that of IS613 (100%) and the −35 to IS612 (100%) indicating the plasticity of these regions.
Conclusion: This is the first report of a *Bacteroides* spp. possessing an active cfiA gene within Scandinavia and the unique insertion sequences associated with this gene testifies to the plasticity of these genetic resistant elements.

P695 Molecular analysis of linezolid resistance in *Staphylococcus aureus*

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Objectives: Linezolid, the first oxazolidinone in clinical use, is effective in the treatment of severe staphylococcal infections. Resistance of *S. aureus* to linezolid recently has been shown to be associated with point mutations within the domain V region of the 23S rRNA gene, which is present in multiple copies in the genome of this pathogen. Here we studied whether there is a correlation between the level of linezolid resistance in *S. aureus* and the presence of mutations in the different copies of the 23S rRNA gene.
Methods: Linezolid-susceptible parental strain was subjected to daily serial subcultivation in Mueller–Hinton broth containing increasing linezolid concentrations for 40 days. The minimal inhibitory concentration (MIC) of linezolid was determined for the parental strain and for descendants by the agar dilution method. Domain V of the different 23S rRNA gene copies of the parental strain and of derivative isolates were amplified by PCR and sequenced.
Results: The linezolid MIC was 2 mg/L of the parental strain and increased gradually to 128 mg/L within 40 days of linezolid selection. Derivatives with elevated linezolid MICs revealed the substitution G2447T in the 23S rRNA gene. The level of linezolid resistance within derivatives correlated with the number of the 23S rRNA gene copies carrying this mutation.
Conclusions: The data suggest that the level of linezolid resistance in *S. aureus* is dependent on the number of the 23S rRNA gene copies carrying a specific resistance-associated point mutation. As linezolid-resistant *S. aureus* mutants can be selected *in vitro* under selection pressure, a cautious and judicious use of linezolid *in vivo* is strongly recommended in order to maintain the valuable status of this class of antibiotic agents.

P696 Characterisation of the first CTX-M14-producing *Salmonella enterica* serotype Enteritidis isolate

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Introduction: We describe the first infection caused by a CTX-M-14-producing *Salmonella enterica* serotype Enteritidis isolate. Although reports of ESBL associated with *Salmonella* are relatively rare, the number of reported cases in this organism has been increasing in recent years in numerous countries.
Materials and Methods: Clinical samples were processed according routine and identification was carried out with VITEK 2 system. The screening of ESBL was achieved by disk diffusion with discs of third cefalosporins with and without clavulanic acid according to manufaturer's instructions. Cefotaxime-resistant transconjugants were obtained. MICs were evaluated for the isolates and their transconjugants by microdilution in broth according to NCCLS and 4 mg/mL fixed concentration of beta-lactamase-inhibitor was added to third generation cephalosporins, aztreonam and piperacillin. PGFE was performed for *Salmonella* strains using XbaI endonuclease. Isoelectrofocusing was realised with Phast System. bla CTX-M gene was amplified from all cefotaxime-resistant isolates and their trasnconjugants and sequence was analysed.
Results: An susceptible *Salmonella* isolate (no. 1) was isolated from a blood culture from a 10-month-old girl admitted in Paediatric wards Then, two control faeces samples from the same patient yielded a cefotaxime-resistant *Salmonella* (isolate nos 2 and 3) and a cefotaxime-resistant *Escherichia coli* (isolate no. 4). The three resistant isolates and their transconjugants showed synergism with clavulanic acid compatible with ESBL phenotype and they also produced beta-lactamase with a pI of 8.0, which suggests a CTX-M9-type enzyme. It was possible to amplify the blaCTX-M fragment and the analysis of deduced amino acid bla sequences showed that the corresponding gene encoded the CTX-M-14 beta-lactamase. The three *Salmonella* isolates belonged to serogroup D, serotype Enteritidis, phage type PT1 and showed identical PFGE-patterns except for one extra band of 75 kb in the cefotaxime-resistant isolates 2 and 3.
Conclusions: Our findings provide the first evidence of CTX-M-14 associated with *Salmonella enterica* serotype Enteritidis. The molecular epidemiologic study showed that the child was infected with the same *Salmonella* strain, which developed ESBL phenotype during the episode. The finding of a intestinal *E. coli* strain with the same ESBL indicates a possible intestinal bla gene transmission between both species of Enterobacteriaceae.

P697 OmpK37 and reduced susceptibility to imipenem and meropenem in *Klebsiella pneumoniae*

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Objectives: The aim of the study was to investigate the reduced carbapenem susceptibilities in clinical isolates of *Klebsiella pneumoniae*.

Methods: *K. pneumoniae* strains were initially isolated from blood obtained from two patients at Groote Schuur Hospital, Cape Town. The isolates were susceptible to amoxicillin-clavulanate, imipenem (MIC, 0.125 mg/L), and meropenem (MIC, 0.032 mg/L) but resistant to amoxicillin, cefuroxime, cefotaxime, ceftazidime, cefepime, and cefoxitin. Extended spectrum beta-lactamase activity was detected using the double disc technique. Both patients were treated with meropenem. Subsequently, *K. pneumoniae* was isolated from faeces from the same two patients. These isolates displayed a similar susceptibility profile, except that they were resistant to co-amoxiclav, and had reduced susceptibilities to meropenem (MIC, 3–6 mg/L) and imipenem (MIC, 1–2 mg/L). The relatedness of the strains was investigated using pulsed field gel electrophoresis (PFGE). *E*-test strips were used to detect the presence of metallo-beta-lactamases (MBLs) with activity against carbapenems. To study the porin content of the strains, outer membrane proteins (OMPs) were extracted, separated by SDS-PAGE and, where necessary, identified by Maldi-TOF analysis.
Results: Each of the strains had an identical PFGE profile, showing that the strains were related. No MBLs were detected in the strains with reduced susceptibility to the carbapenems. A comparison of the porin profiles identified a 40 kDa protein in the strains with reduced carbapenem susceptibilities, which was not present in the susceptible isolates. Maldi-TOF analysis indicated that the protein was most similar to the *E. coli* OmpG. The most likely counterpart of OmpG in *K. pneumoniae* is OmpK37, the absence of which probably accounts for the reduced susceptibility to meropenem and imipenem. OmpK35 and OmpK36 were not observed in any of the preparations. This finding is consistent with the cefoxitin resistance phenotype of all four strains.
Conclusion: Meropenem susceptible and resistant *K. pneumoniae* were isolated from blood and faeces, respectively, from two patients. Reduced susceptibility to meropenem and imipenem was associated with the loss of OmpK37. Both patients were treated with meropenem, which may have influenced the selection of OmpK37-deficient strains.

P698 *In vitro* sequential development of gene mutations in fluoroquinolone-resistant salmonellae

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Objectives: (1) To investigate the development of fluoroquinolone-resistant salmonellae *in vitro*; (2) to characterise the sequential accumulation of target gene mutations leading to high-level fluoroquinolone-resistance.
Methods: Fluoroquinolone-resistant mutants of *Salmonella enterica* serotype Typhimurium and *S. Hadar* were obtained by plating sensitive strains on agar containing increasing twofold concentrations of ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, norfloxacin or ofloxacin. Mutants thus obtained were again subjected to selection to obtain nth-step mutants (where n stands for the number of times the mutants had been subjected to selection). Susceptibilities of the mutants were determined and mutations of target genes were detected by multiplex PCR amplimer conformation analysis and confirmed by direct DNA sequencing.
Results: First-step mutants of *S. Typhimurium* usually harboured a gyrA mutation (Asp87→Asn/Gly/His/Tyr), the MICs were 4–16 times higher than those for the parent strains and were below the sensitive breakpoint of fluoroquinolones. One mutant harboured a gyrB (Glu466→Asp) mutation and the MICs were 2–8 times lower than those for the parent strain. Only three second-step mutants harboured an additional gyrA mutation (Ser83→Phe) while two 2nd-step mutants from the parent with a gyrB mutation harboured a gyrA mutation (Asp87→Gly or Ser83→Phe). Mutations in parC (Glu51→Asp, Gly78→Asp, Ser80→Arg/Ile or Glu84→Gly/Lys) were first observed in third-step mutants. Although most fourth-step mutants were resistant to high concentrations of fluoroquinolones as were third-step mutants, no additional mutation was found.Fewer mutations were detected in mutants of *S. Hadar:* First-step mutants harboured a gyrA mutation (Asp87→Asn/Gly/Tyr or Ser83→Phe) and a gyrB (Glu466→Asp) mutation. No additional mutation was found in second-step mutants although MICs were higher than those for the parents.
Conclusions: Low-level fluoroquinolone-resistant salmonellae which evolved by development of a gyrA mutation became high-level resistant by developing additional gyrA and parC mutations. Such sequential development of fluoroquinolone-resistance varied among *Salmonella* serotypes.

P699 Biochemical characterisation of IMP-16, a novel IMP variant harboured in a class I integron of a *Pseudomonas aeruginosa* clinical isolate from Brazil

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Objectives: Several variants of IMP-type enzymes have been identified and they are from 1 to 17% amino acid residues divergent from IMP-1. In some cases, they also present with different kinetic properties. Here we report the biochemical characterisation of a new IMP variant, IMP-16, that was found in *Pseudomonas aeruginosa* index strain (101-4704) clinical isolate from Brasilia (Brazil).
Methods: Primers targeting the 5′CS and 3′CS regions of class 1 integron were used to amplify the blaIMP-16 containing integron. These primers yielded PCR products, which were sequenced on both strands using DuPont Automated systems. After integron sequence analysis, the blaIMP-16 gene was subcloned into expression vector pPCRScriptCam SK+ and overexpressed in *Escherichia coli* DH5alfa. The IMP-16 protein was purified by Fast Performance Liquid Chromatography. Kinetic properties were determined with several beta-lactam substrates measuring hydrolytic activity under initial rate conditions.
Results: The N-terminus of the IMP-16 showed typical features of bacterial signal peptides that target proteins to the periplasmic space and the most likely cleavage site is located between the alanine at positions 18 and glycine at position 19. This produces a mature protein of 25 266 Da with a theoretical pI of 6.5. IMP-16 is a new IMP variant that differs from IMP-1 by 15% amino acid residues, being one of the most divergent variants so far identified. It is more similar to IMP-8 and IMP-11 (89.8 and 90.3%, respectively) at the level of mature protein. *E. coli* DH5alfa harbouring the blaIMP-16 recombinant plasmid showed a similar beta-lactam resistance profile when compared with the index strain (101-4704), being resistant to nearly all beta-lactam tested, apart from aztreonam and carbapenems. The IMP-16 enzyme was overproduced in *E. coli* and purified (>95%). Preliminary kinetic analysis revealed K_m values of 38, 98, 0.05 and 10 μM, and K_{cat} values of 0.05, 0.24, 2.0 and 1.2 /s for meropenem, imipenem, penicillin and nitrocefin, respectively.
Conclusions: IMP-16 is a new highly divergent IMP variant detected in *P. aeruginosa*. Kinetic parameters showed the great structural and functional plasticity among this group of clinical important enzymes.

Molecular bacteriology: detection and identification of agents

P700 Pyrosequencing at the Health Protection Agency, UK

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One of the remits in the Genomics, Proteomics and Bioinformatics Unit (GPBU) of the UK Health Protection Agency is to utilise genomics in infection control by the use of accelerated genomic information and enhanced biotechnological methods to streamline the traditional approaches used in classical molecular genetics to instigate advances in Public Health Protection.Alteration in gene expression patterns or DNA sequence can have profound effects on biological functions and pathological processes. In GPBU, systems are developed for identifying genetic variations and gene expression and our key developmental objectives are to reformat sequence-based typing into fast, high throughput systems, to explore microbial genome sequences for VNTRs and SNP targets as tools for high resolution strain comparison and to provide tools for molecular-based surveillance of high priority infections.To this end, Pyrosequencing technology has been evaluated for its utility in SNP characterisation (including antibiotic/drug resistance) and genotyping in Public Health Microbiology in the UK. An overview of applications will be presented including:
HIV-1
– drug resistance
– subtyping
Hepatitis C virus
– genotyping
– minor genotype analysis
Antibiotic resistance
– *Neisseria gonorrhoeae*
– *Salmonella enterica*
– *Mycobacterium tuberculosis*
Mycobacterium tuberculosis
– screening from sputum samples
– VNTR typing
H antigen typing of *Salmonella*

P701 Universal 16S rDNA PCR and sequencing in the diagnosis of infective endocarditis directly from heart valve tissue

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Background: The microbiological diagnosis of infective endocarditis (IE) is based on positive culture of heart valve tissue or blood culture but cultures remain negative when IE is caused by fastidious micro-organisms or antimicrobial treatment is started before cultures are obtained.
Objective: To evaluate the usefulness of a universal 16S rDNA PCR method followed by direct sequencing in heart valve tissue for IE diagnosis in the routine of a clinical microbiology laboratory, compared with traditional heart valve culture (HVC) and blood culture (BC).
Methods: Heart valves received for culture over a ten-month period were studied by 16S rDNA PCR with primers PSL and P13P. Positive samples were subsequently sequenced for identification. HV were cultured by conventional methods. BCs were performed by the BACTEC 9240 system. Sensitivity of the assay was assessed by obtaining DNA from 10-fold dilutions of *Streptococcus oralis*. After molecular analysis, clinical records of patients and results of conventional cultures were consulted.
Results: Twenty-four samples of HV (24 patients) were studied. In 10 patients IE was clinically rejected and their valves were included in the study as negative controls. Their HVC, BCs and PCR were negative. The remaining 14 cases had either proven (13) or possible (1) IE. Overall, BCs were positive in 11 patients but HVC remained positive at the moment of resection in only four patients. Of the 14 cases, 12 were microbiologically documented by conventional cultures. PCR was positive in the 12 confirmed cases. Micro-organisms identified by PCR matched those cultured by conventional cultures except in one case in which the valve was inadequately remitted to the microbiology laboratory. In the 2 cases with IE and with no micro-organisms demonstrated by conventional cultures, PCR was also negative. The median time of analysis to a PCR result was 1 day and to a sequence and bacterial identification in PCR-positive samples, 3 days. The analytical sensitivity of this assay was 100 CFU/mL.
Conclusions: Universal 16S rDNA PCR followed by sequencing applied to resected heart valves seems to be a reliable test for diagnosis of IE. Our study suggests its applicability to patients with conventional negative microbiology, mainly those studied during the course of antimicrobial therapy.

P702 Clinical importance of PCR diagnosis in bacterial meningitis and sepsis

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Objectives: The importance of the detection of bacterial DNA by PCR in laboratory diagnosis of invasive bacterial infections has been increasing. PCR represents the rapid, sensitive and specific method, which provides the possibility to detect bacterial DNA in cases of application of few doses of ATB therapy. In such cases the classical microbiology methods often fail.
Methods and Materials: After DNA extraction PCR for determination DNA of *N. meningitidis*, *Haemphilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes* was used. In 2001–2003, 92 patients (CSF, blood and urine) with suspected invasive bacterial infection (sepsis or bacterial meningitis) were examined for the presence of mentioned bacterial DNA in case of negative microscopy and agglutination examinations.
Results: Invasive meningococcal disease (IMD) was diagnosed by clinical and laboratory methods in 20 of 60 examined patients. In 10 patients *N. meningitidis* was detected by classical microbiology methods, in 10 patients *N. menigitidis* DNA was found only by PCR (7x in CSF; 3x both in CSF and blood; 1 only in urine). In 50% of patients with the diagnosis of IMD the presence of *N.meningitidis* was not detected by classical microbiology methods, only by PCR. Of the rest of 72 patients bacterial DNA was detected in 28 patients (4x *Haemophillus influenzae*, 15x *Streptococcus pneumoniae*, 6x *Listeria monocytogenes* and 3x *Staphylococcus aureus*).
Conclusion: The decline of positive findings of bacterial DNA by classical methods (microscopy, agglutination, cultivation) may be caused by the administration of the first doses of ATB in the onset of non-specific symptoms of disease. The detection by very sensitive PCR enables to detect DNA not only from live but also disintegrated bacteria. The significance of PCR diagnosis in these cases has increased very much because of its speed, sensitivity and specificity.

P703 A new scoring method for molecular diagnostic kit users

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Objectives: A proposed score provides informative, intuitively appealing feedback to molecular diagnostic kit users. The colour-coded score [green (= highly satisfactory) to red (highly

unsatisfactory)] is presented for individual samples and overall performance. Simple graphical presentations compare performances within and between amplification methods.
Methods: The score uses the log copies/mL reported from quantitative assays. Analysis of Variance using the amplification method for each panel sample is used to: check for normality, identify outliers and estimate the standard deviation. The difference between the participant's result and target value is divided by the standard deviation. A participant's score for an individual sample is defined as the integer part of the absolute value of this value (max 3). Scores of 0, 1, 2 and 3 are colour-coded green, yellow, amber and red, respectively, for participant feedback. Assuming independence, normality and no amplification method effect, the distribution of scores are approximately 68, 27, 4.5 and 0.5%.The overall performance for an individual participant is the sum of the individual samples scores. Monte Carlo methods determine the probability distribution of the overall performance score. These scores are colour coded from green to red in proportions approximately equal to individual samples (e.g. 68% green). Feedback for a participant's overall performance is the same as for individual samples.
Results: Scores have been found for the 2002 QCMD panels for hepatitis B, C and HIV. For each sample log results were normally distributed within each amplification method with few outliers detected.Scores frequencies varied amongst individual samples within each panel. For many samples, the proportion of scores were not consistent with that predicted by independent observations from a normal distribution. This was confirmed by ANOVA that showed significant differences in the mean log observations amongst amplification methods. Graphical representations showed pronounced differences amongst the methods within and across panels.
Conclusions: The new scoring scheme is based on well-known statistical properties and techniques. The colour coded performance scores are readily interpretable and simple graphical output also allows participants to gauge their performance against other users of the same method and to help choose amongst methods.

P704 Application of TaqMan probes in end-point fluorimetry for detection of pathogenic bacteria by polymerase chain reaction

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Objectives: 5′-Nuclease polymerase chain reaction in a conventional thermal cycler and a subsequent fluorescence measurement in a 96 well fluorimeter were developed and successfully carried out in flat-bottom microtubes.
Methods: Complete reaction system was simply transferred from real-time PCR to new instrumental conditions. Specific primers and specific TaqMan probes (fluorescent dye 6-FAM, quenching dye TAMRA) for *Salmonella* sp. and *E. coli* strains were used. When finishing the PCR, the fluorescence was measured in endpoint mode in fluorescence reader equipped with an excitation filter with a pass maximum of 492 nm and an emission filter with a pass maximum of 520 nm from the bottom orientation. To define the positivity threshold, three negative control samples (containing no DNA template) were used. Mean value and the standard deviation (SD) were calculated and the positivity threshold was set to (mean + 2 SD).
Results: In these conditions, consistent results were obtained when PCR was done with purified *Salmonella enteritidis* and *E. coli* DNA or with culture lysates. When lysates of series of decimally diluted cultures were analysed a detection limit of 10exp4 CFU/mL was determined, the same sensitivity as with real-time PCR and the same or one order of magnitude higher than with the gel electrophoresis were determined.
Conclusion: The method proved to be a fast and contamination-free alternative to gel electrophoresis and an inexpensive alternative to real-time PCR.

P705 Usefulness of the MicroSeq 500 16S rDNA-based method for identification of bacterial isolates unidentified by commercial automated systems

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Objective: Reliable automated identification and susceptibility testing of clinically relevant bacteria is very important for routine microbiology laboratories, thus improving patients' care. Examples of automated identification systems are: the Phoenix (Becton&Dickinson) and the Vitek2(bioMérieux). Both systems claim to provide accurate and rapid identification as well as susceptibility results with substantially easy workflow. However, more and more frequently microbiologists isolate 'difficult' strains, particularly those isolates from patients who have undergone repetitive antibiotic treatment and which consequently exhibit biochemical characteristics that do not fit into patterns of any known genus and species. The identification of these pathogens is normally failed by automated systems. An alternative could be the genetic identification; the latter based on the 16S rDNA sequencing and analysis, as 16S is highly conserved within a species and among species of the same genus. Aim of the present work is to evaluate the possible use of the MicroSeq (Applera), sequencing 16S rDNA, as the new gold standard for identification of isolates whose identification is not obtained or results inadequate with conventional systems.
Methods: In the present work we have analysed 83 difficult isolates: 25 Gram+ and 58 Gram− strains. The isolates were contemporaneously identified by using both automated systems such as: Vitek2 and Phoenix. The phenotypic identifications were confirmed by genetic analysis performed by using MicroSeq system.
Results: The results have shown that the phenotypic identification provided by Vitek2 and Phoenix was remarkably similar, in particular: 74% of the identification obtained for the Gram−, and 80% for the Gram+ resulted to be in concordance with both systems, and was also concordant with genetic characterisation. The exception was represented by 15 Gram− and nine Gram+ isolates whose phenotypic identifications were contrasting or not conclusive. For these strains the use of MicroSeq demonstrated to be fundamental to achieve the species identification.
Conclusion: The use in clinical microbiology of MicroSeq, particularly for those strains with ambiguous biochemical profiles (including slow-growing strains), results to be helpful allowing to better achieve identifications as compared with conventional systems. Moreover, the system appears easy to use and cost effective, making MicroSeq reasonably applicable also in clinical laboratory.

P706 Facts, feasibility and future of bacterial load of bloodstream infections

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Real-time PCR is becoming more and more a standard technique in many laboratories for the detection of micro-organisms in clinical samples. This closed PCR system enables rapid clinical molecular diagnostics in the medical microbiological laboratory. In blood samples, pathogen-specific PCR is of additional value to conventional blood culture in case of prior use of antibiotics or when slow-growing organisms like mycobacteria are involved. Although there are many reports on pathogen-specific applications, a more general approach with broad-range detection based on the 16S gene has only been described a few times, with interesting results. None of these broad-range studies has been described so far with real-time PCR that enables quantification of initial DNA with deduction of a 'bacterial load' that can possibly be used for monitoring antimicrobial therapy.In this study real-time PCR (TaqMan 7000) was combined with an automated DNA isolation robot (MagNA Pure LC) in order to standardise en optimise DNA isolation and subsequent ampification. Ninety

blood samples from patients with fever were evaluated. PCR showed amplification of 10/13 samples with positive concurrent blood cultures; the three that remained negative in PCR all grew coagulase-negative staphylococci. Other PCR results were in concordance with blood culture outcome and/or clinical data. However, sequence-analysis showed cross-contamination with Burkholderia species DNA that could be traced to buffers of the DNA isolation kit, although a second (mixed) sequence could be recognised. The concordance of clinical data and PCR results may be explained by the presence of blood components related to infection that function as PCR-enhancer, like serum-proteins.In conclusion, real-time PCR seems a promising addition to the spectrum of diagnostic possibilities for bloodstream infections, especially in case of pathogen-specific detection of bacteraemia. To develop a real-time broad-range PCR for detection of bloodstream infections with determination of a 'bacterial load', attention should be given to optimisation of DNA isolation and DNA-free nucleic acid isolation kits and PCR reagents.

P707 Broad-range polymerase chain reaction based bacterial and fungal molecular assays improve the diagnostic ability of the Duke scheme in suspected cases of infective endocarditis

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Objectives: The diagnosis of infective endocarditis (IE) may become sometimes a difficult one, particularly when blood cultures are negative or in cases in which the course of the disease is insidious. The Duke scheme has improved the diagnostic ability of clinicians dealing with suspected cases of IE. However, reaching a precise microbiological diagnosis may be a challenge in clinical situations such as those mentioned above. Molecular approaches have helped on this task and a few reports have been published regarding the clinical validation of these methods and techniques in IE settings. We hypothesised that the molecular microbiological diagnosis of IE may improve the diagnostic ability of the Duke scheme.
Methods: To test this hypothesis, we defined a group of IE patients in which microbiology results from traditional methods (blood and valve cultures) as well as from molecular testing (broad range PCR-based 16S rDNA bacterial detection and 28S-5.6S-IS2 rDNA fungal detection assays from valve samples) were available. Then we compared the diagnostic performance (in terms of Duke classification) of the Duke criteria alone and after the inclusion of microbiological molecular data. Forty-nine suspected IE patients were included in the study.
Results: The Duke scheme alone classified the patients as follows; 25 (51%) as 'definite IE', 22 (45%) as 'possible IE', and two (4%) as 'rejected IE'. When we applied the molecular microbiological data as an additional major criterion, 19 'possible IE' cases were reclassified as 'definite IE' and the two 'rejected IE' cases were reclassified as 'possible IE'. Overall, 43 patients (88%) were considered as 'definite IE' and six as 'possible IE', while no cases remained classified as 'rejected IE'.
Conclusions: Broad-range bacterial and fungal PCR-based assays, when considered as part of the Duke scheme improve its diagnostic ability and might be a useful tool in the microbiological diagnosis of infective endocarditis. This study was supported by the grant of MZ CR No. CEZ MZ 00000209775.

P708 Direct detection of the most important bacterial pathogens from blood cultures using nucleic acid based techniques

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Introduction: Bacterial bloodstream infections are cause of high mortality rates in patients. Therefore rapid and accurate detection of the bacterial pathogens is of high importance for successful patient therapy. We developed a nucleic acid based hybridisation assay using a set of oligonucleotide probes targeting different DNA-regions for the differentiation of 15 Gram-negative species and 18 different Gram-positives including the van and mecA antibiotic resistance-associated genes. A challenge set of 220 bacterial isolates belonging to the genera staphylococci, streptococci, enterococci, members of the family of the Enterobacteriaceae and nonfermenting Gram-negative rods were selected to evaluate the specificity of the selected primers and the oligonucleotide probes. The strains were chosen from routine samples and reference strains provided by different collections (ATCC, USA; DSMZ, Germany; RKI, Germany). Species confirmation of the routine samples was done using the apistrep and the VITEK2 -system (bioMérieux, France). Discrepancies were resolved by direct sequencing a 16S rDNA fragment. Van- and mecA genotype confirmation was performed by an in house PCR-assay. A total of 75 bacterial DNAs were directly extracted from positive BACTEC 9260 (BD Diagnostic Systems, USA) blood culture bottles, randomly collected at our laboratory.
Methods: DNA-Isolation from bacterial cultures: A loop of bacterial cells or up to five colonies from an agar plate were suspended in 150 μL distilled water and 15 min heated at 95°C and sonicated 15 min. DNA isolation from blood cultures: 15 μL of the positive blood culture was spotted on paper. After drying of 10 min at 95°C, 100 μL distilled water was added. DNA was released by a 15 min heating step at 95°C and 10 min sonification.
DNA-Amplification: PCR-amplification was done using a Perking-Elmer 9600 thermocycler (Applera, Germany) with one cycle 5 min at 95°C, 30 cycles 30 s at 95°C, 30 s at 45°C, 30 s at 72°C and a final elongation step with 7 min at 72°C.
Hybridisation: Hybridisation and detection were performed on an automated system (Tecan, Germany).

Target Probe	IS FAM	CTR JOE	CPN ROX	CPS+ Cy5	Detection limit, copies/reaction
IS	+	–	–	–	600
C. trachomatis	–	+	–	–	1–2
C. pneumoniae	–	–	+	–	2–4
C. psittaci	–	–	–	+	2–4
C. abortus	–	–	–	+	2–4
C. felis	–	–	–	+	2–4

Results/conclusion: With regard to the 220 bacterial isolates and the 75 isolates from blood cultures tested the hybridisation assay so far showed excellent results. Differentiation results were obtained in about 4 h from the time the blood cultures gave a positive signal, including DNA extraction, PCR and the hybridisation procedure.

P709 Development of a multiplex real-time PCR for detection and differentiation of Chlamydiaceae species which are pathogenic for humans

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Objectives: Real-time PCR (RT-PCR) offers many advantages over conventional PCR methods for detection of microbial pathogens. The aim of our study was to develop and evaluate the performance of a multiplex 5'-nuclease-based RT-PCR assay for direct detection and differentiation of *Chlamydia trachomatis* (CTR), *Chlamydophila pneumoniae* (CPN) and zoonotic agents [*Chlamydophila psittaci* (CPS), *Chlamydophila abortus* (CAB) and *Chlamydophila felis* (CFE)] in clinical specimens.

Methods: The 5′-end sequence of the omp1 gene which is well characterised in all chlamydial species was selected as a PCR target. It was amplified using the family-specific primers CM1 and CM2 (H. Yoshida *et al.*, 1998) on a Rotor-Gene 2000 system (Corbett research). Three probes containing different fluorescent dyes, JOE, ROX and Cy5 (Biosearch Technologies) were designed to target the signature sequences in the amplified omp1 region, which are highly conserved within CTR, CPN and zoonotic agents and are distinctive between them. The fourth FAM-labelled probe was used for the detection of a heterogeneous internal standard (IS). The analytical sensitivity of the assay was determined by testing peripheral blood leucocyte specimens spiked with chlamydial elementary bodies or recombinant plasmids containing omp1 fragments of the following strains: CTR L2, CPN Kajaani 7, CPS 6BC, CAB B577 and CFE FePn. In addition, a panel of 219 genital swab specimens was used to assess the sensitivity and specificity of CTR detection using RT-PCR in comparison with a commercial PCR assay targeting the cryptic plasmid of this species.
Results: As shown in the table, the multiplex RT-PCR was able to detect specifically and reprodce single DNA copies of each chlamydial species in the presence of IS and excess of human DNA. When compared with monoplex PCRs, multiplexing of four probes did not decrease sensitivity, while no cross-detection between CTR, CPN and zoonotic species was observed. In testing clinical specimens, RT-PCR detected CTR DNA in 44 of the 46 samples that were positive and in one sample that was negative by commercial PCR. The lack of amplification of IS indicated the presence of inhibitors in two samples. Consequently, with commercial test used as a reference, the sensitivity and specificity of RT-PCR were 95.7 and 99.4%, respectively.
Conclusion: The developed method enables rapid, sensitive and specific detection of all members of Chlamydiaceae which are pathogenic for humans.

P710 Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Tripath SurePath™ Pap samples using the Gen-Probe Incorporated APTIMA® Combo 2 Assay

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Objectives: To determine the suitability of TriPath SurePath™ (TriPath Imaging) medium for detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) using the Gen-Probe APTIMA® Combo 2 (AC2) Assay.
Background: Because the cervix is a site of infection for CT and GC, Pap specimens may also be appropriate for detection of these sexually transmitted bacteria. By testing Pap specimens for CT and GC with AC2, multiple test results (CT, GC, Pap) can be obtained from one specimen. SurePath medium was evaluated for the detection of CT and GC with the AC2 assay using a protocol in which an aliquot is transferred to a tube containing Gen-Probe transport medium. The AC2 assay was then performed on the SurePath/transport medium sample using the standard AC2 protocol for endocervical swab and urine specimens.
Methods: For analytical sensitivity, 14 CT serovars and 20 GC clinical strains were diluted in the SurePath medium to 0.01 CT IFU and 0.5 GC CFU per AC2 reaction. Specificity was evaluated with three *Chlamydia* and 51 *Neisseria* nontarget species. Stabilities of samples in SurePath vials and in the SurePath/transport medium mixture were monitored at 4–35°C. Cross-contamination due to Pap sample processing on the TriPath PREPmate™ instrument was determined by processing alternating negative and high titre GC samples and then running them in the AC2 assay. Potentially interfering substances evaluated included whole blood and commonly used feminine hygiene products at usage levels higher than those expected in normal usage.
Results: All CT serovars were detected at 0.01 IFU/AC2 reaction and all GC strains were detected at 0.5 CFU/AC2 reaction. Specificity was 100%, with no cross-reactions observed with non-CT and non-GC species evaluated. Stability study results indicated that CT and GC can be detected from SurePath vials stored for 7 days at 35°C and for 30 days when stored at 4–10°C. CT and GC were detected in SurePath/transport medium mixtures stored at 4–35°C for 30 days. Following processing of high titre positives, false-positive results in known negative samples were <1%. None of the potentially interfering substances affected assay results.
Conclusion: These results indicate that the SurePath Pap medium is compatible with the APTIMA Combo 2 Assay for CT and GC detection.

P711 Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Cytyc Preservcyt® Pap samples using the Gen-Probe Incorporated APTIMA® Combo 2 Assay

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Objectives: To determine the suitability of Cytyc Preservcyt (R) (Cytyc Corp.) medium for detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) using the Gen-Probe APTIMA (R) Combo 2 (AC2) Assay.
Methods: Because the cervix is a site of infection for CT and GC, Pap specimens may also be appropriate for detection of these sexually transmitted bacteria. By testing Pap specimens for CT and GC with AC2, multiple test results (CT, GC, Pap) can be obtained from one specimen. Preservcyt medium was evaluated for the detection of CT and GC with the AC2 assay using a protocol in which an aliquot is transferred to a tube containing Gen-Probe transport medium. The AC2 assay was then performed on the Preservcyt/transport medium sample using the standard AC2 protocol used for endocervical swab and urine specimens. For analytical sensitivity, 14 CT serovars and 20 GC clinical strains were diluted in the Preservcyt medium to 0.01 CT IFU and 0.5 GC CFU per AC2 reaction. Specificity was evaluated with three *Chlamydia* and 51 *Neisseria* nontarget species. Stabilities of samples in the Preservcyt vial and in the Preservcyt/transport medium mixture were monitored at 4–35°C. Cross-contamination due to Pap sample processing on the Cytyc ThinPrep2000 (TP2K) instrument was determined by processing alternating negative and high titre GC samples and then running them in the AC2 assay. Potentially interfering substances evaluated included whole blood and commonly used feminine hygiene products at usage levels higher than those expected in normal usage.
Results: All CT serovars were detected at 0.01 IFU/AC2 reaction and all GC strains were detected at 0.5 CFU/AC2 reaction. Specificity was 100%, with no cross-reactions observed with non-CT and non-GC species evaluated. Stability study test results indicated that CT and GC can be detected from Preservcyt and Preservcyt/transport medium mixtures stored at 4–35°C for 30 days. Following TP2K processing of high titre positives, false-positive results in known negative samples were 1%. None of the potentially interfering substances affected assay results.
Conclusion: These results indicate that the PreservCyt Pap medium is compatible with the APTIMA Combo 2 Assay for CT and GC detection.

P712 *Chlamydophila pneumoniae*-specific mRNA is present in aortic wall biopsies of patients suffering from stable or unstable angina pectoris

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Objectives: *Chlamydophila pneumoniae* (Cp) might somehow be involved in the pathogenesis of atherosclerosis. Several studies have demonstrated a serological association between Cp and coronary artery disease and DNA from the bacteria has been found in various atheromatous vessels. However, only a few investigators have managed to culture Cp from atheromatous plaques. Instead of culturing, viable bacteria could be demonstrated by reverse transcriptase PCR (RT-PCR) against bacterial mRNA. We investigated

the presence of Cp DNA and mRNA in aortic wall biopsies, obtained at surgery, from 24 patients with stable angina pectoris (SAP) and 20 patients with unstable angina pectoris (UAP).
Methods: DNA and RNA were extracted from the same biopsy using the RNA/DNA mini kit (Qiagen). cDNA was made using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Real time PCR directed against the MOMP gene was used to detect the presence of Cp DNA and mRNA in the biopsies. Patient sera were tested for Cp-specific IgM, IgG and IgA antibodies by the microimmunofluorescence technique.
Results: Thirteen (30%) of the biopsies (six SAP; seven UAP) were positive for Cp DNA, eight (18%) were positive for Cp mRNA (five SAP; three UAP) and six (14%) were positive for both DNA and mRNA (4 SAP; 2 UAP). All biopsies but one were positive for human cDNA encoding the GAPDH gene. That biopsy was negative also for Cp DNA and Cp mRNA. Results from the serology will be presented on the poster.
Conclusion: We have demonstrated the presence of Cp in the aortic wall of patients suffering from stable (SAP) or unstable (UAP) angina pectoris. Also, we have demonstrated Cp mRNA in 17% of these patients indicating that the bacteria were metabolically active. There were no significant differences in the frequencies of positivity for Cp DNA or Cp mRNA between the SAP and UAP groups. The results support the hypothesis of an active role for Cp in the pathogenesis of atherosclerosis.

P713 Rapid identification of HACEK group of bacteria by 16S rRNA gene PCR and restriction fragment length polymorphism analysis

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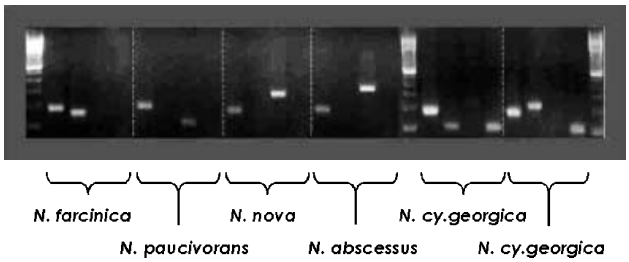
Objectives: Although infective endocarditis due to HACEK group of bacteria (*Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* spp.) is a rare occurrence, the identification of the organisms is still important diagnostically for the specific therapy. The HACEK bacteria are classified as fastidious Gram-negative coccobacilli, and the biochemical characteristics resemble each other. Thus, the identification of HACEK bacteria has been rather hard and sometimes inconclusive. In this study, we developed a rapid and highly sensitive identification method for HACEK bacteria by means of 16S rRNA gene PCR amplification followed by restriction fragment length polymorphism analysis (PCR-RFLP).
Methods: *H. aphrophilus* ATCC 33894, *A. actinomycetemcomitans* ATCC 33384, *C. hominis* ATCC 12826, *E. corrodens* ATCC 23834 and *K. kingae* ATCC 23330 were used. DNA samples were prepared by a DNA purification kit. After PCR amplification using the primers corresponding to *Escherichia coli* 16S rRNA gene, the PCR products were digested with 4 U of either f *Hin*fI and f *Msp*I at 37°C for 1.5 h. The samples were then separated on 1.8% agarose gel, and the restriction patterns were recorded.
Results: The RFLP patterns of five species of HACEK bacteria obtained by combing use of HinfI and MspI digestion were readily distinguished from each other and from other pathogens of infective endocarditis including viridans streptococci. Furthermore, the PCR-RFLP analysis yielded a definitive identification of *C. hominis* from one of the blood samples of the patients with infective endocarditis in which causative pathogens could not be unidentified by biochemical identification kits. The result was confirmed by a 16S rRNA gene sequence analysis of the isolate.
Conclusion: The PCR-RFLP analysis developed in this study was a rapid and highly sensitive identification method for HACEK group of bacteria, and could be applicable for a definitive diagnostic detection of HACEK group of bacteria.

16S-23S rDNA multiplex-PCR *Nocardia*



P714 Same day molecular diagnostic test result for atypical and difficult to grow respiratory pathogens including *M. tuberculosis* complex by combining automated DNA extraction with real-time PCR

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Background: Real-time PCR is a powerful method for detecting bacteria in clinical samples, but DNA extraction is still a crucial and cumbersome step when performing such tests.
Objectives: (i) Establish a 1-day TAT result for the molecular diagnosis of tuberculosis using a combination of an automated DNA extraction method with real-time PCR (RT) (ii) test its performance for other bacterial respiratory pathogens (*M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *B. pertussis* and *B. parapertussis* (iii) assess its performance on clinical samples for *M. tuberculosis*.
Methods: (i) The automated DNA extraction procedure, MagnaPureR, Roche (MP) was compared with a conventional homemade extraction procedure (Boom *et al*.) using silica particles (SI) for 10 stored specimens positive by culture for *M. tuberculosis*. The extracted DNA were amplified and the amplicons detected by real-time PCR (RT) using the ABI 7700, Applied Biosystems; (ii) MP and SI were compared for diluted positive-specimens for the other bacteria; (iii) to assess the performance for the diagnosis of tuberculosis on clinical samples, two different periods of 14 months were compared in terms of sensitivity, specificity, PPV and NPV using culture results as gold standard. A: (March 2001–May 2002) 710 specimens (513 patients) with 76 positive cultures for *M. tuberculosis* (10.7%) with SI-RT; B: (May 2002–July 2003) 817 specimens (619 patients) with 88 positive cultures for *M. tuberculosis* (10.7%) with MP-RT.
Results: (i) Eight of 10 specimens were positive for *M. tuberculosis* with SI-RT and nine of 10 with MP-RT (the three discordant results were weak-positive specimens upon culture); (ii) 100% concordance between the two procedures for the five different bacterial respiratory specimens, even better for the MP-RT when considering the cycle threshold (Ct) results; (iii) the A vs. B (A/B) results were very similar (%): global sensitivity: 90/89; sensitivity for the Ziehl-Neelsen (ZN)-positive samples: 100/100; sensitivity for the ZN-negative samples: 77/ 80; specificity: 99/ 99; PPV: 95/ 95 and NPV: 99/ 99.
Conclusions: One-day TAT molecular diagnostic test results are now possible for several atypical or difficult to grow respiratory bacterial pathogens including *M. tuberculosis* complex by combining automated DNA extraction with real-time PCR.

P715 Reliable and rapid detection of clinically relevant *Mycobacterium* and *Nocardia* species using a multiplex-PCR with genus- and species-specific primers

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We report a rapid and reliable multiplex PCR assay to identify 13 clinically relevant *Mycobacterium* species (including *M. bovis*) and seven of the most frequently occurring *Nocardia* species. The method includes two genus-specific and six species-specific primer mixtures for identification to the species level. Additional genus-primers for closely related actinomycetes such as *Streptomyces* and *Tsukamurella* were included also. The primers were designed from nucleotide sequences of the 16S rDNA, the 16S–23S rDNA intergenic spacer region and part of the 23S rDNA. The established multiplex PCR identification scheme was applied to the identification of 131 reference strains and clinical isolates that were previously identified to the species level by 16S rDNA sequencing or 16S–23S rDNA spacer RFLP. The new scheme was very reliable and specific concerning the intra-species stability of the primers: all strains were identified correctly, although not all strains within a few genetically heterogenous taxa such as *M. kansasii* or *N. abscessus* were detected by this PCR method. This will make the inclusion of further primers necessary for some groups. Furthermore, we tested this multiplex PCR with 45 specimens from the respiratory tract from patients with suspected mycobacteriosis or nocardiosis (42 specimens were positve for acid fast rods, three contained branched Gram-positive rods). Since only microscopically positive specimens were tested, the negative predictive value and the sensitivity were very high. This multiplex-PCR represents a cost-effective, rapid and easy to perform method for the identification of mycobacteria and nocardia from cultures and for the direct detection of these rare but important pathogens in microscopically positive clinical specimens.

P716 Identification of *Mycobacterium gordonae* clinical isolates with the use of four molecular methods

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Objective: The aim of our study was the evaluation of the PCR-RFLP analysis of hsp65 and rpoB genes, a PCR-based assay and the Accuprobe tests (Gene-Probe Inc.) for the identification of clinical *M. gordonae* isolates.
Methods: Thirty seven, of the 42 *M. gordonae* isolates studied, were recovered from clinical specimens of different patients and five strains obtained from the collection of the Greek National *Mycobacterium* Reference Unit. A segment of the RNA polymerase gene (rpoB) was amplified by PCR and the products (342 bp) were digested with the restriction enzymes *Hin*dII, *Hae*III, *Mva*I and *Acc*II. A segment of the heat shock protein gene (hsp65) was amplified by PCR and the products (440 bp) were digested with the restriction enzymes *Hae*III and *Bst*EII. The digested products were electrophoresed and the results were analysed using the PCR-RFLP algorithms. PCR-assays were performed using a pair of *M. gordonae*-specific primers that amplified a fragment of 152 bp of the internal transcribed spacer region. The results of identification were compared with those obtained by conventional biochemical and AccuProbe tests.
Results: All the 42 *M. gordonae* isolates included in this study have been correctly identified by the four molecular methods tested. The PCR-RFLP analysis of rpoB gene generated the typical pattern of type A, B and D for 12, and 12 isolates respectively. However, the PCR-RFLP analysis hsp65 gene generated the typical pattern of type I, II, III, IV for 27, and isolates,respectively. After the combination of patterns 25 isolates had patterns of type A and I, three isolates had patterns of type A and II, three isolates had patterns of type B and II, three isolates had patterns of type D and III and nine isolates had patterns of type D and IV.
Conclusions: The PCR-based assay method is faster than the conventional methods, technically more simple than PCR-RFLP analysis, less expensive than Accuprobe tests. However, the use of PCR-RFLP analysis methods we discover heterogeneity between the isolates of our collection indicating that the combined use of these molecular markers would be useful for typing of *M. gordonae* isolates. Therefore, we recommend the use of the PCR based assay or Accuprobe tests for the rapid identification and the combined use of PCR-RFLP analysis methods for typing of *M. gordonae* isolates and for identification of other clinical mycobacterial isolates.

P717 Evaluation of the BD ProbeTec™ ET *Mycoplasma pneumoniae* amplified DNA assay

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Objectives: New assays for diagnosing atypical pneumonia caused by Chlamydiaceae family, *Legionella pneumophila* and *Mycoplasma pneumoniae* from throat swabs and lower respiratory samples are currently under evaluation on the BD ProbeTec™ ET system. These assays are based on real-time homogenous strand displacement amplification (SDA) and detection technology. It has been reported that molecular methods have increased sensitivity and shorter time to results vs. culture. For this study, we took part in the evaluation of BD ProbeTec ET *Mycoplasma pneumoniae* (MP) Assay with previously collected throat swabs expressed in 2SP medium and stored frozen.
Methods: Specimens (78) were included in this study – 15 specimens were negative by PCR and 63 specimens were previously confirmed positive for *Mycoplasma pneumoniae* by our in-house PCR method. Nineteen of the PCR-positive specimens were culture positive for *Mycoplasma pneumoniae*. Sensitivity of the MP assay was calculated against the gold standard (culture) and against our in-house PCR method. For samples with discrepant results, the PCR assay was repeated from the original 2SP media if available and also from the BD ProbeTec ET processed samples.
Results: Gold standard (culture): Of 19 culture-positive specimens, 16 were MP Assay positive (sensitivity 84.2%). The three culture-positive MP Assay negative specimens could not be tested by PCR on the original 2SP media due to insufficient volume, but were tested from the BD ProbeTec ET processed sample. Only one of the three specimens was positive by PCR.
PCR: Of 63 PCR-positive specimens, 52 were MP Assay positive (sensitivity 82.5%) and 11 were negative. Three of these 11 specimens were culture positive, but could not be tested by PCR as mentioned previously. Eight of the 11 PCR-positive MP Assay negative specimens were tested by PCR on the original 2SP media and six were found to be positive. When PCR was performed on the BD ProbeTec ET processed sample, only four of the 11 specimens with PCR positive, MP Assay negative results were positive. All 15 PCR-negative specimens were also negative by the MP Assay (specificity 100 %).
Conclusion: The BD ProbeTec ET MP Assay is a sensitive and specific assay for the rapid identification of *M. pneumoniae* in throat swabs expressed in 2SP media. The MP assay may be useful in situations where culture is time consuming and difficult to perform.

P718 Quantification of *Salmonella* by 5′-nuclease polymerase chain reaction targeted to fimC gene

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Objectives: A new 5′ nuclease PCR system for the quantification of *Salmonella* spp. using the primers and the probe oriented to *Salmonella*-specific region of the fimC gene was developed.
Methods: The sequence of the fimC gene was carefully checked and *Salmonella*-specific region was identified, and primers and a 5′-nuclease probe oriented to it were designed with a theoretical melting temperature of 60°C. To determine the exclusivity of the primers, 45 non-*Salmonella* strains were tested by conventional PCR. To determine the inclusivity of the PCR system consisting of the primers and the probe, 48 *Salmonella* clinical and food isolates of 34 various serotypes were tested by real-time PCR. For quantification purposes, calibration lines were constructed for three *Salmonella* strains pure cultures and with other bacteria background, respectively.
Results: The PCR system is specific and sensitive with 100% inclusivity and 100% exclusivity. Calibration lines constructed for three *Salmonella* strains were very similar to each other and facilitated quantification in the range from 10^3–10^9 CFU/mL. *Escherichia coli* (10^6) and *Citrobacter freundii* (10^6) background had no effect on *Salmonella* quantification by the system.
Conclusion: Presented highly specific real-time PCR system represents a good tool for quantification of *Salmonella* sp. in clinical, food and environmental materials.

P719 Experiences with Chlamydia testing using the BD ProbeTecET and BD Viper focusing on inhibition in urine samples

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Objectives: On June 1, 2003 our laboratory changed the *Chlamydia trachomatis* (CT) testing platform from PCR/EIA to real-time strand displacement amplification (SDA) using the BD Viper sample preparation robot and two BD ProbeTecETs. The lab performs 70 000 CT analysis per year. The wet swab for cervix and urethra, and urine testing for men was introduced. We evaluated the inhibition rate in different specimen types and analysed the effect of washing urine samples prior to testing.
Methods: We evaluated the first 6 months of using SDA (33 000 samples). Information was obtained from our LIS. A patient was only included once and a positive test result had priority over negative results. We calculated changes in gender sampling, sampling site, age-specific positive sample rate and age-specific percentage of women tested in Copenhagen. After washing 91 negative urine samples and 90 positive urine samples were retested without the washing step.
Results: The average positive sample rate increased from 4.4 (EIA) to 6.4% (SDA). The age-specific positive rate for women increased for all ages over 18 years. The positive rate from female urine $n = 130$ was 5.4% and from male urine, $n = 2220$, 15%. CT samples from men were increased by 53% and positive rate from 12.5–13.7%. Inhibition was seen in 0.7% from cervix, 0.16% from female urethra, 0% from female urine, 0.24% from male urethra and 0.14% from male urine.Reportable results were obtained from 80/91 (88%) unwashed urines that were negative after washing. 11/91 had inhibition (12%) and one of 80 was borderline positive. Reportable results were obtained from 88/90 (97.8%) unwashed urines that were positive after washing. Two of 90 had inhibition (2.2%) and three of 88 were negative.
Conclusion: The positive sample rate was increased by 45% in women and 9.6% in men with male sampling increasing by 53%. Inhibition was only seen in 0.46% of samples, primarily from cervical samples. Washing of urine samples removes virtually all inhibition. Inhibition rate for unwashed urines was significantly higher in negative urines than positive urines $P = 0.02$. Omitting the washing step (with a CT prevalence of 15%) will reduce the time spent on washing by about 66%. This will delay laboratory answers on the inhibited samples by one day due to washing and re-analysis of approximately 10% of the urine samples.

P720 Development of a PCR test-system to detect the DNA of *Bacillus anthracis* (pXO1 and pXO2) by using a hybridisation method for considering amplification results

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Anthrax is the dangerous zooanthroponotic infection. Sporadic and group cases and infection outbreaks are recorded every year. Given such an unstable epidemiological situation, there is need for constant control over this infection. The effectiveness of such control is largely dependent on the availability of highly specific and sensitive diagnostic systems. PCR is currently a widely used method of a rapid diagnosis. There is a possibility of nonspecific DNA fragments, similar in size to the one sought far, forming during amplification. Separating such fragments electrophoretically is very difficult. Our aim was to develop novel PCR test-systems to detect *B. anthracis* DNA in two plasmid replicons (pXO1 and pXO2), on the basis of hybridisation principle of considering amplification results.A total of 29 *B. anthracis* strains and 20 strains of nine closely related species, two *Y. pestis* strains and two *F. tularensis* strains were used. *Bacillus* DNA was extracted by boiling 18-h agar cultures.The structural genes pagA (pXO1) and capB (pXO2), ensuring the synthesis of the toxin and the capsule, were chosen as DNA targets in developing the test system. Were selected following primers:PA1 – 5′-CCCACCAATATCAAAGAACG-3′PA2 – 5′-ATCACCAGAGGCAAGACACC-3′CA1 – 5′-AGA-ACGCAGGCTTAGATTGG-3′CA2 – 5′-TGGGATTGATGAGG-AAACAG-3′A 214-bp fragment of the pagA gene is amplified with the primers PA1–PA2. The primers CA1–CA2 ensure the synthesis of a 104-bp region within the capB gene.For hybridisation, we chose two specific probes. The P probe is complementary to the pagA gene, and the C probe is specific towards the capB gene.P – 5′-GCACTTCTGCATTTCCATGTACTTCA-3′C – 5′-ACGTGTAA-TTCTCATTGCTCCTGGA-3′The study included several stages: amplification of a specific DNA-target with biotinylated primers, immobilisation of the PCR product on a streptavidin-coated microplate, hybridisation of fluorescently labelled probes with the complementary inner regions of the amplicons formed, and signal detection with the fluorimeter.The test-systems developed for the detection of plasmids pXO1 and pXO2 were tested using a variety of strains of *B. anthracis* and heterologous species. The *B. anthracis* strains used were natural isolates from various areas in Russia and the former Soviet Union during anthrax outbreaks. Not a single false-positive or false-negative result was obtained during out study. The analytical sensitivity of the test system was 10 CFU/mL.

P721 Early diagnosis of leptospirosis by polymerase chain reaction (PCR)

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Leptospirosis is a worldwide zoonosis that affects wild and domestic animals and humans. The disease is characterised by various clinical manifestations, ranging from asymptomatic disease to fatal icterohaemorrhagic forms, while the diagnosis is mainly based on the detection of serum-specific antibodies (serological methods).The aim of the study was the contribution of PCR amplification in early diagnosis of leptospirosis and its evaluation in parallel to the results of the serological tests.
Material and methods: In this study, 58 patients (78 whole blood and sera samples) with probable leptospirosis were examined over a 4-year period, 2000–2003. Another group of five patients (five whole blood and sera samples) with fever of other aetiology (rickettsiosis, syphilis, borreliosis, brucellosis, tuberculosis) was assessed in order to evaluate the specificity of PCR. 20 healthy persons (20 whole blood and sera samples) were included in the study, as a control group. PCR methods were performed in all samples using two pairs of primers that amplify the rrs (16S) gene and insertion sequence of IS1533 region in *Leptospira interrogans* genome. An enzyme-linked immunosorbent assay (ELISA) was used for the detection of specific antibodies against *Leptospira* spp. (IgM, IgG). Only PCR techniques were performed in samples of control group.
Results: The two PCR methods produced positive results in 25 of 58 patients (rate 43%) while serological tests gave positive results in 22 patients of 58 (rate 38%). The remaining 33 patients were all negative by PCR and only two patients demonstrated low serological reactivity (cross-reaction). All patients of other infections and the control subjects were found negative by PCR and ELISA. Both PCR methods revealed identical results in all groups.
Conclusions: The diagnosis of leptospirosis is often based on serological tests, but the specific antibodies against *Leptospira* spp. are detected 8–10 days after the onset of the disease, while cross-reactions may be present. PCR is a highly sensitive and specific method that contributes to the early diagnosis of the disease. The combination of PCR and serological tests can substantiate the definitive diagnosis of leptospirosis.

P722 Rapid detection and identification of *Aspergillus* spp. and *Candida* spp. by real-time PCR

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Objectives: To develop and evaluate a real-time PCR assay, based in the Light Cycler technology, amplifying a highly conserved sequence of the multicopy 18S rRNA gene and using specific probes for genus-level identification of *Aspergillus* spp and *Candida* spp.
Methods: *Aspergillus* and *Candida* strains obtained from the Spanish Collection of Type Cultures (CECT) including: *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus* and *C. albicans*, *C. dublinensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis* and *C. sake*, were used. Specificity of the assay was assessed by using DNA extracted from a collection of pathogenic and nonpathogenic bacteria and fungi. The analytical sensitivity of the process was evaluated with of different inocula (10^1–10^5 CFU/mL), and serially diluted DNA of *A. fumigatus* and *C. albicans*.
Results: Reactions using genomic DNA from other species resulted in negative results, indicating that specificity of that assay was a 100%. Analytical sensitivity was 60 fg using DNA and 15 conidia using conidial suspensions for *Aspergillus fumigatus*; while for *Candida albicans* were 100 fg and three cells. The linear range of the assay was from 6 to 6×10^7 fg for *Aspergillus* DNA and from 10 to 10^7 fg for *Candida* DNA. Species identification was determined by analysing the melting curves obtained with the specific probes, the Tm ranged from 67.34°C to 70.7°C for *Aspergillus* spp. and from 51.3°C to 64.5°C for *Candida* spp.
Conclusions: We therefore developed a rapid, quantitative, sensitive, and specific real-time PCR assay to detect *Aspergillus* and *Candida* species. The PCR assay designed and tested as described here provides a high sensitivity and specificity for the detection of fungal DNA and rapidly identifies most of clinically relevant *Candida* and *Aspergillus* species.

P723 Molecular genetic identity of blood and oral isolates of viridans streptococci

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Prevention of haematogenic dissemination of oral bacteria belongs to the significant issues of current emergency care of health-compromised individuals. The aim of study to ascertain the molecular genetic relationships of oral and blood culture isolates of viridans streptococci (*S. salivarius*, *S. mitis*, *S. mutans* and *S. sanguis* groups).
Methods: An attempt was made to test the similarities of the relationships in a group of 30 patients with positive haemocultures harbouring these organisms. Isolates were preliminarily identified by means of colonial morphology and biochemical properties and then genotyped by means of the PCR (spacer 16S and 23S rDNA) and AP-PCR.
Results: Strain identity of blood and oral isolates has been proved in *S. salivarius* in two of four cases, in *S. mitis* in eight of 10 cases, in *S. mutans* in two of two cases and in *S. sanguis* in 15 of 16 cases. Clonal identity of blood and oral isolates has been proved in 50% of isolates. Oral microbial flora is thus the significant source of viridans streptococci in positive haemocultures.
Discussion: Based on these findings it should be recommended, when managing oral health care of a patient at risk, to eradicate not only the oral infectious foci but also to introduce the oral home-care regime reducing substantially plaque and mucosal oral flora.
Supported by the grant Min. of Health, Czech Republic No. 0002377901.