The abstracts in this section have been accepted for publication only and will not be presented at the congress.

Health care issues, public health, pharmaco-economics

R1897 Lack of compatible hospital room at a reference infectious disease unit in Northern Italy

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Bologna, I

Objective and methods: A 4-year retrospective survey was carried out at our infectious disease (ID) ward, to point out discrepancies between immediately hospitalised patients (p), and those referred to us for admission but needing transferral elsewhere due to lack of suitable hospital rooms.
Results: Since the year 2000, 493 p of 2073 needing hospitalisation (23.8%), could not find an appropriate place at our ward. Paralleling the increased room number at our division since June 2002 (16–35 beds), the described phenomenon had a sharp drop in frequency, but a subsequent stabilisation followed, with around 14% of ID p sent elsewhere for hospitalisation, until October 2003. Starting from the year 2000, the percentage of denied hospitalisations decreased from 34.3% in the year 2000, to 26.9% of 2001, to 12.9% of 2002, until 13.1% registered during the first 10 months of 2003 ($P < 0.0001$). HIV disease represented the most frequent diagnosis (44% of cases), followed by acute-chronic-complicated infectious hepatitis, central nervous system infection, undetermined hyperpyrexia, respiratory tract infection, infectious exanthems, paediatric infectious diseases and gastroenteritis. Suspected-confirmed tuberculosis represented the only illness who showed a significant increase of hospitalization requests, from year 2000 to October 2003 ($P < 0.02$), with large contribution given by p recently immigrated to Italy (>80% of overall episodes). P with severe, transmissible diseases are of particular concern, when the accepting ID unit is located 40–120 km far from our city. This occurrence involved telephone contacts and search of an ID ward, and ambulance transfer (with medical assistance when warranted) and happened in around 25% of p denied by our ID and Hospital, since the year 2000.
Conclusion: The role of ID wards represent a 'moving target' which needs of a continuous fitting to economic, technical, human, and professional health care resources, on the ground of ever-changing predominant disorders and/or problems. A strict monitoring of needs of hospital admission in the ID setting is strongly needed, to improve patient's managed care in our metropolitan area in the next future.

R1898 Sepsis in a department of infectious diseases in a general hospital, Celje, 2000–2003

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Celje, SI

Objectives: The aim of our study was to establish the incidence of sepsis, and to identify the causes and source of infection in patients treated at the Department of Infectious Diseases and Febrile Conditions of General Hospital Celje in the years 2000–2002.
Methods and results: In our retrograde study, sepsis was established in 496 (9.3%) of 5316 admitted patients. Our definition of patients with sepsis was based on additional and stricter criteria than those reported in the literature. Thus, the source of sepsis was known in all patients, while the causative agent was proved in 277 (55.8%) of them, of these only in 41 (8.3%) patients it was isolated from haemoculture, while in 236 (47.6%) patients the causative agent was isolated from other infectious cultures. Among the patients with known cause of infection, those with isolated Gram-positive bacteria are prevailing (201 patients, i.e. 62.2%).
Conclusions: As in Slovenia we still have not reached an agreement on the definition of sepsis, there is a great discrepancy between the number of patients diagnosed with sepsis and the number of patients registered by the Epidemiological Service. According to our relatively strict definition, at least one tenth of all the admissions to our Department are attributable to sepsis, while in the period 2000–2002, only 57 cases were reported to the Epidemiological Service from Celje health region with a population of 220 000, which represents only slightly over 10% of all patients treated for sepsis at our Department. The data reported call for an urgent consensus on definition, reporting and registration of sepsis in our country.

R1899 An investigation on frequency of mental disorders amongst TB victims in the city of Zahedan

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Objectives: Tuberculosis has been known for many years. It mainly affects human beings and usually involves the lungs although in one third of the cases other organs are also affected. Hence, the main target group in this project was pulmonary TB victims, who comprise 1.7 billion throughout the world and more than 90% of the victims are observed in underdeveloped and developing countries. As a result the main consequences of chronic TB are anxiety, stress and fatigue. Nonetheless, the dangers of psychological effects of the patient along with mental, cardiovascular disorders, long-term disabilities and social problems all together threaten the victim at home. The highest incidence of TB in Iran exists in Sistan and Baluchistan province (14 times more than state average), this might be due to proximity of the province with Afghanistan and Pakistan, the incidence is uncontrollable. Hence, this research study was designed to investigate the frequency of mental disorders amongst TB sufferers in Zahedan.
Methods: This cross-sectional study comprised of 200 victims who were suffering from TB for at least 11 years referring to Zahedan Bou-Ali hospital and other centres combating TB for diagnosis and continuation of treatment. All the patients were evaluated by standard questionnaire of scl-90-R regardless of their gender. The obtained data were analysed by bio-statistical procedures.
Results: The results showed significant differences (with the help of *T*-test) between male and female TB sufferers i.e. mental disorders amongst women were more than men (55.5%). Furthermore, it was revealed that somatic disorders as well as stress were more within females than males (47.7, 39.3%, respectively). The least occurrence of violent behaviours was observed among victims between 15 and 19 years of age and the highest was attributed to individuals >50 years (the figure was even more for men than women).
Conclusions: According to the findings of this study the frequency of mental disorder within TB affected women was higher that correlates with the other researches. The latter might be due to gender discrimination, socioeconomic conditions, more stressful life, limited sociability within women of this particular area and their cultural attitudes are all encountered as reasons. It is concluded that, as high incidence of mental disorders within women is evident, in parallel with treatment of TB certain centres for counselling are required to reduce their ordeals.

R1900 Behavioural attitudes on the topic of prevention and treatment approach of hospital-based physicians in Greece

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Rio Patras, GR

Objectives: The aim of this investigation was to evaluate preventive practices, attitudes and behaviours of hospital-based physicians of different specialties with regard to their own health and to identify areas that can be targeted for improvements.
Methods: A total of 208 physicians of four specialties groups (general practitioners, internal, surgical and laboratory specialties) in two tertiary care hospitals in Athens and one University hospital in Patras answered in the presence of the interviewer a single-page written questionnaire with eight items. Analyses were made by using the statistical programme SPSS.
Results: The overall response rate was 100%. Of the 208 physicians, 36.5% were internal specialists, 28.4% surgical specialists, 26% laboratory specialists and 9.1% general practitioners. A total of 108 (51.9%) were males and 100 (48.1%) females with a median age of 32 and 31 years, respectively. 62.5% of the total documented had an adequate immunisation for their age group, whereas 10.6% reported not to have been vaccinated and 26.9% declared ignorance. Females and laboratory specialists were significantly more likely than males and the other specialists to be vaccinated. On average, 67.8% reported to always take proper protective measures and 28.4% declared to often take such measures. Factor associated with greater use of preventive strategies was the specialty (laboratory assistants 79.6, surgeons 74.6, internists 60.5 and general practitioners 42.1%). About 50% (37% males, 62% females) of the respondents reported that they face early critical health situations. More than 90% are always (38.5%) or most times (53.4%) in compliance with the guidelines and advices of their specialists colleagues when facing health problems. A high medication compliance is reported for all specialties. 66.8% reported avoiding polypharmacy, whereas 31.3% declared to take more drugs when indicated. Only 2.4% reported having no confidence in other physicians, whereas the great majority trusts their colleagues, based on different criteria.
Conclusion: This study shows that hospital-based physicians have in general a relatively high level of preventive and personal health care at work. Although more than 60% reported a sufficient immunisation, there is still a need for improvement of the effectiveness by ensuring that vaccination schedules are completed and by targeting physicians, especially those who declared having no vaccination or ignorance.

R1901 Intramuscular antibiotics for outpatient treatment in Italy: is it time for change?

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Verona, Brescia, I

Objectives: Among the profound variation of use of different classes of antibiotics in the European Countries, consistent prescription of intramuscular third generation cephalosporins (3GC) is registered in Italy since many years. Such a difference is unlikely to be caused by frequency and severity of bacterial infections, but it is probably linked to physician's and patient's attitudes, historical background, cultural and social factors.
Methods: Literature analysis and market data review allows evaluating the major prescription pattern of antibiotics in Italy and costs for the National Healthcare System.
Results: Nearly half of the prescriptions of 3GC are related to lower respiratory tract infections, but upper respiratory tract and urinary-tract infections are also included. The major driver for the 3GC use seems to be the severity of infection and the clinical conditions of the patients and a perceived higher clinical efficacy and speed of action by either General Pratictioners (GP) or Specialists (mainly pneumologists, ENT and urologists). Patient's request as a reason for 3GC treatment is often reported. A trend of increasing use of 3GC is observed from the North to the South of Italy. The 3GC costs expressed as per DDD are consistently high.
Conclusion: Recent pharmacokinetic/dynamic concepts have definitively provided evidences that the probability of clinical success is linked to specific drug/MIC ratios that oral antibacterials with high (>80%) bio-equivalence and proper *in vitro* activity can safely reach. Educational programs for both GPs and selected specialists are awaited to reduce inappropriate prescriptions of antibacterials.

R1902 Surveillance of antimicrobial resistance at a tertiary hospital in Tanzania

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Objectives: Antimicrobial resistance is particularly harmful to infectious disease management in low-income countries since expensive second-line drugs are not readily available. The objective of this study was to implement and evaluate a computerised system for surveillance of antimicrobial resistance at a tertiary hospital in Tanzania.
Methods: A computerised surveillance system for antimicrobial resistance (WHONET) was implemented at the national referral hospital in Tanzania in 1998. The antimicrobial susceptibilities of all clinical bacterial isolates received during an 18 month period were recorded and analysed.
Results: The surveillance system was successfully implemented at the hospital. This activity increased the focus on antimicrobial resistance issues and on laboratory quality assurance issues. The study identified specific nosocomial problems in the hospital and led to the initiation of other prospective studies on prevalence and antimicrobial resistance of bacterial infections. Furthermore, the study provided useful data on antimicrobial patterns in bacterial isolates from the hospital. Gram-negative bacilli displayed high rates of resistance to common inexpensive antibiotics such as ampicillin, tetracycline, trimethoprim-sulphamethoxazole and sulphonamides, leaving fluoroquinolones as the only reliable oral drugs against common Gram-negative bacilli. Gentamicin and third generation cephalosporins remain useful for parenteral therapy.
Conclusion: The surveillance system is a low-cost tool to generate valuable information on antimicrobial resistance, which can be used to prepare locally applicable recommendations on antimicrobial use. The system pinpoints relevant nosocomial problems and can be used to efficiently plan further research. The surveillance system also functions as a quality assurance tool, bringing attention to methodological issues in identification and susceptibility testing.

R1903 Bacteraemia in a Portuguese hospital: a 9-year analysis

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Lisbon, P

Objectives: The aim of this study was to analyse the bacteriaemia rate and evolution of the most frequent microbiological agents isolated at the laboratory of a central hospital in Lisbon, between 1994 and 2002.
Methods: We studied 40 039 blood cultures and we analysed the most frequent agents responsible for bacteriaemia. Until 1996, blood cultures were processed with Oxoid blood bottles, manually. Since 1997, BactAlert was introduced and microbiologic identification and susceptibility tests were performed by Vitek system.
Results: From the 40 039 blood cultures we have obtained a positivity rate of 9.7% (3871 bottles) and a contamination rate of 6·3% (2508 bottles). Coagulase-negative staphylococci were the most frequent isolated agent (mean: 16.3% with a slightly decrease in

the last years. It was followed by methicillin susceptible *Staphylococcus aureus* (MSSA) (15.6%), which has a tendency to decreased, and *E. coli* (12.7%) that seems to be increasing. Specially in the last years, it is important to notice the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) (8.2%), *Enterococcus faecalis* (7.3%) and *Candida* spp (4.8%). Other organisms had a low incidence and their emergence was intermittent (*Streptococcus pneumoniae*, *Klebsiella pneumoniae*).
Conclusions: The elevated contamination rate alert us for the importance of a correct sample collection as goal of results quality. Although *Staphylococcus epidermidis* is the most commonly isolated agent, as expected, we notice a pattern change in which *E. coli* is more often isolated and reached the top at 2000 and 2002. It is also concerning the increasing incidence of MRSA and *Enterococcus faecalis*. Since 2001, yeasts started to assume an important role as an etiologic agent as a result of the more and more agressive antimicrobial therapies that are prescribed to the patients.

R1904 Cost-effectiveness analysis of miniVITAL automated system vs. routine blood culturing procedure

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Objectives: To evaluate the rate of positive blood cultures, incubation time to detect positive results from inpatients using an automated miniVital system (bioMerieux) (AS) vs. routine blood culture procedure (RBCP) and to determine if AS is a more cost-effective then RBCP.
Methods: Consecutive blood cultures were obtained from polyvalent ICU patients with sepsis (ACCP Consensus Conference, 1992) in three Smolensk city hospitals from June 2002 through December 2002 and included in prospective comparative observational study. Incidence rate of positive results for AS and RBCP was evaluated. Time to detection of positive results for both methods was determined and compared. Direct costs of comparable alternatives were calculated (the hospital perspective was studied) and cost-effectiveness ratios were collated.
Results: A total of 221 blood samples (55 – AS, 166 – RBCP) were obtained from 180 patients. Positive results with AS were obtained in 12 (21.8%) patients and RBCP – 15 (9.0%) patients ($P = 0.017$). Mean time for positive results for AM was 5.3 + 2.1 days, for RBCP – 6.9 + 1.6 days ($P = 0.0001$). Two vials were used in 52.7% (29) in AM and 12.7% (21) in RBCP. Direct costs of culture procedure for AS and PBCP were 8.7 USD and 4.8 USD, respectively. Cost-effectiveness ratio for AS amounted to 0.4 USD and for RBCP to 0.5 USD.
Conclusions: As method has proved to be a more sensitive procedure for evaluating blood cultures in comparison with RBCP. Processing of blood cultures with AS reduces time to positive results thereby guiding rational antibiotic therapy. AS is a more cost-effective then RBCP for blood culturing.
Acknowledgement: The study was performed under the APUA Small Grants Programme

R1905 Microbial investigation of the possible association between pre-term birth and early periodontitis

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Szeged, HUN

Objectives: According to many studies, generalised periodontitis caused by complex anaerobic bacterial flora can be a risk factor for preterm birth (PB). A microbiological study was carried out to examine if early localised periodontitis could be a risk factor for adverse pregnancy outcome.
Methods: Postpartum women without any systemic disease were included into the study. Similar numbers of patients belonged to the case (85) and to the control (80) groups. A PB case was defined if a patient had a threatening premature labour during pregnancy, preterm premature rupture of membranes, or spontaneous preterm labour, and/or the weight of the newborn was <2499 g. Control women had delivery after the 37th gestational week and the newborn's weight was >2500 g. For the microbial investigation the periodontal areas are being sampled directly with sterile paper points that were placed in the gingival crevice. Samples were transported in reduced transport medium to the microbiology laboratory immediately after collection. After gentle dispersion the suspensions were diluted (10-1-10-6) in reduced broth and the suspensions were plated on selective and nonselective media to enumerate the total cultivable bacterial flora. For the isolation of anaerobic organisms, cultures were performed in an anaerobic chamber for 6 days at 37°C. After incubation, total aerobic and anaerobic colony counts were determined. Isolated bacteria were identified by using commercial methods and/or ATB identification procedures.
Results: A significant association was found between PB and early localised periodontitis of the patient with the criteria having bleeding at 50% of the examined sites and having at least at one site 4-mm probing depth. The average weight of the newborns in the periodontitis group was less than in the control group, the difference being significant ($P = 0.047$). There was considerable variation in terms of the total bacterial counts, it was generally higher of the patients belonged to the case group. The total anaerobic count varies from 1×10^6 to 1×10^9/mL and *Prevotella intermedia/nigrescens*, *Porphyromonas gingivalis*, *Actinomyces* sp. and *Peptostreptococcus* sp. were found in greater proportion in the patients suffered from early localised periodontitis.
Conclusion: The results indicate that early localised periodontitis caused by significant number of anaerobic bacteria of the patient during pregnancy can be regarded as an important risk factor for PB.

R1906 Economical consequences of an outbreak of bullous impetigo in Viborg County, DK, 1999–2003

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Viborg, DK

In August 1999 Department of Clinical. Microbiology. suddenly recorded a manyfold increase in the number of swabs taken from patients with bullous impetigo in general practice. The causative microorganism was a toxine producing *Staphylococcus aureus*. Most of the patients were preschool children, evenly distributed from both sexes. The epidemic never ceased and in 2001–2003 there was each year a marked peak in August/September. The incidences were up to 1200 yearly in a population of 235 000. Intra-institutional and inter-family clusters were often noted. Few relapses occurred despite otherwise effective treatment with oral dicloxacillin. All strains were penicillin resistant, but none of the strains were methicillin resistant. In the start of the epidemic only 10% of the strains were resistant to fucidic acid raising to 60% in autumn 2002. Despite that more stringent hand hygiene was recommended to the institutions by the health authorities the epidemic now continues unchanged on the fifth year. Concurrent with the epidemic of bullous impetigo in the community more than 10 babies with staphylococcal scalded skin syndrome (SSSS) were admitted each year to the local paediatric department. Average admission 8 days. SSSS was not diagnosed every year before 1999. Rough calculations of the expenses to visit practitioners, microbiological analyses, prescriptions, drugs and parents lost income as they had to stay home to care for the children amounts to >2 million Euro in the 4-year period. Extrapolated to whole Denmark the epidemic has so far cost >40 million Euro.
Conclusions: It has so far not been possible to stop the country wide, expensive epidemic of bullous impetigo by recommending general hygienic precautions for children alone. To elucidate the true transmission risks some carefully conducted studies should be carried out.

R1907 A targeted approach to fluoroquinolone use improves appropriateness and reduces cost of therapy

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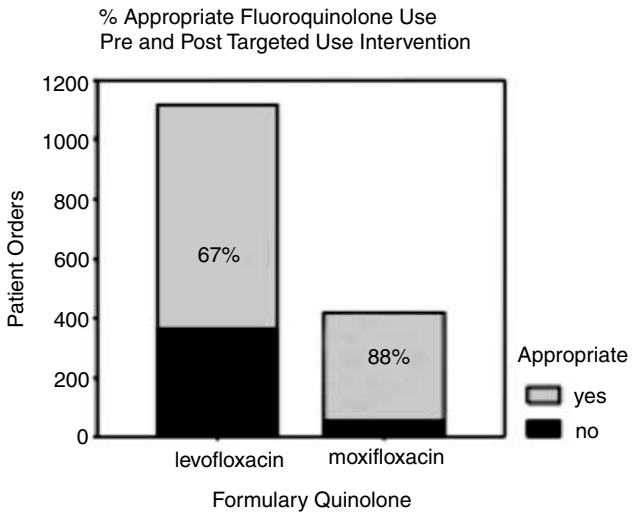
Background: Increasing fluoroquinolone (FQ) use has been described nationally and was observed locally at an urban trauma center, coinciding with increasing FQ resistance rates among *P. aeruginosa*. An antimicrobial management team assessed FQ use daily, reporting results quarterly, and noted an increase in inappropriate levofloxacin (L) prescribing. Excessive use of quinolones can contribute to development of resistance and increased antimicrobial costs. We implemented a formulary conversion from L to moxifloxacin (M) and ciprofloxacin(C). M use was targeted to CAP only, with C reserved predominantly for *P. aeruginosa*.
Objectives: The objective was to assess the impact of the conversion on appropriateness of FQ use, cost of FQ use, cost of all antibiotics, and resistance.
Methods: We conducted an interventional study comparing the 12 months prior to and after the formulary conversion. Appropriateness of FQ use was defined based on criteria defined by the antimicrobial subcommittee. Endpoints assessed were FQ use, FQ appropriateness, FQ costs, total antibacterial use, and total antibacterial costs.
Results: After conversion, total fluoroquinolone use decreased from 49 DDDs/1000 pt days to 33; respiratory FQ use from 25 to 10, and ciprofloxacin use remained stable, 22 to 24. Adherence to criteria improved for all FQs (65% increased to 76%, $P < 0.001$), respiratory FQs (67 up to 88%, $P < 0.001$), and for ciprofloxacin (54 up to 62%). Formulary conversion also corresponded with a decrease in FQ expenditures: pre-intervention = \$607, Post = \$327 (USD per 1000 pt days). A modest increase was seen in costs of cephalosporins (\$2085 up to \$2542) and macrolides (\$1109 up to \$1277). These data correspond to an estimated annual savings of \$20 000 our hospital, or \$132 500 when extrapolated to our entire 2000-bed medical center. Total antibacterial expenditures for the hospital decreased from \$646 000 to \$594 000 for the two study periods. Resistance analysis will be performed and reported once 2003 data is complete.



Conclusion: A targeted approach to formulary FQ management improved appropriateness of FQ use and decreased cost.

R1908 A food-borne outbreak of *Salmonella enteritidis* in Ankara, Turkey

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Objective: To investigate a foodborne outbreak with clinical and laboratory features which was detected in a restaurant of a workplace.
Methods: A cluster of cases with same symptoms associated with gastroenteritis had been identified among the employees of the same organisation who had eaten lunch and dinner produced in the same central kitchen by a food company, was investigated. *Salmonella enteritidis* was isolated from stool samples of six patients out of nineteen by conventional culture methods with serotyping and antimicrobial susectibility tests were done by disc-diffusion method. Interviewes with the related people including the managers, responsible people from kitchen and the food handlers were done. A questionnaire was applied to the people who had eaten suspected meal in order to obtain information for descriptive epidemiology and the food histories.
Results: All foods was prepared at the same kitchen and were delivered five restaurants for lunch and two restaurants for dinner on the day of outbreak. The precooked materials which were all fresh have been stored in healthy conditions and the expire dates were suitable. The lunch consisted of soup with meatball, dolma, yogurt, macaroni with sausages and tomato paste sauce and desert. Except the soup and macaroni recooked, the dinner menu was the same with lunch. Dolma, yogurt and sauce were stored in refrigerator until the dinner. The questionnaire were conducted to 90 people who had eaten from the same kitchen. 20/31 (64.5%), 15/15 (100.0%) and 36/44 (81.8%) of people got ill who had eaten only lunch, only dinner and lunch and dinner respectively. The incubation period ranged from 3 to 32.5 h with a mean incubation period of 17.4 h. The age ranged between 17 and 60 years and the median of age was 32 years. The highest ratio of the infection was observed as 84.3% (70/83) among the people who had eaten macaroni with sausages and tomato paste sauce. The antimicrobial susectibility tests of the six *Salmonella enteritidis* strains revealed that all were uniformly intermediately resistant to tetracycline, and were uniformly sensitive to ampicillin, chloramphenicol, trimethoprim, trimethoprim/sulpfamethoxazole, ciprofloxacin, streptomycin, kanamycin, gentamycin, amikacin, nalidixic acid.
Conclusion: According to those descriptive epidemiological results several hypothesis have been developed and in order to test them an analytical epidemiological study is still on going.

R1909 Antimicrobial prescribing for outpatient adults with acute otitis media

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Objectives: Acute otitis media (AOM) is a common infection requiring physician visits and prescription of systemic antimicrobials (AM) in outpatient departments all over the world. The objective of the study was to evaluate the frequency and patterns of AM prescribing for outpatient adults with AOM in European part of Russia in comparison with previously published recommendations.
Methods: The study was carried out in randomly chosen public outpatient departments in six regions of Russia. In each department consecutive cases of AOM, registered in 2001, were selected for clinical chart evaluation. The appropriateness of AM choice was assessed in accordance with the available guidelines.
Results: The study included 1115 patients with AOM (501 males, 614 females) aged from 17 to 83 years, average age 39.9 ± 13.8

years. AM were prescribed to 78% of patients with AOM. The recommended first-or second-line AM were used in 14.8% of cases (6.7, 7.0, 7.8, 15.5, 22.4 and 43.8% in Volgograd, Rjazan, Smolensk, Ekaterinburg, Yaroslavl and N. Novgorod, respectively). Among 33 different AM frequently prescribed as initial therapy were doxycycline, amoxicillin, ampicillin, ciprofloxacin, co-trimoxazole and lincomycin (19.5, 19.3, 16.7, 11.5, 7.2 and 6.7% of cases, respectively). Smolensk and Ekaterinburg were characterised by predominant use of ampicillin (41.7 and 30.3%, respectively), Yaroslavl–doxycycline (31.9%), Rjazan–ciprofloxacin (24.7%), Volgograd–co-trimoxazole (21.3%). The average course of AM treatment was 7.2 ± 3.4 days and varied from 5.7 ± 3.2 days in Rjazan to 9.0 ± 6.1 days in Volgograd.

Conclusions: AM therapy is still the mainstay of AOM management in outpatient adults despite the lack of substantial evidence of their benefits. The AM choice differs significantly from current recommendations. The appropriate educational efforts should be undertaken to improve AM treatment of AOM in outpatient adults.

R1910 Aetiology and risk factors of infective endocarditis

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Objective: The purpose of our study is to investigate the aetiology and risk factors of infective endocarditis (IE) in our geographic area.

Methods: In a retrospective chart review all 125 patients who fulfilled the Duke criteria for IE between January 1998 and December 2003 were included in the study. For reasons of accuracy for the aetiological diagnosis we have used the automatic method for bacterial detection (BacT/Alert system). The strains were identified by API system.

Results: The persistent bacteraemia was demonstrated in 63.2% cases. The majority of endocarditis were caused by gram-positive cocci (78.5%): *Streptococcus viridans* (25.3%), *Streptococcus bovis* (5%), *Enterococcus* spp. (6.3%), *Staphylococcus aureus* 34% (MSSA24%, MRSA 10%). Gram-negative bacilli were detected in 19% cases. Viridans streptococcal endocarditis were associated with dental procedures ($P = 0.000$) and staphylococcal endocarditis were associated with prosthetic valve or intravascular devices. Frequency of Gram-negative endocarditis was correlated with urinary and gastrointestinal diseases or procedures.

Conclusions: A large variety of microorganisms was implicated in IE, but staphylococci and streptococci remained the most common etiologic agents. The streptococcus aetiology is associated with stomatological invasive procedures or periodontal infections. The prosthetic valve represents an important risk of staphylococcus endocarditis. The endocarditis prophylaxis is not adequate at such time, especially in situations of stomatological or genitourinary invasive procedures.

New drugs

R1911 Novispirins – a new class of antimicrobial peptides with potent *in vivo* activity

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Objectives: The novispirins are a new class of antimicrobial peptides originally developed by Robert Lehrer and coworkers. They resemble a sheep cathelicidin, SMAP-29, and are linear, amphipathic, α-helical peptides. *In vitro*, the novispirins have broad and potent activity against Gram-positive and Gram-negative bacteria, certain yeasts and fungi while exhibiting limited haemolytic and cytotoxic activities. The objective of this study was to determine the *in vivo* therapeutic potential of the novipirins in reducing the bacterial burden and lung pathologies associated with pulmonary infections.

Methods: A rat pneumonia model has been employed. In brief, the rats were anaesthetized and via intra-tracheal delivery inoculated with 10E9 colony forming units of a stable mucoid clinical isolate of *P. aeruginosa*. Immediately after infection and 3 h postinfection, the rats were administered 200 μL of a 0.1 mg/mL solution of novispirin G10 or 200 μL PBS intratracheally to the left lung. The rats were divided in eight groups and analysed for lung pathology, pulmonary bacteria, and histopatholgy at days 3, 5, 7 and 10.

Results: The pathology results showed a large and significant decrease in several macroscopic lung indexes (e.g. lung index of macroscopic pathology -LIMP) on days 3, 5, 7 and 10. In addition, the number of bacteria recovered from the lung was significantly reduced during the initial course of the experiment (day 3 and 5). However, the infections were almost resolved in both treated and untreated animals by day 7 and 10.

Discussion: The results presented here shows that the novispirins holds great promise as therapeutics to combat or limit pulmonary infections, such as *P. aeruginose* in patients diagnosed with cystic fibrosis. Interestingly, several AMPs have been shown to boost various functions of the immune-system. It has still to be determined whether the observed effects are a direct effect of reducing the bacterial burden in the lungs or if it is an indirect effect mediated through stimulation of the immune-system.

R1912 Safety and tolerability of outpatient caspofungin therapy

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Objectives: Outpatient (OP) parenteral therapy may offer a safe, convenient, and cost-effective approach for some fungal infections. We reviewed our experience with OP administration of caspofungin (CAS) for esophageal candidiasis (EC).

Methods: We evaluated the use of CAS as OP therapy in four phase II/III studies of the primary treatment of EC. The doses of CAS included 35, 50 and 70 mg given intravenously once daily. The recommended duration of CAS was 7–21 days. Adverse events (AE) judged by the investigator as definitely, probably, or possibly related to study drug were considered drug-related (DR). OP therapy was used at the discretion of the investigator. For the purposes of analysis, inpatients (IP) were defined as receiving their entire treatment in the hospital; all other patients were counted as OP.

Results: A total of 141/272 (52%) EC patients were OP, of whom 125 (89%) were treated entirely as OP; the remaining 16 received part of their CAS therapy as OP (nine received $\geq$50% of doses as OP). The 131 IP and 141 OP with EC were comparable in age, gender, and baseline endoscopic grade. The large majority of both IP and OP had advanced HIV infection. Frequencies of DRAE in IP and OP are shown in the table. The most common DRAE in OP were fever (10%), infusion site complications (11%), phlebitis (6%), headache (6%), nausea (6%), elevated aspartate transaminase levels (12%), and elevated alkaline phosphatase levels (7%), and were similar to DRAE seen in IP. Four OP (3%) were admitted to the hospital during CAS therapy, but no admission resulted from a DRAE or treatment failure.

	IP [$n = 131$]	OP [$n = 141$]
Duration of CAS therapy, median [range] in days	10 [1–18]	10 [1–20]
Clinical AE		
DRAE	76 (58%)	58 (41%)
Serious DRAE	0 (0%)	0 (0%)
D/C due to DRAE	2 (2%)	1 (1%)
Laboratory AE		
DRAE	66 (50%)	44 (31%)
Serious DRAE	0 (0%)	0 (0%)
D/C due to DRAE	2 (2%)	1 (1%)

Conclusions: CAS was generally well tolerated as OP therapy for EC. There were no serious DRAE and DRAE leading to discontinuation were infrequent in OP. Under appropriate conditions, CAS can be safely administered in an OP setting.

R1913 Novispirins – novel antibiotics with potent activity in wounds

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Objectives: The novispirins are a potent class of antibiotics with broad antimicrobial activities against bacteria, yeasts and fungi. Despite their broad activity, they show limited *in vitro* toxicity. The objective of this study has been to investigate the potential of the novispirins in reducing the bacterial burden in a pig wound model.
Methods: A number of small wound chambers were installed on the dorsal sides of Göttinger mini-pigs. The wound chambers were kept sterile and monitored for 8 days before inoculation with test bacteria. At day 8, each wound chamber was infected with 5×10^8 colony forming unit, and the infection was allowed to establish for 4 days. Clinical isolates of either *Staphylococcus aureus* or *Pseudomonas aeruginosa* were employed in two separate experiments. At day 2 after bacterial inoculation, all wound chambers were infected. At day 4 after initial inoculation, the wound chambers were washed, freshly inoculated with 10^8 bacteria and treated with different doses of novispirin. The number of viable bacteria in the wound chambers were monitored at $T = 0$, 1, 2, 3 and 4 h posttreatment. The wound-chamber CFU was determined by plating of serial dilutions of the samples.
Results: The data revealed a fast and dramatic decline in the number of bacteria in the wound chambers. As reflected *in vitro*, the fastest and most potent response was seen with *P. aeruginosa*, where 99.99% of the bacteria were eliminated within minutes. The same degree of reduction was seen with *S. aureus*, albeit with a little slower kill kinetic.
Discussion: The data shows that the novispirins are a potent alternative to current antibiotic therapies directed against wounds and other topical infections. The potency, antimicrobial spectrum, lack of spontaneous resistance and the speed of bacterial elimination makes this class of antibiotics an ideal candidate for further clinical development.

R1914 The *in vitro* activity of a new oxazolidinone (linezolid) against fluoroquinolone-resistant enterococci isolated in Romania from January 1999 to June 2003

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Objective: To study the antibiotic resistance in *Enterococcus* spp. isolated in Romania.
Methods: A total of 553 enterococci isolated between January 1999 and June 2003 were collected from: urine, surgical wounds, blood, drain and others: bile, synovial and peritoneal fluid. The strains were characterised by using standard protocols (NCCLS). The enterococci were studied for susceptibility to seven antibiotics: amoxicillin (Amx), ciprofloxacin (Cip), gentamicin (Gn), streptomycin(Str), vancomycin(Va), norfloxacin(Nor), linezolid(Lzd) by three methods: disc-diffusion, screening agar plates and agar dilution according to NCCLS recommendations.
Results: The data were analysed according to NCCLS 2002 and showed the following aspects: 77% *E. faecalis*, 22% *E. faecium*, 1% *E. durans*. Amx resistance was seen only in *E. faecium*: 83% in urine, 100% drain, 100% surgical wounds and 98% in blood. No β-lactamase producers were detected. Concerning *E. faecium* high level resistance (HLR) to Gn was 100% in drain, 89% in surgical wounds and 77.8% in blood and HLR to Str was 38% in drain, 57.5% in surgical wounds and 14.3% in blood. For *E. faecalis* HLR to Gn was 7.9% in surgical wounds and 26.3% in blood and HLR to Str was 31.6% in drain, 24% in surgical wounds and 12.9% in blood. Only one strain (*E. faecium*) resistant to Va was found. Concerning Lzd intermediate resistance among fluoroquinolone (FQL)-resistant *E. faecium* (72.9%) only one strain was found. FQL resistant *E. faecalis* (25.7%) showed the same aspect (only one strain Lzd intermediate resistant.)
Conclusion: The Lzd susceptibility pattern showed an excellent and > nearly complete *in vitro* activity against the Romanian enterococci.

Pharmacokinetics, pharmacodynamics, drug interactions, tolerability

R1915 *In vitro* activity of iclaprim (formerly AR-100) against lower gastrointestinal tract isolates

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Objectives: Antibiotic use in respiratory tract infections is frequently associated with bowel flora alterations and selection of antibiotic resistant flora. Iclaprim is a novel diaminopyrimidine in development that reportedly possesses activity against antibiotic-resistant pathogens. This study examined the activity of Iclaprim to over 2000 isolates recovered from the lower GI tract (bowel) of 60 volunteers receiving antimicrobials.
Methods: During 2002/3, 60 healthy volunteers in groups of 15 received four different antimicrobial regimens. Quantitative stool cultures performed over 12 weeks yielded multiple Gram-negative/positive isolates. The activity of Iclaprim was determined utilizing NCCLS specified microbroth dilution MIC testing.
Results: The $MIC_{50,90}$ (μg/mL) and range of Iclaprim vs. bowel flora isolates is shown below:

Organism (*n*)	$MIC_{50,90}$	Range	Organism (n)	$MIC_{50,90}$	Range
Enterobacteriaceae (790)	1/8	0.12–64	*Enterococcus* sp. (330)	0.06/0.5	0.06–64
E. coli (444)	1/8	0.12–64	*E. faecalis* (162)	0.06/0.5	0.06–64
K. pneumoniae (103)	1/8	0.5–64	*E. faecium* (104)	0.06/0.5	0.06–64
E. cloacae (59)	1/4	1–64	*S. aureus* (23)	0.06/0.12	0.06–32
C. freundi (42)	1/2	0.5–16	*S. agalactiae* (32)	0.12/0.25	0.06–32

Conclusions: Iclaprim demonstrates good activity against lower GI Gram-negative bacilli and enhanced activity against lower GI gram positive cocci.

R1916 *In vitro* elution of moxifloxacin by two types of cement: norian versus acrylic bone cement

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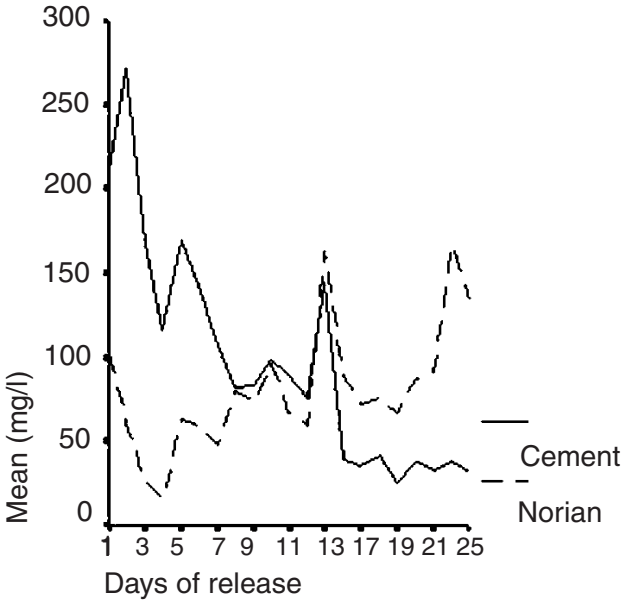
Objectives: Local antibiotic delivery systems for chronic osteomyelitis have evolved in an attempt to reduce the extended use of oral therapy. Moxifloxacin is a novel quinolone active on Gram-positive pathogens of chronic osteomyelitis. Its *in vitro* release of moxifloxacin from Norian and acrylic bone cement was evaluated.
Methods: NORIAN skeletal repair system (Norian) is a novel biocompatible type of bone cement proposed for structural augmentation of cancellous bone after traumatic injury or fractures. Norian and acrylic bone cement were admixed at a 100:3 ratio to moxifloxacin at room temperature in sterile vials. Five samples were prepared per type of material. Mueller–Hinton broth (1 mL) was added over the free surface of the mixture and replaced every 24 h for 28 days; samples were incubated at 37 °C Concentrations of moxifloxacin were determined in daily specimens by an HPLC assay with a Nucleosil-5 150 μM × 2.4 μM column with a 50:50 buffer:acetonitrile mobile phase. A pH: 3 phosphate buffer was applied with a 1 mL/min flow rate.
Results: They are given as means of five samples each day.



Conclusions: Elution of moxifloxacin is higher by Norian after the first days compared with acrylic bone cement so as to be promising for the management of bone infections.

R1917 Penetration kinetics of moxifloxacin in human pancreas

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Objectives: Acute necrotizing pancreatitis is still related to an extremely high mortality rate, based on local infectious complications, particularly in necrotising areas. Limited penetration of antimicrobial drugs in these areas is considered to be a major cause of failure of therapy of severe infections. However, fluoroquinolones have been shown to penetrate sufficiently into pancreatic tissue. On that score, the value of new quinolones such as moxifloxacin (MXF) has not been investigated yet. Using a rat model of acute necrotizing pancreatitis MXF has been demonstrated to penetrate rapidly and efficiently into pancreatic tissue, even into the inflamed and necrotic pancreas.
Methods: Addressing the penetration capability of MXF with respect to the human pancreas, a prospective clinical trial was designed using a single oral dose of 400 mg MXF for antimicrobial prophylaxis in 40 patients (diagnose: chronic pancreatitis or pancreas carcinoma) undergoing pancreas surgery. Samples were taken from blood at 1.7, 3.7 and 5.3 h after application and from resection area of pancreatic tissue at 3.7 and 5.3 h. Concentrations of MXF were determined by HPLC/UV using ofloxacin as internal standard.
Results: Mean plasma concentrations of MXF at 1.7 h after application were 1.4 ± 0.8 μg/mL, at 3.7 h 1.2 ± 0.6 μg/mL and at 5.3 h 1.0 ± 0.5 μg/mL. Corresponding mean concentrations in pancreatic tissue were two to three times higher (at 3.7 h 2.7 ± 1.3 μg/g and at 5.3 h 3.1 ± 1.8 μg/g). The MIC90 of the relevant pathogens encompassed by MXF (e.g. *E. coli*, *Klebsiella* spp. and *Staph. aureus*) were exceeded for at least 5 h in pancreatic tissue. However, the MXF concentration achieved did not reach the MIC of *Pseudomonas* spp. and *Enterococcus* spp.
Conclusion: MXF has been demonstrated to penetrate rapidly and efficiently into human pancreatic tissue. From the pharmacokinetic point of view MXF may have a potential clinical benefit in patient with infectious complications of acute necrotising pancreatitis.

R1918 Bactericidal activity of moxifloxacin against *Haemophilus* species

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Objectives: *Haemophilus influenzae* is frequently isolated in respiratory infections. Pharmacokinetics suggest that macrolides are not as effective against *H. influenzae* as fluoroquinolones. Time-kill kinetics were used to compare the bactericidal rate of moxifloxacin (MXF) to levofloxacin (LEV), azithromycin (AZ), clarithromycin (CLA) and telithromycin (TEL) against strains of *H. influenzae* and *H. parainfluenzae* with elevated macrolide MICs.
Method: Two strains of *H. influenzae* and *H. parainfluenzae* were tested in *Haemophilus* test medium (HTM). Antibiotics were tested at concentrations of 4×, 2×, and MIC. The tubes were incubated in ambient air. Readings were at 10, 20, and 30 min, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 6 h. Plate counts were done in HTM and incubated for 48 h at 35°C in 5% CO_2. Activity was measured by log 10 U (CFU/mL) reductions over time.
Results: For both strains of *H. influenzae* at 4 × MIC MXF there was a 1 log 10 decrease within the first 10 min, and a 3 log 10 decrease (99.9% killing) by 1 h. MXF had the fasted killing rate. A 3 log 10 decrease for both strains was seen at 1.5–2 h for 4 × MIC LEV, and at 4.5 h for one strain at 4 × MIC AZ. TEL and CLA were static. By 2.5 h 2 × MIC MXF achieved 99.9% killing for both strains; it took LEV 3.5 h. AZ, CLA and TEL had no activity until after 2 h. At MIC, MXF was again the most active. For *H. parainfluenzae* a 3 log 10 decrease was achieved by 4× and 2× MIC MXF for both strains, but for only one strain for LEV. MXF had activity within the first 10 min; AZ, the most active macrolide, did not show any activity for the first hour. CLA and TEL were the least active.
Conclusion: The time-kill kinetics of MXF were superior to AZ, CLA and TEL against *H. influenzae*. MXF had a faster killing rate than LEV at all concentrations tested. The bactericidal activity of MXF was better than AZ, CLA and TEL against *H. parainfluenzae*. The killing kinetics of TEL mimicked the macrolide CLA. MXF demonstrated excellent time-kill kinetics against these respiratory pathogens.

R1919 Stability and compatibility of temocillin for administration by continuous infusion in intensive care patients

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Brussels, B

Objective: β-lactams are time-dependent antibiotics and administration by continuous infusion (CI) appears, therefore, a meaningful approach on a pharmacodynamic basis while affording pharmacoeconomic advantages compared with conventional, multiple-daily dosing. Previous studies, however, showed that the stability of individual β-lactams, and their compatibility with other drugs, must be carefully checked under the practical conditions of their projected clinical use. The present study aims at defining the conditions of safe use of temocillin (TMO), a narrow spectrum β-lactam, in this context.
Methods: We mimicked the conditions foreseen for the use of TMO by CI in intensive care patients, namely a daily dose of 4 g dissolved in 48 mL (8.3%) and (i) maintained at appropriate temperatures for up to 24 h (stability studies) or (ii) mixed with drugs commonly used in intensive care patients at mass ratios corresponding to those observed if TMO and the companion drug are administered through the same infusion line (compatibility studies). TMO concentration was determined by HPLC and defined as the sum of its R and S isomers. Criteria for acceptance were (i) chemical stability ($\geq$90% for each isomer and for the sum of both) and (ii) no evidence of physical incompatibility (inspection against a black and white background using an Industry-standard viewer).
Results: TMO was stable for 24 h at temperatures from 20°C through 37°C, and compatible with amikacin (borderline), gentamicin, tobramycin, clindamycin, flucloxacillin, erythromycin, moxifloxacin, ceftazidime, and fluconazole, and with adrenaline, dopamine, dobutamine, isosorbide dinitrate, furosemide, uradipil, theophyllin, omeprazole, insulin, methylprednisolone, ketamine, morphine, sufentanyl, phenytoin, tramadol, penthotal, paracetamol, valproic acid, N-acetyl-cysteine, and aminoacid solutions. In contrast, clarithromycin, ciprofloxacin, meropenem, imipenem, piperacillin/ tazobactam, vancomycin were all found incompatible, and propofol, piritramide and midazolam.
Conclusion: Temocillin can safely be used under the conditions foreseen for the use by CI in intensive care patients and is compatible with many of the drugs commonly used in intensive care patients. Clinical studies of temocillin given by CI are warranted.

R1920 Stability and compatibility of vancomycin for administration by continuous infusion in intensive care patients

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Objectives: Continuous infusion is a potentially useful mode of administration for time-dependent antibiotics in severely ill patients both on pharmacodynamic and pharmacoeconomical bases. In this context, many investigators have advocated the administration of vancomycin (VAN) by continuous infusion (CI). No detailed study, however, is available concerning VAN stability and compatibility with other drugs when used under the practical conditions required for intensive care patients. The present study aims at defining the conditions of safe use of VAN in this context.
Methods: All experiments were made with VAN available on the Belgian market as VANCOCIN®. We mimicked the conditions foreseen for its use by CI in intensive care patients, namely a maximal daily dose of 4 g in 48 mL (8.3%) (i) maintained at appropriate temperatures for up to 72 h (stability) and (ii) mixed with a series of drugs at mass ratios with VAN similar to those that would be observed if VAN and the companion drug would be infused through the same line (compatibility). Criteria were a chemical stability $\geq$90% (as determined by HPLC) and no visible evidence of physical incompatibility (visualisation-inspection against a black and white background using an industry-standard viewer).
Results: VAN was stable at temperatures ranging from 4°C through 37°C for up to 72 h [significant degradation (>10%) was seen at 50°C]. VAN was compatible with cefepime (up to 12.5%), ceftazidime (up to a concentration 0.1%), amikacin, gentamicin, tobramycin, erythromycin, clarithromycin, ciprofloxacin, fluconazole, and insulin, isosorbide dinitrate, nicardipin, uradipil, dopamine, dobutamine, adrenaline, sufentanyl, midazolam, ketamine, morphine, piritramide, N-acetyl-cysteine, and aminoacid solutions. In contrast VAN was incompatible with temocillin, meropenem, imipenem, piperacillin/tazobactam, flucloxacillin, moxifloxacin, and concentrated solutions of with cefepime (>12.5%) and ceftazidime (>0.1%), and propofol, valproic acid, phenytoin, theophyllin, furosemide and methylprednisolone.
Conclusion: VAN is stable in 8.3% solutions maintained up to 72 h at up to 37°C and is compatible with some but not all drugs commonly used in intensive care patients. Special care is required if VAN is co-administered with many β-lactams currently used against Gram(−) organisms.

R1921 Cerebrospinal fluid penetration and pharmacokinetic/pharmacodynamic parameters of cefepime in serum and cerebrospinal fluid

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Seville, E

Objectives: To assess the cerebrospinal fluid (CSF) penetration and the pharmacokinetic/pharmacodynamic parameters in serum and CSF of intravenous cefepime in adults with external ventricular drains (EVD).
Patients and methods: Five patients with EVD and normal renal function. Two patients (cases 1 and 2) with *Pseudomonas aeruginosa* meningitis (MIC of cefepime 1.5 and 1 mg/L, respectively) received cefepime 2 g i.v./8 h. Three patients with nosocomial pneumonia without central nervous system infection, were treated with cefepime 1 g i.v./8 h (cases three and four) or 2 g i.v./8 h (case five). Serial CSF and serum samples were obtained on day 3 of treatment, following administration of i.v. cefepime (0, 30, 60, 120, 180, 240, 360, 480 min). Serum and CSF cefepime concentrations were determined by HPLC assay. Proportion of CSF/serum of cefepime in the CSF Tmax and time of cefepime concentration above MIC (T/CMI) were calculated.
Results: Tables 1, 2 and 3.

Table 1 Patients with meningitis. Cefepime Concentrations (mg/l)

	0′	30′	60′	120′	180′	240′	360′	480′
Case 1 (serum)	7.3	107	73.5	47.6	30.5	25	14.7	11.9
Case 1 (CSF)	12.5	11.9	14.3	15.8	15.5	15	13.6	13
Case 2 (serum)	6.9	71	44.7	28.1	28.5	10.6	4.9	2.3
Case 2 (CSF)	1.9	5	7.4	6.4	5.9	5.5	3.5	2.1

Table 2 Patients with nosocomial pneumonia. Cefepime Concentrations (mg/L)

	0′	30′	60′	120′	180′	240′	360′	480′
Case 3 (serum)	4.5	55.8	33.8	21.4	16.2	12.8	7.7	6.5
Case 4 (serum)	1.7	32.8	22.6	15.3	9.3	7.1	4.3	2.9
Case 5 (serum)	2.4	76	42.2	20.8	12.9	9.5	5.4	3.3
Case 3, 4, 5 (CSF)	0	0	0	0	0	0	0	0

Table 3 Patients 1 and 2. Pharmacodynamic parameters

	Δ T/MIC (serum)	Δ T/MIC (CSF)	Proportion CSF/CSF
Case 1	100%	100%	33.1
Case 2	100%	100%	16.6

Conclusions: In patients with meningitis intravenously administered cefepime 2 g/8 h, penetrated CSF at levels sustaining above de MIC during the total time between dosing. In patients without meningeal inflammation cefepime did not penetrate in CSF.

R1922 Concentrations of cefotaxim and its metabolite desacetylcefotaxim in infants and children during continuous infusion

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Purpose: Despite its widespread use surprisingly few data exist on pharmacokinetics of cefotaxim and its metabolite in children. PK/PD relationships indicate that continuous infusion of β-lactam antibiotics may be advantageous. We determined concentrations of cefotaxim and its metabolite during continuous infusion in a routine hospital setting.
Materials and methods: Cefotaxim is routinely given as a continuous infusion in all hospitalised children requiring antimicrobial chemotherapy in our hospital. As part of a therapeutic drug monitoring program, serum samples were taken on day 1, and day 3 and 5 if applicable, as a routine workup. The first blood sample was taken at least 18 h after start of infusion, 100 mg/kg/day after a loading dose of 33 mg/kg. Samples were stored at -70°C until assayed. Concentrations of cefotaxim and its principal metabolite were performed by HPLC in one run using an assay developed to that purpose.
Results: Evaluable serum samples were obtained from 77 children. Of those with samples at both day 1 and 3 ($n = 53$) mean (SD) cefotaxim concentrations were 23.4 (15.1) and 16.8 (8.7) mg/L, respectively ($P < 0.01$). Desacetylcefotaxim concentrations amounted to 33.5 (26.0) and 22.6 (19.9) mg/L ($P < 0.01$). The variation between patients was high. 10 children had cefotaxim concentrations <10 mg/L. Correlation between sample 1 and 2 was 0.6 (pearson *r*). Concentrations on day 5 ($n = 22$) were in the same range 19.9 (11.3) and 19.9 (13.8), respectively.
Conclusions: Although the mean concentrations of cefotaxim and its metabolite were within expectations based on data from adults, there were large variations between individual children. In nearly 20% of children concentrations could be regarded as suboptimal, probably due to a high elimination rate. More detailed pharmacokinetic studies in children are much needed.

In vitro activity to antimicrobial agents

R1923 Nearly unchanged susceptibility against piperacillin/tazobactam, 10 years after its introduction

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Weingarten, Munster, D

Objectives: To determine the antimicrobial susceptbility rates for piperacillin/tazobactam and selective bacterial pathogens in the first decade of piperacillin/tazobactam use in clinical therapy.
Methods: The *in vitro* activity of piperacillin/tazobactam was checked by means of a metanalysis of published MIC distributions in the period 1993–2003. Papers concerning unselected *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Proteus mirabilis*, *Morganella morganii*, *Pseudomonas aeruginosa* and *Bacteroides fragilis* were enrolled, only. The interpretation of the MIC data occurred on the basis of NCCLS-recommended MIC breakpoints.
Results: The percentage of susceptibility against piperacillin/tazobactam is undulatory over the time. The figures reported in the first and last two papers published up to now were as follows:

	Year	*n*	% Susceptibility	Year	*n*	% Susceptibility
E. coli	1993	2933	98.81	2003	1349	96.89
C. freundi	1996/1997	54	92.59	2003	148	77.70
K. pneumoniae	1996	615	97.24	2003	467	92.72
K. oxytoca	1996	327	89.91	2003	298	91.61
E. cloacae	1996/1997	161	83.23	2003	496	77.62
P. mirabilis	1993	593	99.83	2003	419	98.57
M. morganii	1996/1997	60	100	2003	156	97.44
P. aeruginosa	1993/1994	3895	83.52	2003	1296	87.73
B. fraqilis	1993/1994	938	98.08	2003	504	99.80

The only species showing moderate decrease in susceptibility to piperacillin/tazobactam is *C. freundii*. Minimal decreases in susceptibility were found for *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis* and *M. morganii*. However, minimal increases in susceptibility were found for *K. oxytoca*, *P. aeruginosa* and *B. fragilis*.

Conclusion: Piperacillin/tazobactam remains the antibiotic of first choice in empirical clinical chemotherapy because of its broad spectrum of activity and its extremely low potential to develop increasing resistance.

R1924 Comparative *in vitro* activity of cefepime with third-generation cephalosporins against Gram-negative bacilli isolated from different clinical specimens in a clinical centre, Serbia

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Objectives: This study was performed to compare *in vitro* activity of cefepime (FEP), with that of the third-generation cephalosporins–cefotaxime (CTX), ceftriaxone (CRO) and ceftazidime (CAZ) against extended spectrum β-lactamase (ESBL) nonproducing Gram-negative bacilli isolated over 6 months.
Methods: During the study period, 1898 isolates of *Pseudomonas* spp.(465), *Escherichia coli* (456), *Stenotrophomonas maltophilia* (254), *Klebsiella* spp.(234), *Acinetobacter* spp. (202), *Proteus mirabilis* (137), *Serratia marcescens* (76), *Proteus vulgaris* (24), *Providencia stuarti* (20), *Citrobacter freundii* (18), *Enterobacter* spp.(nine) and *Morganella morganii* (three) were examined. The strains were isolated from various specimens from May 2003 to November 2003. Antibiotic susceptibility test was done by standard Kirby–Bauer disc-diffusion method. ESBL production was determined by double-disc synergy test with CTX, amoxicillin-clavulanate (AMC) and CAZ.
Results: None of the investigated strains manifested ESBL activity. Cefepime inhibited *Serratia marcescens*, *Pseudomonas* spp., *Escherichia coli* and *Klebsiella* spp. isolates in 91.6, 85.5, 82.1 and 70.7%, respectively. The highest resistance was noted in *Acinetobacter* spp. 23.8% and *Stenotrophomonas maltophilia* 17.1%. These species were also intermediate susceptible to FEP 28.6 and 36.6%, respectively. Twenty-six per cent of strains were susceptible to FEP and resistant to third-generation cephalosporins. *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Stenotrophomonas maltophilia* were resistant to CAZ in 21.4, 75.6, 44.7, 90.6 and

87.8%, respectively, to CRO in 42.8, 85.4, 61.8, 95.2 and 97.5%, respectively, and to CTX in 46.4, 92.7, 93.4, 100 and 100%, respectively. Gram-negative strains, which appeared to be complete resistant to all tested cephalosporins (12.1%), were susceptible to carbapenems.
Conclusion: These data showed the high level of sensitivity of the most detected isolates of bacteria to FEP, thus it seems to be a promising antimicrobial agent for the therapy of infections due to ESBL nonproducing Gram-negative bacilli resistant to third-generation cephalosporins. The overall susceptibitiy rates to third-generation cephalosporins in our study indicate the increasing problem of resistance to these antibiotics in our centre.

R1925 Antimicrobial resistance among *Streptococcus pneumoniae* isolates causing invasive disease in Crete, Greece

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Streptococcus pneumoniae remains an important pathogen causing severe infections in both children and adults, annually resulting in significant morbidity and mortality.
Objective: To determine the antimicrobial resistance of *Streptococcus pneumoniae* isolated from clinically significant specimens in the University Hospital of Crete, Greece.
Material and methods: Eighty-two *S. pneumoniae* isolates were studied. The origin of these was blood ($n = 33$), cerebrospinal fluid ($n = 8$), middle ear aspirates ($n = 22$) and other sites ($n = 19$). Susceptibility testing was performed using the Etest method according to NCCLS guidelines. The following antibiotics were tested: penicillin G, cefuroxime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, clarithromycin, azithromycin, roxithromycin, clindamycin, ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin, chloramphenicol, tetracycline, trimethoprim/sulphamethoxazole, and vancomycin.
Results: Among the 82 isolates, 54 (65.9%) were susceptible to penicillin, 15 (18.3%) were intermediate and 13 (15.8%) had high-level resistance to penicillin. Resistance rates observed to other antibiotics were as follow: erythromycin 24.4%, clarithromycin 24.4%, roxithromycin 24.4%, azithromycin 26.8%, clindamycin 7.3%, chloramphenicol 6.1%, tetracycline 15.8%, and trimethoprim/sulphamethoxazole 17%. Resistance to multiple (at least three) antimicrobial agents was observed among 13 strains. All isolates tested were sensitive to vancomycin, ciprofloxacin, levofloxacin, and moxifloxacin.
Conclusion: These findings highlight the need for continuous surveillance and susceptibility testing of all pneumococcal isolates recovered from patients with invasive disease.

R1926 Transferable resistance to β-lactam and aminoglycoside antibiotics in ESBL-producing clinical isolates of *Klebsiella pneumoniae*

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Objectives: We studied transferability of resistance to β-lactam and aminoglycoside antibiotics in two sets of ESBL-producing *Klebsiella pneumoniae* clinical isolates obtained from University Hospital Ruzinov, Bratislava (Slovakia) in 2002 and 2003, respectively.
Methods: The level of resistance to selected β-lactam and aminoglycoside antibiotics was determined using the standard agar dilution method according to the NCCLS guidelines. The antibiotics included in the study were penicillins (ampicillin–AMPI), second-generation (cefoxitin–CFOX), third-generation (cefotaxime–CTAX, ceftazidime–CTAZ, ceftriaxone–CIAX) and fourth-generation (cefepime–CEPI) cephalosporins, monobactams (azthreonam–AZTR), carbapenems (meropenem–MERP) and aminoglycosides (gentamicin–GEN, tobramycin–TOB, netilmicin–NET, amikacin–AMI, isepamicin–ISE). Transferability of β-lactam and aminoglycoside resistance determinants was studied by bacterial conjugation with *Escherichia coli* K-12 3110 as a recipient strain. Transconjugants were selected as resistant to ampicillin.
Results: All clinical isolates tested were resistant to AMPI and none of them was resistant to MERP, NET, AMI and ISE. In the first set of 25 clinical isolates obtained in 2002, 36% of clinical isolates were resistant to CFOX, 72% to CTAX, 96% to CTAZ, 80% to CIAX, 4% to CEPI, 80% to AZTR, 40% to GEN and 64% to TOB. In the second set of 21 clinical isolates obtained in 2003, 71% of clinical isolates were resistant to CFOX, 95% to CTAX, 95% to CTAZ, 90% to CIAX, 86% to CEPI, 90% to AZTR, 29% to GEN and 95% to TOB. Determinants of resistance to AMPI, CTAX, CTAZ, CIAX, CEPI, AZTR, GEN and TOB were transferable by bacterial conjugation to the recipient *Escherichia coli* K-12 3110. The frequency of tranfer ranged from 7.3×10^{-6} to 1.7×10^{-3}.
Conclusions: The antibiotics MERP, NET, AMI and ISE had high efficiency in ESBL-producing *Klebsiella pneumoniae* clinical isolates. All β-lactam and aminoglycoside resistance determinants present in clinical isolates (donors), with exception of CFOX, were transferable by bacterial conjugation to the recipient *Escherichia coli* K-12 3110. Our results point to the fact, that β-lactam and aminoglycoside resistance in tested clinical isolates is plasmid-determined and this contributes to the spread of resistance in the mentioned hospital.

R1927 Studies on *in vitro* antimicrobial activity of silicon oil against major causative agents of endophthalmitis

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Purpose: Silicon oil (PDMS 5000) has been widely used as an internal tamponading agent in vitreous surgery, yet after internal eye surgery bacterial or fungal endophthalmitis, blindness causing complication, may develop. The aim of the study was to evaluate antimicrobial properties of silicon oil *in vitro* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, which are considered the major causative agents of postoperative endophthalmitis.
Materials and methods: The clinical isolates of representative microorganisms (*S. aureus*, *P. aeruginosa*, *C. albicans*) were selected. The bacteria and the fungus were separately inoculated in PDMS 5000 (produced by AcriMed, Germany). Control inoculations in physiological saline and sugar bouillon were performed. The samples of 0.01 mL from each medium were diluted according to serial dilution procedure and plated in Petri dishes with 5% sheep blood agar for bacteria and Sabouraud broth for *Candida albicans*. After 24 h incubations for bacteria and 48 h incubations for fungus CFUs were counted.
Results: All the microorganisms revealed an apparent decrease in CFUs in PDMS 5000. The total elimination was observed for *S. aureus* after 5 days. For *P. aeruginosa* solitary colonies (<25 CFUs) were observed up to 7 days, after 7 days of incubation no growth of *P. aeruginosa* was observed. High *C. albicans* CFU values were counted up to 3 days of the incubation. After 5 days single fungal colonies were observed. CFUs of the examined microorganisms declined slightly in physiological saline. A growth pattern similar to the growth curve of microorganisms was observed in sugar boullion.
Conclusion: Our study indicates that silicon oil could have an antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, which are considered the major causative agents of postoperative endophthalmitis.

R1928 Isolation rate and sensitivity to antibiotics of *Moraxella* (*Branhamella*) *catarrhalis* isolated from adult's expectorated sputum samples. A 5-year experience in a tertiary Greek hospital, 1998–2002

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Objective: To investigate the isolation rate of *M. catarrhalis* from adult's expectorated sputum samples (ESS) and to determine the sensitivities of the strains to common used antibiotics.

Material and methods: A total number of 7056 ESS were examined during 1998–2002. The identification of *M. catarrhalis* was performed by Gram stain, observation of typical colonies growth on 5% human blood agar after 48 h of aerobic incubation at 37°C and positive oxidase and DNAase production. Sensitivities were obtained in Muller–Hinton agar according to proposed bibliography. β-lactamase was examined by nitrocefin sticks (Oxoid).
Results: The overall isolation rate of *M. catarrhalis* from adult's ESS was 2% (1998, >1.3%; 1999, >1.5%; 2000, >3.1%; 2001, >2.5%; 2002, >2%). The sensitivities to antibiotics were as follows: ampicillin 2%, amoxicillin/clavulanic 100%, erythromycin 96%, telithromycin 100%, cefaclor 97%, cefuroxime 100%, trimethoprim/sulphamethoxazole 97%, chloramphenicol 100%. Almost all strains (98%) produced β-lactamase.
Conclusions: In contrast to old reports from other countries the isolation rate of *M. catarrhalis* to our study was low (2%). All ampicillin-resistant strains produced β-lactamase. Resistance to erythromycin, which constitutes the drug of choice for *M. catarrhalis* respiratory infections, was remarkable (4%).

R1929 Trends in resistance of *P. aeruginosa* isolates from lower respiratory tract in adults over a 5-year period (1998–2002) in a tertiary hospital

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Objective: To monitor trends in the antimicrobial behaviour of *P. aeruginosa* isolates from bronchial secretions and sputa of patients hospitalised in ICU and pneumological departments (PD) of a tertiary Greek hospital over a period of 5 years.
Material and methods: A total number of 3497 strains (ICU 1715, PD 1782) were examined during 1998–2002. Isolation and identification were performed according to conventional microbiological methods. Sensitivities were obtained by microdilution method (Wider I, Fransisco Soria Melguizo SA) guidelined by NCCLS.
Results: The annual resistance (%) (1998–2002) for PD/ICU were respectively: Ceftazidime 25/78, 28/70, 28/70, 24/65, 25/69, Piperacillin/tazobactam 18/67, 22/59, 26/62, 30/52, 15/40, Aztreonam 35/86, 42/78, 41/87, 46/70, 34/68, Amikacin 24/79, 31/58, 34/77, 24/46, 12/42, Ciprofloxacin 31/84, 40/68, 40/78, 40/70, 38/69, Imipenem 15/20, 8/55, 2/55, 28/61, 19/67, Cefepime 38/65, 40/68, 43/73, 42/71, 34/74.
Conclusions: (i) The resistance in both departments to Ceftazidime and Cefepime showed a stable route. (ii) An increasing resistance rate to Imipenem was observed over time in ICU while. (iii) The resistance to Piper/tazobactam, Amikacin and Aztreonam (ICU) showed a decreasing trend during the last 2 years of the study.

R1930 Susceptibility of *H. influenzae* isolated from adult lower respiratory tract infections. A 5-year experience in a tertiary Greek hospital, 1999–2003

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Objective: To survey the antimicrobial susceptibility of *H. influenzae* strains over a period of 5 years, isolated from patients who were suffered from community acquired lower respiratory tract infections and had admitted in the outpatient's ward of our hospital.
Material and methods: A total number of 742 (1999, 136; 2000, 158; 2001, 173; 2002, 139; 2003, 136) *H. influenzae* strains were isolated on chocolate agar and incubated in CO_2 7% at 37°C. The identification was performed by Gram stain, catalase and oxidase tests and by X and V requirements. The susceptibilities were obtained in *Haemophilus* test medium (HTM) agar, according to NCCLS guidelines. Nitrocefin sticks (Oxoid) were used for β-lactamase determination.
Results: The overall resistance (%) to ampicillin was 8.3 (6.6, 7.6, 6.9, 10.1, 10.2), to Cefaclor 2.9 (2.2, 2.0, 3.5, 4.0, 3.0), to cefuroxime 0.8 (0, 0, 1, 2, 1), to trimethoprim/sulphamethxazole (T/S) 20.5 (24.2, 26.7, 28.5, 14.3, 8.8) and 0% for imipenem, cefotaxime, ciprofloxacin, moxifloxacin, amoxicillin/clavulanic acid. All resistant to ampicillin strains produced β-lactamase.
Conclusions: An increasing resistance of *H. influenzae* strains to ampicillin was observed during the last 2 years of our study ($P > 0.10$). The decreasing resistance to T/S was remarkable but not statistically significant ($P > 0.10$). All *H. influenzae* strains appeared to have stable sensitivities to quinolones and imipenem.

R1931 *In vitro* interaction of β-lactams and ciprofloxacin on multiresistant *Pseudomonas aeruginosa*

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Objectives: Increased prevalence of multidrug-resistant (MDR) *P. aeruginosa* in the nosocomial setting raised the need for the study of any probable susceptibility to the interaction of β-lactams and ciprofloxacin.
Methods: A 6 log 10 inoculum of 21 isolates resistant to ceftazidime (CZ), imipenem (IM), meropenem (MM), aztreonam, ciprofloxacin (CIP), amikacin and colistin were *in vitro* exposed over-time to CZ, IM, MM, CIP and to their interaction in tubes with Mueller–Hinton broth. All β-lactams were applied at a concentration of 16 mg/L and CIP at 2 mg/L; these concentrations are equal to the mean serum levels of the applied antimicrobials. Tubes were incubated at 37°C. Bacterial growth was estimated at regular time intervals and any equal or more than a 2 log 10 decrease compared with the most active single agent was considered indicative of synergy.
Results: Synergy between CZ and CIP was documented in one (4.76%), three (14.3%) and four (19%) isolates at 4, 6 and 24 h of growth respectively. Respective results for synergy between IM and CIP involved eight (38.1%), seven (33.3%) and seven (33.3%) isolates and between MM and CIP involved two (9.5%), two (9.5%) and three (14.3%) isolates.
Conclusions: Results of *in vitro* synergism between β-lactams and ciprofloxacin suggest the application of imipenem and ciprofloxacin for the management of infections by MDR *P. aeruginosa*.

R1932 Antibiotic susceptibility of the *Acinetobacter* species isolated from clinical specimens

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Introduction: Members of the genus *Acinetobacter* are implicated in a wide spectrum of infections, including nosocomial bacteraemia, pneumonia, secondary meningitis, skin and soft tissue infections and urinary-tract infections.
Methods: The susceptibilities of *Acinetobacter* strains to twelve antibiotics isolated during the period of January 2001–December 2003 were determined by the disc-diffusion method as recommended by NCCLS.
Results: A total of 259 *Acinetobacter* spp. were collected and 77 of them were isolated from blood samples, 62 from wound infections, 58 from the respiratory tract, 40 from urine, 12 from cathaters, eight from the peritonium and two from cerebrospinal fluid. The resistance of *Acinetobacter* species to the 12 antibiotics tested were respectively: Chloramphenicol 82%, ceftazidime 77%, cefotaxime 76%, gentamicin 70%, trimethoprim/sulphamethoxazole 66%, amikacin 61%, ciprofloxacin 75%, piperacillin 56%, cefoperazone 54%, tobramycin 53%, cefepime 40%, netilmicin 29%, imipenem 22%.
Conclusion: *Acinetobacter* strains represent a real problem especially in nosocomial infections as they are routinely more resistant. Surveillance programmes and infection-control protocols are essential to control this problem.

R1933 *In vitro* activity of cefepime and cefpirome in comparison with third-generation cephalosporins against Enterobacteriaceae

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Objectives: To determine the *in vitro* antimicrobial activity of fourth-generation cephalosporins (cefepime and cefpirome) in comparison to third-generation cephalosporins against Enterobacteriaceae.
Methods: During a 2-year period (November 2001–October 2003) a total of 696 Enterobacteriaceae strains (371 *E. coli*, 75 *Klebsiella* spp., 69 *Proteus* spp., 67 *Enterobacter* spp., 62 *Serratia* spp., 25 *Citrobacter* spp., 17 *Salmonella* spp., eight *Morganella morgannii* and three *Providencia* spp.) were isolated in our laboratory. The identification and the *in vitro* activity to cephalosporins were carried out by the Vitek system (Biomerieux).
Results: The susceptibility for all isolates of Enterobacteriaceae to fourth-generation cephalosporins was: cefpirome 95% (662/696) and cefepime 94% (652/696). The susceptibility for all isolates of Enterobacteriaceae to third-generation cephalosporins was: Ceftazidime 90.4% (629/696), Ceftriaxone 87% (606/696), Cefotaxime 86% (598/696) and Cefixime 80% (555/696).
Conclusions: Both of the fourth-generation cephalosporins had significantly better *in vitro* activity than all the third-generation cephalosporins against Enterobacteriaceae. Cefpirome was found to be more active *in vitro* than Cefepime against Enterobacteriaceae.

R1934 *In vitro* activity of five third-generation cephalosporins against Enterobacteriaceae

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Objectives: To compare the *in vitro* activity of five third-generation cephalosporins against Enterobacteriaceae since these drugs are commonly used to treat these infections.
Methods: During a 2-year period (November 2001–October 2003) a total of 696 strains belonging to Enterobacteriaceae (371 *E. coli*, 75 *Klebsiella* spp., 69 *Proteus* spp., 67 *Enterobacter* spp., 62 *Serratia* spp., 25 *Citrobacter* spp., 17 *Salmonella* spp., eight *Morganella morgannii* and three *Providencia* spp.) were isolated from clinical specimens in our laboratory. The identification and the *in vitro* susceptibility to third-generation cephalosporins were performed by the Vitek system (Biomerieux). Tested cephalosporins were: cefotaxime, ceftriaxone, ceftazidime, cefixime and cefpodoxime.
Results: The susceptibility for all isolates of Enterobacteriaceae to third-generation cephalosporins was: ceftazidime 90.4% (629/696), ceftriaxone 87% (606/696), cefotaxime 86% (598/696), cefpodoxime 82% (570/696) and cefixime 80% (555/696). *E. coli*, *Citrobacter* spp and *Providencia* spp strains had the same susceptibility to *Ceftazidime*, *Ceftriaxone* and *Cefotaxime* (97.3, 96.0 and 100%, respectively). *Klebsiella* spp strains had the same susceptibility to ceftriaxone, cefotaxime and cefixime (96%). *Enterobacter* spp. strains had the same susceptibility to cefotaxime and ceftazidime (60%). *Serratia* spp. strains had the same susceptibility to cefixime and cefpodoxime. *Salmonella* spp strains and *Morganella morgannii* strains had the same susceptibility to all third-generation cephalosporins (100 and 80%, respectively).
Conclusions: All third-generation cephalosporins had an excellent *in vitro* activity to the majority of Enterobacteriaceae (80% to 90%). Ceftazidime proved to be the most active of the tested cephalosporins. Cefixime and Cefpodoxime were the less-active of third-generation cephalosporins against Enterobacteriaceae.

R1935 Impact of the incubation atmosphere on the susceptibility of *Streptococcus pyogenes* isolates to erythromycin and azithromycin determined by disc-diffusion test

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Objectives: *Streptococcus pyogenes* is an important cause of upper respiratory-tract infections.
Methods: During the 8 months period (since September 2001 till May 2002.) a total of 579 strains of *S. pyogenes* were isolated and tested against penicillin, erythromycin and azithromycin. The strains were isolated from throat swabs (513), nose swabs (55), wound swabs (seven), vagina swabs (two) and ear swabs (two). Disc-diffusion tests were used for susceptibility testing. Cultures of all 579 isolates were incubated at 37°C in an atmosphere containing 5% CO_2 and at the same time were also incubated in air.
Results: In an atmosphere containing 5% CO_2 all strains of *S. pyogenes* were susceptible to penicillin, 14.3% (83) were resistant to erythromycin and 14.9% (86) were resistant to azithromycin. We had also intermediate strains to erythromycin 0.9% (five) and to azithromycin 0.9% (five). In air all strains were susceptible to penicillin too, 8.1% (47) were resistant to erythromycin and 6.6% (38) were resistant to azithromycin. Here we had also intermediate strains, 11.6% (67) to erythromycin and 9.5% (55) to azythromycin.
Conclusion: All strains were susceptible to penicillin. The activity of macrolides, in case that susceptibility testing was performed in air, was significantly greater than activity of the same drugs in an atmosphere containing CO_2.

R1936 Antifungal effects of griseofulvin and terbinafine against some dermatophytes species

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Objectives: In recently years, antifungal drug resistance has dramatically been increased with resulted in the unsuccessful intreatment of dermatophytic patients. Thus, the emergency of finding new antifungal drugs with low side-effect has recently been noted by several researchers. Fungal infection of the skin and its appendages specially dermatophytosis to be considered as the most prevalent skin disorders. Dermatophytosis is a fungal infection which involve the stratum corneum of skin, hair and nail. This contagious animal infection caused by a special group of fungi called dermatophytes.
Methods: In this study the effect of different concentrations of two known antifungal drugs, griseofulvin (8.25–2000 μg/mL) and terbinafine (0.005–4μg/mL) on *Microsporum canis*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* investigated. The fungal isolates were separately added to different concentrations of above-mentioned drugs (1000 cells/mL) and then mixed with semisolid SDA medium in plates. Then the plates were incubated at 28°C for 7–12 days. The drug free sample was mentioned as control for MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration).
Results: The results show that the MIC, 50–100% for both antifungal drugs. This growth for inhibitory effects of griseofulvin was obtained at 1–31.25, 1–31.25, 2–125, 0.06–0.25 and 0.03–0.25 μg/mL for *Microsporum canis*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*, respectively. These values for terbinafine were measured as 0.007–0.5, 0.007–0.125, 0.005–0.5, 0.06–0.25 and 0.03–0.25 μg/mL for *M. canis*, *E. floccosum*, *M. gypseum*, *T. mentagrophytes* and *T. rubrum* accordingly.
Conclusions: In spite of difference observed in antifungal susceptibility, the examined fungi our results indicated that both drugs can effectively inhibit the growth of some important native dermatophytes and thus, have potential values for treatment of clinical dermatophytosis in human and animals.

R1937 *In vitro* susceptibility of *Candida* species isolated from clinical specimens against some antifungal agents

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Objectives: The aim of this study was to determine the resistance of *Candida* species isolated from hospitalised patients to ketoconazole (KET), fluconazole (FLU), itraconazole (ITRA), amphotericin B (Amp), flucytosine (FCU) and voriconazole (VOR).
Methods: A total of 118 clinical isolates of *Candida* were obtained from a variety sources (urine, blood e.g). Identification was done by germ-tube test and ID 32 C identification system (BioMerieux, France). The most common species of identified *Candida* was *C. albicans* (69), followed by *C. parapsilosis* (16), *C. glabrata* (12) *C. tropicalis* (nine), *C. kefyr* (eight) and one of *C. lusitania*, *C. spherica*, *C. sake*, *C. lambica*, respectively. An adapted NCCLS M 27 – a method was used to evaluate the activity of KET, FLU, ITRA, AmpB, FCU and VOR. The MICs of the strains were evaluated by RPMI 1640 medium with microdilution method.
Results: There were no isolates of tested *Candida* spp. resistant to KET, FLU, ITRA, FCU, AmpB and VOR. Intermediate resistant appeared in 21 isolates to KET and FLU, 10 isolates to ITRA. It is known that the investigational voriconazole have not been assigned interpretive breakpoints.
Conclusion: Among 120 *Candida* isolates were found highly susceptible to KET (MIC; KET ≤ 8 μg/mL) followed by FLU (MIC; FLU ≤ 8 μg/mL), ITRA (MIC; ITRA ≤ 0.125 μg/mL) FCU (MIC; FCU $\leq 4\mu$g/mL) AmpB (MIC; AmpB < 1 μg/mL) and VOR (MIC; VOR ≤ 0.0312 μg/mL), so voriconazole and AmpB were each more active than ketoconazole, flukonazole, itraconazole and flucytosine.

R1938 Inhibition of staphylococcal biofilm formation and eradication of established biofilms by oregano oil

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Objectives: To evaluate the effect of oregano essential oil on biofilm formation and eradication of established biofilms of staphylococci strains.
Methods: The strains (20) were characterised by polymerases chain reaction (PCR) for icaA and icaD detection and adherence to polystyrene microtitre plates for biofilm production. The influence of subinhibitory concentrations (sub-MICs) of oregano oil was evaluated on biofilm producers defined as strong or medium using absorbance values. The biofilm inhibitory (BIC) and biofilm-eradicating concentrations (BEC) of oregano oil were also determined for bacteria grown attached to polystyrene microtitre plates for 56 h and compared with the values obtained for the planktonic cells (MIC and MBC).
Results: Oregano oil influenced biofilm formation, greater inhibition was obtained with levels of 1/2 MIC (65%) than 1/4 MIC (33%) and this effect did not correlate with the initial degree of adherence. For the most strains tested the BIC (0.125–0.250% v/v) and BEC (0.25–0.5% v/v) values were coincident or twofold greater than the concentration required to inhibit growth in suspension (MIC and MBC, respectively).
Conclusions: Oregano essential oil showed a good *in vitro* activity in inhibiting and eradicating biofilm produced by staphylococci strains commonly involved in medical-devices related infection.

R1939 Reproducibility of Sensititre *M. tuberculosis* broth microdilution susceptibility procedure

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Objective: To measure reproducibility of three QC strains, *M. tuberculosis* ATCC 25177 (H37Ra), ATCC 27294 (H37Rv) and T32948 (clinical strain resistant to rifampicin, isoniazid and ethambutol) in a commercial broth microdilution panel containing first and second line tuberculosis drugs after 7, 14 and 21 days incubation compared with a frozen panel.
Method: Freezer stocks were thawed and inoculated on 7H11 agar slants. Cultures were incubated for 7–14 days until good growth. Cultures were suspended in tubes containing saline, 0.2% Tween and glass beads. Vortexed for 5 min and allowed to settle for 30 min before removing the top 1–3 mL. Transferred aliquot to a fresh tube and turbidity adjusted in a nephalometer to be equivalent to a 0.5 McFarland. 75 μL transferred to 10 mL 7HSF broth. 100 μL dispensed into each well of a panel containing dried antimicrobics. Plates were sealed and incubated at 35°C and examined for growth after 7, 14 and 21 days. Five replicates per strain were put up each day for 10 days. Frozen plates contained 50 μL of broth and drug per well. Plates were thawed and dosed with 50 μL of broth containing a 2× concentration of organism. Five replicates were put up each day for 2 days.
Results: Viable counting a 0.5 McFarland adjusted suspensions for eight replicates of four strains gave a mean count of 9×10^7 CFU/mL (range 2×10^7–3×10^8 CFU/mL). All plates could be read after 7 days although growth was heavier after 14 days and 21 days MICs fell within one dilution of the mode for amikacin, clofazimine, kanamycin, capreomycin, ofloxacin, rifampicin, ethambutol, ansamycin and streptomycin. There was no shift in the modal MIC over time. MICs for ethionamide shifted two to threefold during incubation. There was two well shift between MICs of dried and frozen panels for Isoniazid for the two sensitive strains. This shift was not seen with the resistant strain.
Conclusion: Broth microdilution susceptibility of the three *M. tuberculosis* strains was easy to read, highly reproducible and with the exception of ethionamide not affected by length of incubation. The difference in MICs with isoniazid for sensitive strains between dried and frozen panels will be investigated.

R1940 Comparative evaluation of the resistance of piperacillin/sulbactam and piperacillin/tazobactam to nosocomial pathogens isolated from patients with complicated urinary-tract infections

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Objectives: This study was designed to assess and compare the *in vitro* activity of piperacillin/β-lactamase inhibitor combinations vs. piperacillin against common nosocomial pathogens isolated from patients with complicated urinary-tract infections. We evaluated the *in vitro* activity of piperacillin/sulbactam and piperacillin/tazobactam versus alone piperacillin against 314 clinical isolates (253 Gram-negative and 61 Gram-positive) collected from Izzet Baysal Bolu State Hospital located in Bolu, Turkey in 2001–2003 years.
Methods: A total of 314 strains, isolated at Bolu State Hospital during the period of January, 2001 to September, 2003, made up of *Pseudomonas* spp. (114), *Acinetobacter* spp. (19), Enterobacteriaceae (135), *Staphylococcus* (41) and *Enterococcus* (five) were tested using the disc-diffusion method. Only one sample per patient was included in the study. All consecutive isolates where collected from urine and urinary catheter of patients with complicated urinary-tract infections that considered to occur 3 days later after hospitalisation were tested for their susceptibility to piperacillin/sulbactam, piperacillin/tazobactam and piperacillin, The zone inhibition was interpreted according to NCCLS recommendations.
Results: Generally, piperacillin/β-lactamase inhibitor combinations exhibited greater activity according to piperacillin against all strains. For Gram (+) bacteria, antimicrobial susceptibilities to the three drugs were almost identical. *Pseudomonas aeruginosa* showed extremely high resistance to the three drugs. Piperacillin/tazobactam was most effective compound for Gram (-) bacteria except *Acinetobacter*. Piperacillin/tazobactam retained activity against 89% of Enterobacteriaceae, 79% of *Acinetobacter* and 71% of *Pseudomonas*, while piperacillin/sulbactam was effective against 89% of *Acinetobacter*.

Conclusion: Our results might prove important for the appropriate choice of antibiotic therapy with Piperacillin-β-lactamase inhibitor combinations and sulbactam could be a good therapeutic alternative for the treatment of *Acinetobacter* infections.

R1941 Mutant prevention concentration: a comparison of different quinolones for macrolide-susceptible or resistant *Streptococcus pyogenes* and macrolide-susceptible or resistant *Streptococcus pneumoniae*

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Naples, I

Objectives: Mutant prevention concentration (MPC) represents the lowest antibiotic concentration able to prevent growth of resistant bacterial mutants when a large inoculum (>1010 CFU/mL) is exposed to drugs. The aim of the present study was to compare MPCs of different quinolones such as moxifloxacin (MXF), garenoxacin (GNX), ciprofloxacin (CIP), levofloxacin (LVX) and grepafloxacin (GRX) against *Streptococcus pyogenes* and *Streptococcus pneumoniae*, either susceptible (MS) or resistant (MR) to macrolides.
Methods: Overall, 30 clinical isolates of *S. pyogenes* (MS, $n = 15$; MR, $n = 15$) and 30 *S. pneumoniae* strains (MS, $n = 15$; MR, $n = 15$) were tested towards MXF, GNX, CIP, LVX and GRX. MICs were determined with a microdilution method (NCCLS). MPCs determination was performed by applying 10^9–10^{10} CFU/mL of bacteria to serially diluted drug-containing plate which were incubated at 37°C for 24 + 48 h. MPC was the lowest concentration of drug with no growth at 48 h. All procedures were performed in duplicate to ensure reproducibility.
Results: The MIC90–MPC90 (μg/mL) for *S. pyogenes* and *S. pneumoniae* were as follows: 0.12–0.5/0.25–1 (MXF); 0.06–0.25/0.06–0.25 (GNX); 2–4/4–16 (CIP); 0.5–2/1–4 (LVX); 0.5–2/0.5–2 (GRP). The rank order of potency, based on MPC, capacity of restricting the selection of resistant mutants, was, for both *Streptococcus pyogenes* and *Streptococcus pneumoniae*: MXF = GNX > LVX > CIP = GRX. Time above MPC was >24 h for MXF and GNX. Different susceptibility to macrolides did not affect results.
Conclusions: This study suggests that MIC and MPC consistently predict quinolones activity against macrolide-susceptible and macrolide-resistant *Streptococcus pyogenes* and *Streptococcus pneumoniae*. MPCs determination represents an innovative strategy for preventing selection of resistant mutants as therapeutic behaviours able to achieve and maintain MPC drug concentration could result in restricting the selection of resistance.

R1942 *In vitro* susceptibility of anaerobic bacteria isolated in 2000–2003

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Introduction: The resistance among clinical isolates of anaerobic bacteria increase to antimicrobial agents, frequently to clindamycin. The role of anaerobic bacteria and intra-abdominal sepsis has been extensively examined during the past 25 years. This situation is accompanied by improved cultural techniques, changes in method for susceptibility testing *in vitro* and changes taxonomy.
Materials and methods: The susceptibility of recent clinical isolates of anaerobic bacteria were tested by using broth micro-dilution method by NCCLS recommendation and by E-test. The anaerobic strains were obtained from clinical specimens in Faculty and Town hospitals in Ostrava in years 2000–2003. MIC to penicillin, ampicillin/sulbactam, piperacillin/tazobactam, meropenem, cefoxitin, clindamycin, metronidazole and chloramphenicol were determined.
Results: The table shows MIC 90 (mg/L) for most common organisms.

Test organism	*n*	MIC_{90} (mg/L) PEN	AMP/SUL	PIP/TAZ	MER	CXT	CLI	MET	CMP
Cl. perfringens	222	0.25	0.25	0.5	0.125	1	0.5	1	4
B. fragilis	719	16	1	2	0.125	8	0.5	0.5	4
B. fragilis group *Prev. bivia*	143	1	0.25	1	0.125	4	0.25	1	2
Prev. melaninogenica	381	0.125	0.125	0.5	0.25	2	0.125	0.25	1

Conclusion: The results indicate very good activity against anaerobic bacteria for used antibiotics. The lowest MIC was determined for meropenem. The susceptibility testing of anaerobes is needed for adequate therapy of individual patient, for good choice of empiric therapy and for monitoring susceptibility patterns of anaerobes.

R1943 *In vitro* activity of ertapenem against clinical strains isolated from abdominal infections

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Objectives: Ertapenem has a broad spectrum of activity and is effective against most clinically relevant microorganisms, including Gram-positive and Gram-negative aerobes and anaerobic strains. It has potential for use as empirical treatment of a wide range of infections including intrabdominal infections where the bacteriology is often complex. The aim of our study was to compare the *in vitro* activity of ertapenem with that of other antibiotics with a broad-spectrum of antibacterial activity against aerobic clinical strains isolated exclusively from community acquired intrabdominal infections.
Methods: All antibiotics were tested by E-test method. The antibiotics tested were: ertapenem, imipenem, ceftazidime, cefepime, piperacillin/tazobactam,. ciprofloxacin and amikacin. NCCLS breakpoints values were adopted.
Results: A total 136 microorganisms were isolated and their susceptibility analysed. *E. coli* (37.5%) was the most isolated microorganisms followed by *S. epidermidis* (11.8%) and *S. aureus* (10.5%). Age range of patients was 3–85 years (mean 56.3). Peritonitis was the most prevalent disease (48.5%), followed by visceral abscess (30.2%) and colecystitis (21.3%). Ertapenem and imipenem exhibited essentially the same spectrum of activity. All members of Enterobacteriaceae were susceptible to ertapenem and imipenem, with the former showing lower MIC values than the latter. On the contrary, ertapenem was often less-potent notably against the few strains of *P. aeruginosa* and enterococci. Conversely, the activity of the ceftazidime and cefepime was unreliable against many genera of Enterobacteriaceae and Gram-positive microorganisms. In many cases, tazobactam failed to restore completely the activity of piperacillin against many Enterobacteriaceae. Ciprofloxacin had modest activity against Gram-positive, variable activity against Enterobacteriaceae and low activity against nonfermenting Gram-negative rods. Methicillin-resistance was found in 22.2% of staphylococci, whereas ESBL was detected in 25.9% of Enterobacteriaceae.
Conclusion: Judged on the *in vitro* activity, this study suggests that in intrabdominal infections, ertapenem is an excellent alternative for empirical antibacterial monotherapy of polymicrobic infections, especially when local epidemiology indicates the predominance of multiresistant Enterobacteriaceae.

R1944 *In vitro* susceptibility of fusidic acid in staphylococcal strains during 3 years

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Objectives: In this study, we investigated the suspectibility of fusidic acid (FD) of the methicillin susceptible (MS) and resistant

(MR) 1208 staphylococci isolated from different clinical specimens of hospitalised patients in our hospital in years 2000, 2001, 2002.
Methods: Oxacillin and fusidic acid susceptibilities were determined by disc-diffusion method according to NCCLS standart and Comité de l'Antibiogramme de la Société Francaise de Microbiologie, respectively.
Results: The results are demonstrated in the table.

	FD susceptible	FD resistant	Total
MS coagulase (+)	303	19	322
MR coagulase (+)	419	55	474
MS coagulase (−)	92	14	106
MR coagulase (−)	176	130	306
Total	990	218	1208

Conclusions: Fusidic acid is an antibiotic of choice, particularly for the treatment of methicillin resistant staphylococci. However, in our study the relatively high resistance rate (%48) of fusidic acid was observed in methicillin-resistant coagulase-negative staphylococci.

R1945 Synergy of antimicrobial combinations against pan-resistant *Acinetobacter baumannii* clinical isolates

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Objective: To assess the activity of antimicrobial combinations: amikacin plus cefepime. (AMI + CEF), amikacin plus ciprofloxacin(AMI + CIPRO), amikacin plus imipenem (AMI + IMI), amikacin plus meropenem (AMI + MER) against 14 pandrug-resistant *Acinetobacter baumannii* isolates by Etest.
Methods: The E-test strips (AB Biodisk, Sweden) were used to evaluate the activity of AMI + CEF, AMI + CIPRO, AMI + IMI, AMI + MER combinations using the fractional inhibitory concentration (FIC). E-test regarding antimicrobial combinations has compared with the checkerboard method in previous evaluation with 90% agreement. One antibiotic strip (A) was placed on an agar plate for 1 h and then removed and a second antibiotic strip (B) was placed on top of the gradient of the first agent. The MIC A + B was interpreted after overnight incubation at 35°C as the value at which the inhibition zone intersected the scale on the E-strip. Synergism was defined as an FIC index <0.5. FIC index = FIC of drug A + FIC of drug B, where FIC A = MIC A + B in combination/MIC A alone; and FIC B = MIC A + B in combination/MIC B alone.
Results: AMI + CEF was the most effective combination in seven of 14 (50%) of strains. The combinations of AMI + MER and AMI + CIPRO were synergistic against five (36%) and three (21%) strains of *Acinetobacter baumannii*, respectively. AMI + IMI combination was associated with synergy only in one isolate tested.
Conclusion: Synergy was detected with all combinations tested. The highest rate of synergy were observed with AMI + CEF combination followed by AMI + MER and AMI + CIPRO. These results suggest that these combinations may be useful in the treatment of pandrug-resistant. *Acinetobacter baumannii* clinical isolates in our hospital.

R1946 Antimicrobial activity of root canal sealers – *in vitro* evaluation

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Objectives: According to some researchers, antimicrobial activity is a desirable feature of an endodontic sealer. Bacteriostatic effect can be helpful in preventing a growth of any remaining microbes after chemo-mechanical treatment of root canal. The aim of the study was evaluate antimicrobial activity of sealers such as: zinc oxide–eugenol-based – Canason and Endomethason N, zinc oxide-bismutum-based – Diaket, glass ionomer cement – Endion, urethane methacrylate resin-based – Endorez, zinc oxide–calcium hydroxide-based – Proxiapex, silicon-based -RSA, and Gutta-Percha. Quality of all materials were conformable to Directive 93/42/EEC.
Methods: Freshly mixed sealers were formed to disks and allowed to set for 7 days in 100% humidity at 37°C. The disks were placed on agar plates. After incubation 20 h at 37°C discs were removed and plates were inoculated with standardised suspension of microbial strains: *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus mutans*, *Peptostreptococcus anaerobius*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The plates were incubated up to 48 h at 37°C. The growth of microorganisms were monitored and zones of growth inhibition were measured. Nitrocellulose membranes were applied onto inhibition zone then transferred to sterile agar plate and incubated. The growth of microorganisms were compared with those in previous step and antibacterial activity were estimated.
Results: All the sealers showed different activity depending on the tested strains. Biocompatible sealers such as Endion, RSA and Gutta-Percha did not inhibit growth of bacterial and fungal strains. Endorez were active to *Peptostreptococcus anaerobius* only. Diaket, Canason, Endomethason N and Proxiapex proved to be effective against tested microorganisms. They were bactericidal for Gram-positive bacteria (*Streptococcus* sp. and *Peptostreptococcus anaerobius*, however bacteriostatic effect was observed in cases of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *Peptostreptococcus anaerobius* was the most susceptible to all specimens wherease *Enterococcus faecalis* was resistant.

R1947 Surveillance of erythromycin resistance of *Streptococcus pneumoniae* in Nis, Serbia in the last 5 years

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Nis, CS

Objectives: The surveillance of erythromycin susceptibility of *Streptococcus pneumoniae* in Nis country.
Materials and methods: A total of 1825 strains of invasive and non-invasive *S. pneumoniae* isolated from patients during the years of 1999–2002 were evaluated. Susceptibility testing was performed by disk-diffusion for all isolates, and MICs were determined by E-test and agar dilution (the breakpoints applied were NCCLS 2002), for 330 isolates.
Results: The origin of the 1825 isolates tested was: blood 0.38%, CSF 0.49%, tracheal aspirate 10.19%, sputum 6.68%, nasal swab 77.89%, eye 3.56%, ear 0.65% and tracheostoma 0.16%. Clinical isolates were 27%. 60.05% patients were male and 39.94% were female. Under 1 year were 2.3%, 1 year old 12.6%, 2 years, 16.98%, 3 years, 12.98%, 4 years 10.57%, 5 years 10.46%, 6–15 years 21.97%, 16–60 years 11.12% and over 60 years were 0.98%. Of the total number of tested, there were 87.86% of children and 12.14% adults. Resistant isolates to erythromycin in children were 28.78, 19.83, 14.37, 17.19 and 31.6% from 1999 to 2003, respectively. In adults, this percentages for resistant strains were 30, 16.12, 17.08, 11.11, 31.6% from 1999 to 2003, respectively. The total number of resistant isolates was 27.7, 19.13, 14.91, 16.09 and 34.88% per age. The most of strains were isolated from October to April, 75% of all isolates. A total of 330 strains were tested by using disc-diffusion and Etest.
Conclusions: During the study period we have been observing a significant increase in the incidence of *S. pneumoniae* resistant to erythromycin, especially in children, and generally high per cent of resistant strains. This problem in our region underlines the need for continuous surveillance of antimicrobial resistance profiles of *S. pneumoniae*.

R1948 *In vitro* activities of imipenem, meropenem and piperacillin-tazobactam against *Pseudomonas aeruginosa* and *Acinetobacter* spp. caused infections in an ICU, 2001–2003

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Objectives: To determine *in vitro* activities of carbapenems and piperacillin-tazobactam against nonfermentative Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from the patients hospitalised in the ICU of a university hospital in years 2001 through 2003.
Methods: A total of 109 *P. aeruginosa* and 97 *Acinetobacter* spp. strains isolated from patients having infections in the ICU were studied. The distribution of microorganisms among the isolates is given the Table 1.

The antibiotic susceptibilities were determined by E-test (AB BIODISK) using Mueller–Hinton agar OXOID) according to NCCLS recommendations.
Results: The resistance ratios of studied *P. aeruginosa* and *Acinetobacter* spp. strains are presented in the Table 2.

Table 1. The distribution of studied strains among the samples

Pathogens ($n = 206$)	Samples						
	Blood	Urine	BAL	ETAS	Catheter	Wound	CSF
P. aeruginosa ($n = 109$)	26	11	7	52	3	7	3
Acinetobacter spp. ($n = 97$)	41	3	0	35	6	10	2

BAL: Bronchoalveolar lavage, ETAS: Endotracheal aspiration specimen, CSF: cerebrospinal fluid.

Table 2. The antimicrobial resistance ratios of *P. aeruginosa ve Acinetobacter* spp. strains

Pathogens ($n = 206$)	Antibiotics		
	Piperacillin-tazobactam (%)	Imipenem (%)	Meropenem (%)
P. aeruginosa ($n = 109$)	90	90	74
Acinetobacter spp ($n = 97$)	100	96	84

Conclusions: High levels of carbapenem and piperacillin-tazobactam resistance are observed among the *P. aeruginosa* and *Acinetobacter* spp strains isolated from the ICU patients of our hospital.

R1949 The activity of some chemical disinfectants on *Aspergillus niger*

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The aim of study was to evaluate of fungicidal activity of commercial chemical disinfectants, which are used for surface disinfectant in order to prevent hospital infection. The products used were representatives for different groups of active substances: alcohols (I), aldehydes and QAC (II), compounds releasers chlorine (III). The disinfectants were examined in the specified concentrations and exposure times recommended by The National Institute of Hygiene in Poland (PZH) and German Society for Hygiene and Microbiology (DGHM) for clean surfaces. The ingredients and parameters recommended by PZH and DGHM as fungicidal are described bellow: I (400% ethanol (96%), 10.0% *n*-propanol, 0.05% benzalkonium chloride, 0.0018% glutaraldehyde): undiluted solution–2 min (PZH); 5 min (DGHM); II (9.5% glutaraldehyde, 7.5% glyoxal, 9.6% didecylodimethylammonium chloride): 1%–15 min, 0.75%–60 min (PZH); 1%–15 min; 0.5%–60 min (DGHM); III (99% sodium dichloroisocyanurate: 1.5 g active chlorine in tablet): 1000 ppm of active chlorine—15 min (PZH), DGHM–lack of recommendation. The test was performed according to the method described in European Standard EN 13697, but instead of stainless steel discs the frosted glass carriers were used. The test strain was *A. niger* ATCC 14606. The product according to this method shall demonstrate at least 3 log reduction in viable fungal counts. In our test the alkohol disinfectant (I) reduces fungi strains at rate 99.9% in 5 min. The disinfectant based on aldehydes and QAC (III) were ineffectiveness in recommended parameters: 1% in contact time 15 and 60 min and when was tested at concentration 2% in 15 min. The disinfectant with sodium dichloroisocyanurate, when diluted at concentration 1000 ppm active chlorine was lack of fungicidal activity in 15 min, but at concentration 2000 ppm active chlorine was effectiveness. The *A. niger* is more resistant than other fungi (*C. albicans* ATCC 10231 and *T. mentagrophytes* ATCC 9533) tested in method propagated by PZH or DGHM. The alcohol disinfectant posses fungicidal properties according to strain *A. niger*. The aldehyde disinfectants known as fungicidal products are ineffective on mould spores. The disinfectant with sodium dichloroisocyanurate was effective to tested strain *A. niger* in higher concentration than recommended for disinfection clean surfaces.

Mechanisms of action and of resistance of antimicrobial drugs

R1950 Class 1 and class 3 integrons among *Klebsiella oxytoca* in a paediatric hospital

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The *Klebsiella* genus is responsible for the most frequent human nosocomial infections in the respiratory and urinary tract. The control of emergent klebsiellae in hospital environment is important as they easily acquire antibiotic resistance genes. The main objective of this work was the characterisation of integrons associated to antibiotic resistance among *Klebsiella oxytoca* isolates. Twenty-one *Klebsiella oxytoca* strains, isolated from different biological products in a paediatric hospital, revealed intermediate susceptibility to broad-spectrum cephalosporins and aztreonam, resistance to aminopenicillins and inhibitors, aminoglycosides and fluroquinolones. By isoelectrofocusing it was observed different isoelectric points (pIs) of 5.4, 5.6, 7.0, 7.4, 7.5 and 7.6 indicating the production of different types of beta-lactamases, that was confirmed by PCR reaction, with specific primers for blaTEM, blaOXY e blaOXA genes. These strains showed three class 1 integrons with approximately 1650, 2000 and 3500 bp in size, detected with specific primers 5′CS and 3′CS. Class 3 integrons were detected by amplification of class 3 integrase with specific primers intI3F and intI3R. All isolates generated the same M13 fingerprinting profile, suggesting that they were clonally

related, although they produced different beta-lactamases. The presence of mobile genetic elements, such as plasmids and integrons, which facilitate the gene mobility between strains, increase the necessity of control the dissemination of these strains, *K. oxytoca*, in hospitals.

R1951 Presence of TEM-1 and ROB-1 beta-lactamases in clinical isolates of *Haemophilus influenzae* and *H. parainfluenzae*. Correlation with betalam resistance

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Objectives: Two betalactamases, TEM-1 and ROB-1, have been identified in *H. influenzae* and *H. parainfluenzae*. Given the lack of information on the incidence and the resistance to betalactams conferred by the production of the ROB-1 in our area, we decided to undertake a study to determine if and how often it appears in our isolates.
Methods: We collected 171 consecutive clinical isolates of the genus *Haemophilus*, and we determined their susceptibility to ampicillin, amoxicillin–clavulanate (AC), cefaclor, cefuroxime and cefotaxime by the agar dilution method. Of them, 28 strains were betalactamase positive by the nitrocefin test (16.4%): 22 *H. influenzae* and six *H. parainfluenzae*. We analysed the presence of the genes bla-TEM-1 and bla-ROB-1 in the 28 betalactamase-positive strains by PCR.
Results: In *H. influenzae* the presence of the bla-TEM-1 gene was detected in 20 strains (91%) and the bla-ROB-1 in two (9%). Whereas in *H. parainfluenzae* the bla-ROB-1 gene was detected in three strains (50%) and in one of them together with the bla-TEM-1 gene. All isolates were resistant to ampicillin and susceptible to cefotaxime regardless of the betalactamase present. Two betalactamase positive AC-resistant (BLPACR) isolates were TEM-1 producers. The MIC90 (mg/mL) of cefaclor was 64 for the TEM-1 positive and ROB-1 positive strains. The MIC90 (mg/mL) of cefuroxime was eight for the TEM-1 producers and 1 for the ROB-1 producers.
Conclusions: The presence of the ROB-1 betalactamase among our *H. parainfluenzae* isolates is higher than among the *H. influenzae*. The prevalence of ROB-1 betalactamase in the *H. influenzae* isolates is very similar to previously published reports. Although rare we have found one isolate with both betalactamases. The strains with ROB-1 betalactamase did not have higher MIC than the TEM-1 isolates for the betalactams studied.

R1952 Patterns of macrolide resistance determinants among *Streptoccus pneumoniae* isolates

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It is well established that the prevalence of resistance to antibiotics depends, in part, on their use, as countries with low rates or high rates of penicillin resistance have low or high rates of β-lactam prescription. Similarly, there is an apparent association between prescription and resistance to macrolides. When the data are examined in more detail a more complex situation is revealed. Mocrolide resistance in *Streptococcus pneumoniae* is typically due to acquisition of the erm (B) gene, which mediates ribosomal modification, or the mef gene, which encodes an efflux pump. In this study, we assessed the relationship between various factors and resistance and undertook systematic analysis of multidrug resistance. Betalactam and macrolide resistance was characterised at a molecular level.
Materials: Susceptibilities to macrolides were evaluated in 150 *Streptococcus pneumoniae* isolates of which 100 were from patients with invasive diseases and 50 were from healthy carriers. Susceptibility of pneumococci to erythromycin, was tested by the agar diffusion technique.
Conclusions: The MIC of penicillin and cefotaxime for these isolates had been assayed previously and showed a high rate of penicillin resistance (68%). Compared with erythromycin as an indicator of macrolide activity, 15 (10%) isolates had MIC > 1 μg/mL and thus were classified as resistance. Among these strains, only one (0.3%) had erythromycin MIC > 32 μg/mL. Twenty of these strains, selected were examined by polymerase chain reaction (PCR) for the presence of erm B gene. All strains, which were resistant to erythromycin contained erm B-related genes

R1953 Study of amikacin resistance in *A. baumannii* clinical isolates

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Madrid, E

The aim of this study was to determine the presence of the aph(3′)-VIa gene by PCR, which encodes an inactivating enzyme amikacin in *A. baumannii* clinical isolates.
Methods: A total of 36 clinical isolates of *A. baumannii* (17 amikacin resistant and 19 amikacin susceptible), recovered from patients admitted in Hospital La Princesa from 1999 to 2001 were included. Isolates were identified by MicroScan (Dade-Behring) and amikacin susceptibility performed by agar dilution, according to NCCLS guidelines. All the strains were studied for the presence of the aph(3′)-VIa gene by PCR. *A. baumannii* ATCC 19606 was used as control. The amplified DNA products were resolved by electrophoresis in 1.5% agarose gels.
Results: All resistant clinical isolates had positive PCRs for the aph(3′)-VIa gene (234 bp fragment) and 18 susceptible strains had negative PCRs whereas one susceptible strain had a positive reaction for the amplification of aph(3′)-VIa gene.
Conclusions: This study showed that all resistant isolates had the aminoglycoside-3′-phosphotransferaseVI [APH(3′)-VI], a type of 3′-O-phosphotransferase which inactivates amikacin.

R1954 Accumulation of at least 17 different acquired-resistance genes in a clinical *Escherichia coli* isolate

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Objectives: Recently the rate of acquired antibiotic resistance in *E. coli* has risen. Resistance causes bacteria to grow more slowly due to the additional genetic material they have to replicate and express. Yet the multiresistant strains isolated from hospitals show no such deficits. If bacteria are viable despite so many resistance genes then an evolution of the genes or of their arrangement must have taken place. In order to analyse this evolution the molecular background of such multiresistant strains has to be known. Strain 56 is a clinical *E. coli* isolate resistant to most beta-lactams, quinolones, aminoglycosides, tetracyclines, chloramphenicol and co-trimoxazol. In this work we want to analyse the responsible resistance genes and how they are arranged in the cell.
Methods: Minimum inhibitory concentrations (MICs) of 50 antibiotics were determined. Resistance genes and accompanying mobile structures were detected by PCR and sequencing, MAR-phenotype was confirmed by organic solvent tolerance assay. Conjugation experiments were performed and the resultant transconjugants were analysed to reveal which resistance genes were transferable. The plasmids of donor and transconjugants were also prepared and analysed.
Results: Strain 56 encodes at least 17 different acquired resistance genes on the chromosome and on three different plasmids P1, P2 and P3. On the chromosome are two mutations in gyrA, one in parC, and at least one leading to MAR-phenotype. The nontransferable plasmid P1 encodes the resistance genes catI, tet(B), and aac(6′). Aph(3′)-Ia and TEM-1b are located on the transferable plasmid P2.

Plasmid P3 is capable of transferring the following resistance genes: CMY-2, *dfr12*, *ant(3′)-I*, *qacED1*, *sul1*, *merA*, *flo*, *tet(A)*, and *sul2*, whereby *dfr12*, *ant(3′)-I*, *qacED1*, and *sul1* are arranged in a class1 integron in a Tn21-like structure.
Conclusions: The strain carries at least 17 resistance genes, mostly located on transferable plasmids, conferring resistance to 43 of 50 tested antibiotics. It is remarkable that bacteria with so many resistance genes have viability similar to their susceptible parent cells. What is even more surprising is that despite the presence of integrons and transposons that allow the bacteria to rearrange these genes and give them the potential to reduce redundancy and optimise gene expression we still see such a large redundancy of resistance [*tet(B)* and *tet(A)*, *flo* and *catI*, *sul1* and *sul2*, CMY-2 and TEM-1b].

R1955 Abililty of *Escherichia coli* 0157:H7 EDL933 mutants to develop resistance to ciprofloxacin and other antimicrobial agents

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Objective: Shigatoxin-producing *Escherichia coli* 0157:H7 is an important food-borne human pathogen. The use of ciprofloxacin in treating *Escherichia coli* 0157:H7 is contraversial. *In vivo* experiments in mice demonstrated that ciprofloxacin increases free faecal Shigatoxins, and causes death. However, in humans the administration of fluoroquinolones at early stages of infection caused by *Escherichia coli* 0157 may slow the progression of the disease to haemorrhagic uraemic sydrome (HUS). The objective of our study was to examine ciprofloxacin resistance in *Escherichia coli* 0157:H7 EDL933 and to develop resistant mutants to ciprofloxacin and examine their cross-resistance to other antibiotics.
Method: *Escherichia coli* O157:H7 EDL933 is sensitive to ciprofloxacin with the minimum inhibitory concentration (MIC) of 0.03 mg/L. Mutants were selected at 1 and 2 times the MIC. The process was repeated until high-level ciprofloxacin resistance (MIC >16 mg/L) was detected. After each mutation step, MICs of mutants to ciprofloxacin, nalidixic acid, tetracycline, and chloramphenicol were measured by a standard agar dilution method. The stability of mutants was investigated by serial sub-culturing of the mutants on ciprofloxacin-free MacConkey agar plates for 10 generations, and subsequently retesting their susceptibilities. Mutation frequencies were determined by comparing the number of colonies that grew on plates containing antibiotic with the number of colonies obtained in the absence of the compound.
Results: Four-step mutants were selected. Mutant frequencies were determined to evaluate the rate at which mutations occur, and were found to be in the range of 10e-8 and 10e-7 for each step of the selection process. Mutations led to an increase in the MICs of ciprofloxacin (00.6–32 mg/L), and a 16-fold increase in the MIC of nalidixic acid (MIC > 128 mg/L) followed by a fourfold increase in the MIC of chloramphenicol (MIC 32 mg/L). The MIC of tetracycline was also increased by twofold (MIC 4 mg/L). These results suggest the presence of a multiple resistance (Mar) phenotype.
Conclusion: These findings suggest the ability of *Escherichia coli* 0157:H7 EDL933 to easily develop resistance to ciprofloxacin and other quinolones. This appears to be accompanied by an increase in resistance to nonfluoroquinolone compounds. Patients infected by *Escherichia coli* 0157:H7 when treated and exposed to ciprofloxacin may acquire resistant bacteria in their gastrointestinal tract.

R1956 Extended-spectrum beta-lactamase producing Enterobacteriaceae in a multidisciplinary Indian intensive care unit

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Objectives: Extended spectrum beta-lactamase (ESBL) producing organisms pose challenges to clinical microbiologists, clinicians and infection control professionals especially in the setting of the Intensive Care Unit (ICU). Due to concerns about the rise in antibiotic resistance in the ICU pathogens this study was under taken to determine the prevalence of ESBL production in Enterobacteriaceae, which are common nosocomial pathogens in the ICU. The study was performed in a multidisciplinary ICU in south India during the period March–May 2003.
Methods: One-hundered consecutive clinically significant isolates from patients in the ICU which exhibited resistance to the ceftazidime (<22 mm) or cefataxime (<27 mm) by disc diffusion test were further subjected to phenotypic confirmatory tests namely, double disc synergy and inhibitor potentiated disc diffusion tests. Minimal inhibitory concentrations (MIC) of oxyiminocephalosporins and imipenem were determined by agar dilution method. Detection of beta-lactamase genes specific for TEM and SHV types was performed by PCR.
Results: Sixty-five per cent of all Enterobacteriaceae isolates from ICU were ESBL producers and *Escherichia coli* was the most common organism. Seventy per cent of the isolates exhibited zone enhancement with double disc synergy test. Ninety five per cent of the strains exhibited potentiation with Ceftazidime and clavulanic acid combination and 91% with cefataxime and clavulanic acid. Co-resistance was observed to gentamycin (87%), amikacin (51%), tobramycin (54%) and ciprofloxacin (73%). All the strains had high minimal inhibitory combination values for cephalosporins and were uniformly susceptible to imipenem. A fourfold reduction in the MIC was produced with inhibitor combinations. TEM genes were detectable in 62 strains and SHV in 72 strains by PCR. RFLP and DNA sequencing of the representative strains is underway to study their clonal origin.
Conclusion: There is a high prevalence of ESBL producing Enterobacteriaceae in our ICU and this needs to be addressed by continuous monitoring programmes, antibiotic and infection control policies. High degree of co-resistance to multiple antibiotics limits the treatment options in the critically ill patients. TEM and SHV genotypes are the most common types seen in our strains.

R1957 Characterisation of variant *Salmonella* genomic island 1 harbours 3 integrons from serovar Typhimurium DT104

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Objective: *Salmonella enterica* serovar Typhimurium DT104 (DT104) has become a major virulent pathogen emerged as a world health problem. The organism commonly has resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline, so called pentadrug resistant pattern (ACSSuT). Its five antibiotic genes are located between two integrons and clustered in about 13 kb size of chromosomal region. An antibiotic resistance genes cluster in *Salmonella* genomic island 1 (SGI1) has been previously elucidated in multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 and its partially deleted or replaced gene clusters were found in serovars Agona, Paratyphi B, and Albany. We tried to find another variant antibiotic genes clusters in *Salmonella enterica* serovar Typhimurium DT104 isolates from Korean patients.
Methods: Minimum inhibitory concentration (MIC) tests against ampicillin and chloramphenicol, polymerase chain reaction with specific primers for antibiotic resistance genes cluster in SGI1 and southern analysis with aadA2, blaPSE-1 and floR probes were performed to pentadrug resistant 14 DT104 isolates from Korean patients. The novel 3.9 kb DNA fragment produced by XbaI and XhoI digestion of a DT104 genomic DNA was cloned and sequenced.
Results: We identified a *S. enterica* serovar Typhimurium DT104 isolate from a diarrhoea patient which has two times of MIC values against ampicillin and chloramphenicol comparing with those of other DT104 isolates. This isolate had a novel integron-associated gene cassette as well as a known antibiotic resistance gene cluster in its SGI1. The novel integron-associated gene cluster harboured blaPSE-1 and sul1 carried by a class I integron and floR, and partial tetR in that order.

Conclusion: This finding is the first report that shows multiplicated antibiotic genes associated with integron in SGI1, suggesting the recombinational events that lead to the resistance genes shuffling as well as intragenomic transfer of this integron.

R1958 The prevalence of *pmrA*, the fluoroquinolone efflux pump gene, in clinical isolates of *Streptococcus pneumoniae*

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Objectives: The role of efflux in fluoroquinolone (FQ) resistance in *Streptococcus pneumoniae* (SP) is unclear. It has been suggested that almost 50% of ciprofloxacin (CFX)-resistant SP actively efflux CFX (*Antimicrob Agents Chemother* **42**, 2032). However, 80% of SP efflux CFX irrespective of CFX susceptibility (*J Antimicrob Chemother* **44** (Suppl. A): abst. P381). Both studies relied on reserpine to inhibit efflux, which might not be an ideal screening method. As the FQ efflux gene, *pmrA*, has been identified (*Antimicrob Agents Chemother* **43**, 187) we screened a panel of SP clinical isolates for the presence of *pmrA*.
Methods: 1871 SP isolated between 1999 and 2001 from 11 countries was evaluated for levofloxacin (LFX) MIC by NCCLS microbroth dilution. Total DNA was then extracted from these isolates and a 533-bp region of *pmrA* amplified by PCR (16S rRNA internal primers were also included as a control). PCR products were then subjected to agarose gel electrophoresis to determine the presence of a 533-bp product from *pmrA* and/or a 200-bp product for the 16S control. The whole *pmrA* gene was also amplified using primers 177 bp upstream and 74 bp downstream and sequenced as required.
Results: In summary, LFX produced MIC 50% and MIC 90% values of 0.5 and 1 mg/L, respectively. The percentage susceptible, intermediate and resistant were 98.5, 0.4 and 1.1, respectively. As would be expected from other surveillance data, LFX resistance was rare in SP. Nevertheless, all isolates (including SP R6 control) possessed pmrA. Furthermore, 50 of 1871 SP produced a smaller *pmrA* band than usual. Complete *pmrA* was sequenced from a sample of these isolates and all were found to contain a 28 amino acid deletion (AA 159–186) compared with SP R6. Forty-nine of these isolates were susceptible to LFX but one was intermediate. Twenty-four of 50 were from Germany, which may suggest that the isolate is a short-*pmrA* clone.
Conclusions: It would appear that *pmrA* is a constituent of the 'normal' SP genome and presence of the gene is not associated with LFX MIC. A shorter derivative of *pmrA* was also found in a minority of isolates but this again was not associated with LFX resistance. If efflux has a role to play in LFX resistance it may involve differential expression of the gene, although a recent study has found that *pmrA* expression and FQ-efflux inhibition by reserpine do not correlate (*Antimicrob Agents Chemother* **46**, 808).

R1959 Effect of phenylalanine arginine beta-naphthylamide (PAN) on the activity of quinolones against *Klebsiella pneumoniae* (Kp) strains expressing or not increased active efflux of norfloxacin (IAE-NOR)

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Objectives: To evaluate the effect of the efflux pump inhibitor PAN on the activities of the hydrophilic quinolone norfloxacin (NOR) and two hydrophobic compounds, nalidixic acid (NAL) and pefloxacin (PEF), against 25 clinical isolates of Kp, and to relate this effect with the presence of active efflux of norfloxacin.
Methods: MICs of NOR, NAL, and PEF were determined by microdilution (NCCLS guidelines) in the presence or in absence of PAN (20 mg/L). IAE-NOR was determined previously in our laboratory by a spectrofluorometric assay [*Antimicrob Agents Chemother* (2002) 46, 3926–3932) and defined as a $\geq$50% increase in the accumulation of this quinolone in the presence of carbonyl cyanide m-chlorophenylhydrazone (CCCP) in comparison with the corresponding basal value in the absence of CCCP. Among the 25 strains evaluated, 13 expressed IAE-NOR, and 12 lacked IAE-NOR.
Results: PAN reduced at least four times the MICs of NOR for 67 and 25% of strains expressing or lacking IAE-NOR, respectively. However, PAN caused a reduction of at least four times in the MICs of PEF for all strains evaluated, with independence of IAE-NOR expression. Similarly, in the presence of PAN, the MICs of NAL decreased at least four times for all strains expressing IAE-NOR and for 92% of strains lacking IAE-NOR. PAN caused reductions $\geq$8 and $\geq$16 times in the MICs of PEF against 85 and 69% of IAE-NOR(+) strains and against 67 and 50% of strains lacking IAE-NOR. PAN caused reductions $\geq$8 and $\geq$16 times in the MICs of NAL against 85 and 77% of IAE-NOR(+) Kp, and against 92 and 66% of IAE-NOR(−) Kp.
Conclusions: PAN is able to reduce $\geq$4 times the MICs of NOR against many Kp isolates expressing IAE-NOR, but also against a minority of isolates lacking IAE-NOR. When considering more hydrophobic quinolones, PAN reduces the MICs of PEF and of NAL against 100% and 92–100% of the tested isolates, respectively, with independence of IAE-NOR expression. The synergistic effect of PAN with quinolones is independent of the expression of IAE-NOR.

R1960 First description of TEM-24 beta-lactamase produced by nosocomial strains in Portugal

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Objective: Characterisation of the first extended spectrum beta-lactamase (ESBL) TEM-24 described in Portugal, produced by two nosocomial strains: *Klebsiella pneumoniae* and *Escherichia coli*.
Methods: Two uropathogenic isolates (*K. pneumoniae* – Kp2638 and *E. coli* – Ec1248) were collected from two patients in two hospitals in different geographic areas, on March and April 1999. MICs of 14 different beta-lactams (alone or in combination with beta-lactamase inhibitors) were evaluated by an agar dilution method, according to SFM guidelines. To confirm the ESBL production, the isolates were re-tested with the *E*-test ESBL strips. The beta-lactamases were identified by isoelectrofocusing, a multiplex-PCR method and sequencing.
Results: Kp2638 strain showed only reduced susceptibility to ceftazidime (MIC = 32 mg/L) and the Ec1248 strain presented resistance to aztreoname (MIC > 256 mg/L), ceftazidime (MIC > 256 mg/L) and reduced susceptibility to cefepime (MIC = 32 mg/L). Both strains were ESBL positive: MIC synergy between the third-generation cephalosporins and aztreoname alone and in combination with clavulanate was detected; this synergy was confirmed by *E*-test. Multiplex-PCR method showed that Kp2638 strain presented a blaTEM gene plus a blaSHV gene and Ec1248 strain showed a blaTEM gene plus the ubiquitariy ampC gene. Sequencing blaTEM genes from both strains allowed to the identification of blaTEM-24, with the following nucleotide mutations (and corresponding amino acid substitutions): C317→A (Gln39→Lys), G512→A (Glu104→Lys), C692→A (Arg164→Ser), G911→A (Ala237→Thr) and G917→A (Glu240→Lys). These enzymes are also under the control of the strong PaPb blaTEM-2 gene promoter.
Conclusion: TEM-24 beta-lactamase was firstly reported in Portugal in this study. Antimicrobial-resistant bacteria ESBL-producing in Portuguese hospitals should be carefully monitored for its impact on the nosocomial infections.

R1961 Interaction of ceftazidime and tobramycin with vancomycin against *Pseudomonas aeruginosa*

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Genoa, I

Objectives: *Pseudomonas aeruginosa* is responsible of severe and often lethal infections that can hit immunocompromised hosts. The evolution of resistance in this pathogen to currently available

agents poses major therapeutic threats globally. Vancomycin is a powerful active antibiotic on the Gram-positive bacteria, and they very rarely develop resistance. In Gram-negative the entry of the glycopeptides is prevented by the lipopolisaccaride (LPS). It is assumed that some antibiotics might disorganise the structure of lipopolisaccaride and allow the passage of vancomycin into the bacterial cell. In order to estimate this hypothesis and new therapeutic alternatives, the ceftazidime (CAZ) and tobramycin (TOB) have been tested in association with vancomycin.
Methods: A bacterial suspension of about 109 CFU/mL was seeded on plates containing a fixed concentration of VAN (500 mg/L) and increased doses (2x, 4x, 8x, 16x) of CAZ and TOB. Survivors were counted after 48 h at 37°C. Result were interpreted as synergism (99%), additive (90%) and indifference (no significant change) of the CFU/mL reduction found in the drug combination in comparison to the drug alone.
Results: CAZ in combination with VAN reacted synergistically in 16 of 41 cases, additive was found in 17 of 41 interactions and indifference was noted in eight of 41 tests. When TOB was added to VAN synergism was registered in eight of 41 cases, additive was detected in 15 of 41 cases and the remaining tests (18 of 41) resulted in an indifferent reaction. Further tests were also carried out employing teicoplanin (400 mg/L). Preliminary results (11 tests performed) indicated that this glycopeptide favourably reacted with both CAZ and TOB showing similar incidence as reported for vancomycin.
Conclusions: Glycopeptides favourably interacted with the other drugs against *P. aeruginosa*. This might be an interesting new option in treating the above pathogens especially in situation were the drugs could be administered topically. The possibility of using glycopeptides under less restrictive conditions is under study, because these drugs are characterised by a mode of action that imply a very rare selection of resistant strains.

R1962 Detection of CMY-2 beta-lactamases in clinical *P. stuartii* isolates from Egypt

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Objectives: The nosocomial pathogen *P. stuartii* is naturally susceptible to third generation cephalosporins and aminoglycosides. Resistance to these antibiotics can be due to overexpression of chromosomally encoded AmpC and AAC(2′)-Ia enzymes, respectively. Overexpression arises from mutations in the regulatory mechanisms for expression of the respective genes. The aim of this work is to characterise the molecular mechanism of antibiotic resistance in clinical isolates of *P. stuartii*.
Methods: In this study, three *P. stuartii* isolates from patients with burn infections in a university hospital in Egypt were used. MICs were determined using a microdilution method according to NCCLS guidelines. SDS-PAGE analysis of crude beta lactamase extracts was performed. Plasmid profiles were determined and conjugation experiments were carried out using *E. coli* as recipient. PCR experiments were performed to detect blaTEM, blaCMY and intI1 genes, amplicons of blaTEM and blaCMY genes were sequenced. PCR was also used to detect the aminoglycoside resistance genes aac(6′)-Ib, ant(2″)-Ia, aph(3)-I and aac(3)-IIc.
Results: All isolates were resistant to different aminoglycosides and furthermore to third generation cephalosporins and cefoxitin, but sensitive to cefepime and carbapenems. No clavulanic acid effect was observed. For all strains resistance to beta lactams and aminoglycosides was transferable. Two beta lactamase bands (40 kDa, 28 kDa) were seen on the SDS-PAGE with all strains, including the transconjugants. blaTEM and blaCMY genes were detected by PCR. Sequencing of the amplicons revealed 100% identity with the deduced amino acid sequences of TEM-1 and CMY-2, respectively. Transferable aph(3)-I and aac(3)-IIc genes were detected in two strains. Also, a class I integron with aac(6′)-Ib gencassette was detected in two isolates, the third isolate carried an ant(2″)-Ia gencassette within the integron.
Conclusion: In *P. stuartii* a combined beta lactam and aminoglycoside resistance is possible via independent mutations leading to overexpression of chromosomally encoded resistance genes. This was not observed in the examined clinical isolates. Resistance to beta lactams was due to a CMY-2 enzyme located on a plasmid carrying aminoglycoside resistance genes also. Even for a natural AmpC producer acquisition of a plasmid encoded ampC gene can be beneficial in the respect that multiple resistance can easily arise.

R1963 Isolation and antibiotic resistance of Salmonella from Itsoseng and Mmabatho areas in the North West Province, South Africa

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Background: In most countries (South Africa), antimicrobial drugs are used in food-producing animals for treatment of infectious disease and for growth-promoting effects and leads to the appearance and persistence of resistant strains (Radotitis *et al*., 1995).
Aim: The aim and objective was to detect the prevalence of *Salmonella* in sewage water and the antibiotic resistance of isolates.
Method: Sixteen sewage water samples from Mmabatho and Itsoseng in the North West Province was collected from winter (June–July) and spring (August–November). Sterile plastic bottles were used to collect a litre of water, and were labelled with name, time and place of collection. Water was well mixed and 10 mL was poured into sterile tubes, and was centrifuged at speed of 300 rpm for 10 min. The supernatant was discarded and a pellet was mixed with selenite broth and incubated overnight at 37°C. Selenite broth was sub-cultured onto SS agar plates and incubated for 18–24 h at 37°C. Following incubation, colonies from SS agar were purified on another SS agar. Plates were then incubated for 18–24 h at 37°C. API 20E test was used (according to the manufacturer's instructions) to further characterise colonies suspected to be *Salmonella*. Antibiotic resistance of isolates was performed on MH agar plate (Bauer 1966). The following antibiotic discs were used: streptomycin, chloramphenicol, amoxicillin, oxytetracycline, sulphamethoxazole, erythromycin, trimetroprim, polymyxin B and cephalexin. A zone of inhibition (clear area) around the disk indicated that bacteria were susceptible to the antibiotics used. Serological Test Nonlactose fermenters (pale colony), sometimes with black centres due to hydrogen sulphide (H_2S) production, were picked from *Salmonella shigella* agar to perform serologygical typing.
Results: From 16 samples at Itsoseng, nine *Salmonella* isolates were identified as group B and seven were identified as group E. At Mmabatho, from 16 samples 10 *Salmonella* isolates were identified as group B and six were identified as group E. Antibiotic resistance was also performed. From both places, in winter and spring 100% of *Salmonella* isolates were found resistant to most antibiotics, namely, erythromycin, amoxicillin, sulphamethoxazole, trimethroprim, erythromycin and cephalexin.
Conclusion: Multiple antibiotic resistance was observed for all the *Salmonella* isolates which might be greatly due to the miss use of these drugs by individuals in the area.

R1964 Effect of amount of beta-lactamase on the post-beta-lactamase inhibitor effect of clavulanic acid against isogenic *Escherichia coli* strains producing SHV extended-spectrum beta-lactamases

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Objectives: Post-beta-lactamase inhibitor effect (PLIE) is a phenomenon which enables beta-lactam/inhibitor combinations to remain active *in vivo* throughout the interval between two consecutive doses, even when inhibitor serum concentrations decrease to values below those active *in vitro*. The aim of this investigation was

to determine the effect of increased amount of SHV-extended-spectrum beta-lactamases (ESBLs) on the duration of the postantibiotic effect (PAE) and post-beta-lactamase-inhibitor effect (PLIE).
Methods: The experiments were performed on isogenic *Escherichia coli* strains with strong and weak promoters which produce SHV-2, SHV-3 and SHV-5 beta-lactamases. The strains with weak promoter produced low amount of beta-lactamase (mpb strains) while strains with strong promoter hyperproduce the enzyme (mpa strains). The minimum inhibitory concentrations (MICs) of beta-lactams alone and combined with clavulanic acid were determined by broth microdilution method. PAE and PLIE were induced by exposing the cultures to cefotaxime (8 mg/L) and clavulanic acid (4 mg/L) for 2 h. For PLIE determination clavulanic acid was removed by dilution and the cultures were re-exposed only to cefotaxime. The determination of PAE and PLIE was performed by viable counting method. PAE was calculated as $PAE = T - C$, where T is the time necessary for viable bacteria in the test culture to grow by 1 $\log_{10}$ and C is time necessary for viable bacteria in the control culture to grow by 1 $\log_{10}$. PLIE was calculated as PLIE = total delay – (DG + PAE), where DG is delay of growth due to experimental conditions.
Results: The PLIE lasted 1.4 h for the SHV-2 mpb strain, 2.2 h with SHV-3 -mpb strain and 0.9 h for SHV-5 mpb strain. The strains with strong promoter had shorter duration of PLIE. It ranged from 0.3 h for SHV-3 mpa strain to 0.8 h for SHV-2 mpa strain. No PLIE was detected for SHV-5-mpa strain. The PAE was also of shorter duration in the strains which hyperproduce beta-lactamase. It was not observed with SHV-3-mpa and SHV-5-mpa strains. Mpb strains with lower amount of beta-lactamase had duration of PAE, which ranged from 0.62 to 1.4 h.
Conclusions: The hyperproduction of SHV-ESBLs shortened the duration of PAE and PLIE in isogenic *E. coli* strains. This phenomenon could be responsible for the resistance of some SHV-ESBL-producing Enterobacteriaceae with high-level enzyme production to beta-lactam/inhibitor combinations and for *in vivo* therapeutic failures of such combinations.

R1965 Effect of linezolid on nitric oxide production by lipopolysaccharide stimulation

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Objectives: Low-dose and long-term administration of linezolid into patients with airway inflammatory diseases could favourably modify their clinical conditions. However, the therapeutic mode of action of linezolid is not well understood. Free oxygen radicals, including nitric oxide (NO), are well recognised as the important final effector molecules in the development and the maintenance of inflammatory diseases. The aim of this study was to investigate the influence of linezolide on NO generation *in vivo*.
Methods: Male ICR mice, 5 weeks of age, were orally administered with linezolid once a day for 2–4 weeks. The mice were then injected intraperitoneally with 5.0 mg/kg lipopolysaccharide (LPS) and the plasma NO level was examined 6 h later.
Results: Although pretreatment of mice with linezolid for 2 weeks scarcely affected NO generation by LPS injection, the administration of linezolid for 4 weeks significantly inhibited LPS-induced NO generation. The data in the present study also showed that pretreatment of mice with linezolid for 4 weeks significantly suppresses not only production of pro-inflammatory cytokines interleukin-1beta, interleukin-6, and tumour necrosis factor-alpha, but also inducible nitric oxide synthase mRNA expressions, which are enhanced by LPS injection.
Conclusion: These results strongly suggest that suppressive activity of linezolid on NO generation in response to LPS stimulation *in vivo* may, in part, account for the clinical efficacy of linezolid on chronic inflammatory diseases.

Epidemiology of resistance, antibiotic usage

R1966 *In vitro* susceptibility to antibiotics of *Escherichia coli* isolates in community-acquired urinary-tract infections in 1998–2003

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Objective: To determine the change in antimicrobial susceptibility of *E. coli* isolates during 1998–2003 in community-acquired urinary-tract infections (CA-UTIs) and to suggest currently empirical antibiotic therapy in these patients.
Methods: During January 1998–October 2003, a total of 880 *E. coli* isolates causing CA-UTIs were included this study. Isolation and identification were performed by standard method and susceptibility testing was performed by disc-diffusion method. Susceptibility was tested ampicillin (AMP), amoxicillin–clavulanate (AMC), trimethoprim–sulphamethoxazole (SXT), ciprofloxacin (CIP), gentamicin (CN), cefuroxime (CXM) and nitrofurantoin (F).
Results: The prevalence of resistance among *E. coli* was >50.0% for ampicillin and 40% trimethoprim–sulphamethoxazole for in each year studied. The susceptibility pattern of *E. coli* to nitrofurantoin (5.4, 6.3, 7.2, 5.3, 8.4 and 8.8%), cefuroxime (5.9, 6.9, 7.2, 6.2, 11.3 and 7.5%) and amoxicillin–clavulanate (18.4, 21.5, 25.0, 29.2, 23.9, 11.2%) did not present significant differences in all six years periods. There was a significant increase in *E. coli* susceptibility to ciprofloxacin (14.7, 11.3, 18.5, 25.6, 26.7 and 24.8%) and gentamicin (7.0, 13.9, 15.3, 25.6, 23.2 and 13.8%).
Conclusion: The best *in vitro* susceptibility was shown for cefuroxime and nitrofurantoin, followed by gentamicin, ciprofloxacin and amoxicillin-clavulanate. Strains of *E. coli* show high resistance rate to ampicillin and trimethoprim–sulphamethoxazole. Empirical initial treatment with ampicillin and trimethoprim-sulfamethoxazole is inadequate in approximately one-third of UTI cases in our region. A large number of pathogens can safely be treated with nitroforantoin, cefuroxime and gentamicin and may be empirically treated with amoxicillin–clavulanate and ciprofloxacin.

R1967 Vancomycin intermediate staphylococci in neutropenic patients

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Ankara, TR

Objectives: Since its discovery, reduced glycopeptide susceptibility of *Staphylococcus* species is a growing problem. The aim of this study was to determine the prevalance of vancomycin intermediate *S. aureus* and coagulase-negative (CNS) *Staphylococcus* species isolated from neutropenic patients in Gazi University Hospital.
Methods: A total of 64 CNS and 37 *S. aureus* isolates were agar screened with plates containing 4 μg/mL of vancomycin. If no growth was detected in the end of 48 h of incubation, the strain was considered susceptible. Confluent growers were designated as possible vancomycin intermediate strains. Population analysis was performed for the stains with a countable number (1–30) of colonies in screening plates, to confirm heterogenous vancomycin intermediate susceptibility. In population analysis, criss-cross serial dilutions of vancomycin and bacterial suspensions were tested to determine the frequency of vancomycin intermediate cells in the culture.

Results: Five of 64 CNS strains were found possible hetero-vancomycin intermediate in screening plates. All five strains was confirmed to harbour more than $1/10^6$ vancomycin intermediate cells, thus identified as hetero-vancomycin intermediate. All of the *S. aureus* strains were found susceptible to vancomycin. No confluent growers were detected in screening plates.
Conclusion: This preliminary report indicates that the rise of vancomycin intermediate CNS is an imminent danger.

R1968 Quantity and quality of antibiotic usage in hospitals and in the community in two areas in Indonesia

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Objectives: To gather data on antimicrobial use on different levels of health care in order to develop a reliable method to measure antibiotic use in a developing country.
Methods: Prospective audit in two cities. The quantity and quality-of-use was studied in patients admitted to hospital (group I), patients discharged from hospital after >5 days (group II), patients consulting a health centre (puskesmas) (group III), and household contacts of the patients admitted to hospital (group IV). Patients were included during 4 months in 2001 in Surabaya (SBY) and 2002 in Semarang (SMG). The quantity of antibiotic usage in the community (groups I–III and IV) was assessed by an inquiry made at inclusion, for hospitalised patients (group II) it was recorded from medical records on discharge. This method was validated in a sample by the parallel prospective recording of antibiotic use completed with interviews of nurses and patients. Quality-of-use was analysed in a random selection of hospitalised patients by an audit (Gyssens *et al*, *J. Antimicrob. Chemother*. 1992; **30**, 724–727). Groups of three reviewers assessed indication, choice, dosage, route of administration and duration of treatment.
Results: A total of 3996 individuals, equally distributed over the groups, were enrolled. In SBY, 20% of the patients on admission had used an antibiotic in the last month, 20% of the patients in the puskesmas and 6% of the family members. In SMG this amounted to 23, 14 and 6%, respectively. Total consumption of antibiotic use of adult outpatients was 17.1 and 18.2 DDD/1000 person-days. Both in the community and in hospitals, penicillins were most frequently used. Of all hospitalised patients, 83% were prescribed at least one antibiotic. In SBY, 90.4% of patients in surgery were prescribed antibiotics. At least 48% of these patients were prescribed prophylaxis, and possibly more, since 16% were prescribed for an unknown indication. Total antibiotic use in adult hospitalised patients was 57.8 and 31.8 DDD/100 patient days in SBY and SMG respectively. Results of the validation in SBY showed that actual consumption was about 30% higher. Although the reviewers had a widely different opinion on the quality of individual prescriptions, they judged antibiotics not indicated in 10–80% in SBY and 19–76% in SMG.
Conclusions: In hospitalised patients, high consumption and inappropriate use of antibiotics were prevalent. The study is continued with interventions.

R1969 Adherence to surgical antibiotic prophylaxis guidelines in Italy

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Objective: Aim of the study was to evaluate the adherence to guidelines of antibiotic prophylaxis (AP) in surgery. The study was carried out contemporarily at the publication of the national guidelines on antibiotic surgical prophylaxis (NGAP).
Materials and methods: We carried out a retrospective analysis of AP in consecutive patients discharged from surgical units in five hospitals in Lombardy, Italy. The following data were obtained from the clinical chart: age, sex, duration of preintervention hospitalisation, type of intervention, level of surgical wound contamination, urgent surgery, type and duration of antibiotic prophylaxis, and adherence to local guidelines.
Results: We analysed 526 surgical procedures, performed in 21 U. 18 U (86%) had written local guidelines. 275 patients were males (52%), and had a mean age of 56 + 22 years. Sixty-four procedures were urgent (12%) and in 77 cases (15%) prosthetic material was implanted. Most interventions, 422 (80.2%), were clean, 49 were clean-contaminated (9.6%) and 55 were contaminated/infected. The AP was not performed in 93 cases. First generation cephalosporins were used in 174 patients, second generation in 42, and third generation in 45. Protected penicillins were utilised in 67 cases, and a combination two to three of antibiotics in 64 interventions, while glycopeptide were used as single agents in four cases, and other antibiotics in 27. The mean duration of AP was 4 + 5 doses, or 2 + 3 days, while the median duration was two doses, or 1 day. The AP was correct in 254 cases (48%). In 79 surgical procedures (15%) the AP was started the day before the intervention. Significant differences in adherence were identified among the wards, varying from 3 to 91%. The duration of the prophylaxis was shorter in the wards with written guidelines as compared with those without: 3 + 4 doses (2 + 3 days) and 10 + 8 doses (4 + 3 days) respectively ($P < 0.001$). Wide variations were noted among different wards in the same hospital.
Conclusions: This study, implemented to evaluate the state-of-the-art in antibiotic prophylaxis in some Italian hospitals when the national guidelines (NG) were published, shows wide variations, with adherence rate extremely low in some wards. This indicates a possibility of improvement both on duration and on the choice of the drug. This work may be useful to evaluate which will be the impact of the NGAP.

R1970 *Acinetobacter* spp. bloodstream infections over a 9-year period at an inner city hospital in the USA

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Objectives: The emergence of multi-drug resistant *Acinetobacter* has become a major therapeutic dilemma for many institutions worldwide. We sought to evaluate the trends of *Acinetobacter* infections, including the susceptibility patterns to different antimicrobial agents over a 9-year period at our institution.
Methods: Nonduplicate bacterial isolates from the University of Illinois Medical Center from May 1994 to December 2002 were entered into a relational database [Microsoft Access(R)]. Further data collected included: culture species, date, susceptibility, and patient location.
Results: A total of 89 unique *Acinetobacter* spp. bloodstream infections (ABSI) were identified from approximately 8500 blood isolates. Since the year 2000, we have seen an increase ABSI at our institution. Overall, 83% (74 of 89) were *A. baumannii* and 17% (15 of 89) were *A. lwoffii*. The incidence of ABSIs was slightly higher in the intensive care units (ICU) (55%) than the medicine wards (44%). Most of ABSI were found in the medical ICU

Table 1 Per cent of *Acinetobacter* spp. susceptible to various antimicrobials.

Year	1999	2000	2001	2002
Ceftazidime	100%	40%	29%	31%
Pipercillin	100%	20%	29%	31%
Imipenem	100%	100%	57%	80%
Gentamicin	67%	33%	24%	25%
Levofloxacin	67%	33%	18%	41%

(29%). 18% of ABSI were found in paediatric patients. Between 1999 and 2002, there was a decreasing trend of susceptibilities to gentamicin, levofloxacin, imipenem, pipercillin and ceftazidime.
Conclusions: Overall, there is an increased number of ABSI at our institutions and these were not limited to ICU patients. In the past 3 years, the *Acinetobacter* spp. have had decreasing susceptibilities to most antimicrobials at our institution which is concerning.

R1971 Antimicrobial resistance in *Streptococcus pyogenes* isolates in Moscow, Russia from 2002 to 2003

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Objectives: *Streptococcus pyogenes* (SPY) is responsible for various acute supportive conditions, including the tonsillitis/pharyngitis in children and skin and soft tissue infections. In the last years the resistance rates of SPY have been rising steadily. The aim of this study was the evaluation of the current prevalence of antimicrobial resistance in Moscow.
Methods: Local microbiology laboratories in Moscow collected SPY isolate from January 2002 to October 2003. The isolates were recovered from throat swabs and pus samples. Identification was performed by routine laboratory techniques including latex agglutination test. MICs were determined by the standard microdilution procedure and interpreted according to NCCLS criteria. The following antimicrobial drugs were tested: penicillin G (PEN), erythromycin (ERY), clindamycin (CLI), tetracycline (TET) and levofloxacin (LEV).
Results: A total of 192 SPY isolates were obtained in 2002 and 151 in 2003. Each of the 343 isolates was susceptible to penicillin. ERY resistance rates in 2002 and 2003 were 7 and 3%, respectively (MICs range 1–16 mg/L). Among 19 ERY-resistant isolates only one demonstrated CLI MIC = 1 mg/L. ERY-susceptible isolates were susceptible to CLI. Tetracycline resistance rates in 2002 and 2003 were 44 and 47%, respectively. TET resistance was detected in 18 of 19 ERY-resistant isolates. Decreased levofloxacin susceptibility was found in one isolate (MIC =4 mg/L).
Conclusions: The M-phenotype is predominant among macrolide-resistant SPY isolates in Moscow. Considering the steady rise of resistance to macrolides and other antibiotics, continuous surveillance of this development as the basis for the formulation of optimal treatment regimens for SPY infections, is clearly warranted.

R1972 Antimicrobial resistance trends among *Escherichia coli* and *Klebsiella* spp. from ICU patients: report from MYSTIC Programme (1997–2003) in a Russian centre

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Objective: *Escherichia coli* (EC) and *Klebsiella* spp. (KL) are the main producers of ESBL and the highest ESBL rates were found in ICU patients in Eastern Europe. Here we present the data on resistance patterns of EC and KL, including possible ESBL-producers, from Russian centre participating in MYSTIC Programme.
Methods: From 26 to 43 consecutive, nonrepetitive, clinically significant isolates of EC and KL from ICU patients with hospital-acquired infections were collected prospectively each year from 1997 to 2003. Identification of isolates was performed by routine laboratory techniques; MICs of meropenem, imipenem, ceftazidime, cefepime piperacillin/tazobactam, ciprofloxacin and gentamicin were determined by the standard microdilution procedure and interpreted according to NCCLS criteria. Ceftazidime/clavulanate combination was used for phenotypic confirmation of possible ESBL production according to NCCLS recommendations.
Results: During the study period the prevalence of ESBL producers increased among EC isolates from 20 to 40%. Among KL isolates ESBL production varied from 40 to 70% in different years. ESBL production was strongly associated with gentamicin and ciprofloxacin resistance. No carbapenem resistance was detected among available isolates. Cefepime demonstrated significantly lower MICs against ESBL producers in comparison with ceftazidime. High-level piperacillin/tazobactam resistance varied between 10 and 20%, but intermediate resistance exceeded 40%.
Conclusions: Carbapenems are valuable options in the treatment of EC and KL hospital-acquired infections in ICUs with high prevalence of ESBL production.

R1973 Changes in antibiotic resistance of the most frequent Gram-negative bacteria isolated in intensive care units

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Objectives: The aim of our study was to observe the changes in antibiotic resistance of the most frequent Gram(−) bacteria isolated in the intensive care units (ICU) of AHEPA hospital, Thessaloniki, Greece.
Methods: The material of our study were Gram(−) bacteria isolated from the culture of every pathological specimen which was sent to our laboratory during the years 2000 and 2002. The identification of bacterial isolates and their antibiotic susceptibilities were performed by VITEK 60 analyzer of Bio-Merieux. The most frequent bacteria isolated were: (i) *Pseudomonas aeruginosa* 132 strains during 2000 and 106 during 2002 (ii) *Acinetobacter calcoaceticus* 98 strains during 2000 and 109 during 2002 (iii) *Klebsiella pneumoniae* 53 strains during 2000 and 83 during 2002.
Results : The phenotypical antibiotic resistance of the strains isolated was as follows: *Pseudomonas aeruginosa* to Amikacin (ÁÍ) was 15 and 60% during 2000 and 2002 respectively, to Cefepime (CFP) 45 and 65%, to Ceftazidime (CFZ) 35 and 35%, to Ciprofloxacin (CIP) 40 and 65%, to Imipenem (IMP) 50 and 65%, to Tobramycin (TO) 30 and 60%, to Ticarcillin/CA (T/CA) 50 and 70%, to Piperacillin/Tazobactam (P/T) 10 and 30%. *Acinetobacter calcoaceticus* to AN was 93 and 95% respectively, to CFP 90 and 92%, to CFZ 97 and 99%, to CIP 91 and 95%, to IMP 15 and 67%, to TO 88 and 98%, to T/CA 68 and 70%, to P/T 41 and 72%. *Klebsiella pneumoniae* was to AN 10 and 50%, to CFP 20 and 20%, to CFZ 80 and 90%, to CIP 45 and 50%, to IMP 0 and 0%, to TO 80 and 90%, to T/CA 75 and 95%, to P/T 65 and 90%, respectively.
Conclusion: (i) Acinetobacters have the highest resistance percentage; (ii) a significant increase in resistance was observed in Imipenem and Piperacillin/ Tazobactam of *Acinetobacter* (52 and 31%, respectively). No other changes in resistance of *Acinetobacter* was noticed to the rest antibiotics. (iii) *Pseudomonas aeruginosa* presents a significant increase in resistance to all antibiotics, except ceftazidime. (iv) *Klebsiella pneumoniae* has no resistance to Imipenem but a great increase of its resistance is observed to Amikacin, ceftazidime, tobramycin and b-lactamase inhibitors.

R1974 Analysis of bacteriological results of protected telescopic catheter in ICU patients with ventilator-associated pneumonia

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Objective: The aim of the study was to evaluate the results (identification and antibiotic susceptibility patterns) of the **protected telescopic catheter** (PTCs) performed in ICU patients with the clinical suspicion of VAP.
Methods: During the years 2000–2003, we evaluated 132 positives of a total of 233 samples performed in 233 ICU patients with the clinical suspicion of VAP. Cultures were considered as positives

when bacteria were discovered in at least 1000 CFU/mL. Identification and antibiogram were performed by the Wider Automatic System according to NCCLS guidelines. Duplicates were excluded from the analysis.
Results: The most frequent isolated microorganisms were: *A. baumannii* in 24.2% of the specimens, *P. aeruginosa* in 22%, *K. pneumoniae* in 11.4%, *E. coli* in 6.8%, MRSA in 6.8% patients. In 23 samples, two types of bacteria were isolated, while four others were recorded as contaminates (more than two types of bacteria). Resistance to Imipenem was found in 3.1% of the *A. baumannii* strains and in 27.6% of *P. aeruginosa* strains. Resistance to Meropenem was recorded in 21.9% of *A. baumannii*, 27.6% of *P. aeruginosa* and in 6.7% of *K. pneumoniae* stains. Resistance to Ciprofloxacin was almost exclusively discovered in *A. baumannii* isolates (85%) and less frequently in *P. aeruginosa* and *K. pneumoniae* strains (20.7 and 6.7%, respectively). Additionally, resistance to third generation cephalosporins was recorded in 87.5% of the *A. baumannii* strains, in 55.2% of *P. aeruginosa*, in 46.7% of *K. pneumoniae* and in 22.2% of *E. coli*. Piperacillin/Tazobactam resistance was found in 46.9% of the *A. baumannii* strains, in 17.2% of *P. aeruginosa* and in 20% of *K. pneumoniae*. All MRSA isolates were sensible to Vancomycin and Teicoplanin.
Conclusion: Multiresistant Gram-negative bacteria was the most frequent aetiologic agent in ICU respiratory infections. However, MRSA infections were much less frequent, probably because of the application of a multidisciplinary programme concerning the early detection of MRSA in the ICU patients (upon admission) and hygiene measures against MRSA cross-infections. A prospective study aimed to identify epidemic strains of *A. baumannii* and *P. aeruginosa* is ongoing in our hospital.

R1975 Characterisation of *Haemophilus influenzae* strains isolated from noninvasive and invasive infections in children

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Aims: The aims of the study were to determine the susceptibility, biotype and capsular serotype of *H. influenzae* isolates obtained from children with invasive and noninvasive infections, treated in Children's Memorial Health Institute, Warsaw, Poland in 2003.
Materials and methods: Altogether 371 *H. influenzae* strains were collected from respiratory tract infections, meningitis and intra-abdominal infections between January and December 2003. Susceptibility testing was performed by Etests (AB Biodisk). β-lactamase production was identified by use of nitrocefin disk (BBL DrySlide Nitrocefin; Becton Dickinson). Biotype was determined by use of Api NH (Biomerieux), and serotype – by agglutination tests (*Haemophilus influenzae* Agglutinating sera, Abbott).
Results: Eighty-eight per cent of isolates were β-lactamase-negative and sensitive to ampicillin. One β-lactamase-negative ampicillin-resistant (BLNAR) strain (Hib) was isolated from ovarian fluid. The strain was also resistant to amoxicillin/clavulanate (MIC > 256 mg/L), cefuroxime (MIC > 256 mg/L), cefotaxime (MIC > 256 mg/L), and imipenem (MIC > 32 mg/L); however it was susceptible to azithromycin (MIC = 1 mg/L), co-trimoxazole (MIC = 0.064 mg/L), ciprofloxacin (MIC = 0.008 mg/L) and chloramphenicol (MIC = 0.5 mg/L). All the remaining isolates were susceptible to amoxicillin/clavulanate and cefuroxime. Overall susceptibility to cefaclor, tetracycline, co-trimoxazole and chloramphenicol was 98, 97, 60 and 100%, respectively. Over 99% of isolates were sensitive to azithromycin and 97% – to clarithromycin. All invasive infections were caused by strains of type b, biotype I.
Conclusions: The study shows relatively high prevalence of β-lactamase production in *H. influenzae* strains obtained from our children. Most β-lactamase-positive, antibiotic-resistant strains were isolated from children with chronic diseases such as cystic fibrosis, bronchiectasis and asthma, frequently exposed to antibiotics.

R1976 Microbiological assessment of bacterial septic patients receiving monotherapy with cefoperazone/sulbactam

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Objective: To evaluate source of infection, main pathogens and microbiological activity towards prevalent species in septic patients receiving monotherapy with cefoperazone/sulbactam.
Methods: Samples from septic patients were collected from 18 study sites in 10 Russian cities. Sepsis was diagnosed according to ACCP Consensus Conference, 1992. Collection was done in patients who were assigned monotherapy with cefoperazone/sulbactam (1:1) 4–8 g every 8 h. Antimicrobial susceptibility testing of the Enterobacteriaceae, *Pseudomonas aeruginosa* and Gram-negative nonfermenters was performed in a reference laboratory by broth microdilution with data interpretation according to NCCLS standards (2003). NCCLS recommendations for cefoperazone were applied through absence of susceptibility testing criteria for cefoperazone/sulbactam.
Results: Aetiology was confirmed in 73 patients of 127 enrolled in the study and 119 strains were evaluated. Clinical specimens included: wound discharge – 65 (54.6%), abdominal cavity pus – 25 (21.0%), blood – 13 (10.9%), tracheal aspirate – 5 (4.2%), urine 4 (3.4%), sputum – 4 (3.4%) and pleural cavity drainage – 3 (2.5%). Predominant bacteria were Enterobacteriaceae – 44 (37.0%), *P. aeruginosa* – 22 (18.5%) and *Enterococcus* spp. – 22 (18.5%). Less prevalent were *Staphylococcus aureus* – 19 (16.0%) and Gram-negative nonfermentative bacteria – 12 (10.0%). Cefoperazone/sulbactam showed superior activity against Enterobacteriaceae in comparison to cefoperazone ($P < 0.004$), ceftazidime ($P < 0.017$), cefepime ($P < 0.002$) and similar activity as piperacillin/tazobactam ($P = 0.546$), imipenem ($P = 0.514$), and ciprofloxacin ($P = 0.792$). To cefoperazone 50.0% strains of *P. aeruginosa* and 75.0% of Gram-negative nonfermentative species were resistant. Cefoperazone/sulbactam showed no advantages in activity *in vitro* against latter two groups of pathogens in comparison with cefoperazone alone.
Conclusions: The main pathogens causing sepsis in the studied cohort were Enterobacteriaceae, followed by *P. aeruginosa* and *Enterococcus* spp. Cefoperazone/sulbactam showed favorable *in vitro* activity against majority of pathogens and may be considered as a potential drug of choice for empiric therapy of sepsis. However, further multicentre studies are needed in order to confirm its utility for this indication.

R1977 Antimicrobial resistance in *E. coli* isolates in a large German community-based hospital 1998–2003 – trends correlated with antimicrobial use

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Objectives: *Escherichia coli* is the most commonly found Gram-negative pathogen in humans. It causes a broad range of infectious diseases as sepsis, urinary tract infections, enterogenic and cholegenic infections and pneumonia and sometimes meningitis. Especially in the case of life-threatening infections, empirical therapy is mandatory. For adequate treatment monitoring of antimicrobial resistance is necessary.
Methods: The resistance against fluoroquinolones, aminopenicillines, third-generation cephalosporins and cotrimoxazole in a large community-based German hospital from 1998 to 2003 was evaluated with respect to antimicrobial use. The data for the whole hospital (HOSP) were compared with those an internal medicine department (DIM) caring for a huge number of geriatric patients.
Results: From 1998 to November 2003 10056 *E. coli* strains ($n = 3408$, DIM) were isolated which comprised 23% of all pathogens (DIM: 25%) and tested against levofloxacin (MIC breakpoint 1 mg/L), ampicillin (4 mg/L), cefotaxime (4 mg/L), and cotrimoxazole (32 mg/L) using the Vitek 2 automated bacteriology system. During the observation period antimicrobial

resistance increased against fluoroquinolones (HOSP: 8–16%, DIM: 9–20%), aminopenicillines (HOSP: 39–56%, DIM: 40–61%) and cotrimoxazole (HOSP: 19–27%, DIM: 20–31%) significantly, while nearly all strains were fully susceptible against third-generation cephalosporins (HOSP: 0–1%, DIM: 0–1%). Trends for fluoroquinolones correlated well with the use of group 3 and four which had doubled in the last two observation years where the main increase in resistant strains (9–16% HOSP, 9–20% DIM) occurred. In all other substances tested resistance increased continuously over the period. Invasive (blood culture) isolates had a significantly higher fluoroquinolone resistance (HOSP: 23% in 2003, DIM: 38% in 2003) than other isolates. Strains isolated in 2003 had a significant higher resistance rate compared those strains from published German and European data.

Conclusions: Extent of antimicrobial resistance depends often more on local conditions and specific treatment behaviours than on the nation-wide situation. Therefore, treatment has to rely on local data.

R1978 Monitoring of extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella* sp. at secondary and tertiary care hospitals in central Switzerland

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Objectives: In conferring resistance to all cephalosporins (except cefamycins), penicillins, and monobactams extended spectrum β-lactamase (ESBL) producers represent a serious threat to hospitalised patients.

Methods: During two 4-months periods (September to December 2002; August to November 2003) 1096 strains of *E. coli* and 292 strains of *Klebsiella* sp. have been isolated from clinical specimens (*E. coli*: $n = 522$ in 2002, $n = 574$ in 2003; *Klebsiella* sp.: $n = 131$ in 2002/ $n = 161$ in 2003). Patients were hospitalised at a tertiary care center (Kantonsspital Luzern) and at three secondary care hospitals (Schwyz, Sursee, Wolhusen). In 2002, consecutive screening for ESBLs has been performed by the Kirby-Bauer disc-diffusion method on a separate plate, testing ceftazidime (30 μg), cefpodoxime (10 μg), and ceftriaxone (30 μg) according to the National Committee for Clinical Laboratory Standards (NCCLS; M2-A8 and M100-S13). In 2003, screening for ESBLs has been performed by the same method, but placing the disks containing ceftazidime (30 μg) and ceftriaxone (30 μg) adjacent to one containing amoxicillin-clavulanic acid (20/10 μg) at a distance of 23 mm. The plates were checked for inhibition zones and phantom zones. Suspected presence of ESBLs have eventually been confirmed by applying Etest (R); strips containing ceftazidime and cefotaxime, respectively, with and without clavulanic acid.

Results: In the first period, six ESBL producers have been detected in five clinically unrelated specimens, while in the second, there were six ESBL producers in four clinically unrelated specimens. In 2002, one specimen and in 2003, two specimens contained both *K. pneumoniae* and *E. coli*. All isolates originated from the tertiary care hospital ($n = 1020$). Prevalence of ESBLs ranged from 0.92% in 2002 to 0.81% in 2003 [($P = 0.94$; *E. coli* 0.96% in 2002, 0.35% in 2003 ($P = 0.38$); *Klebsiella* sp. 0.77% in 2002 and 2.40% in 2003 ($P = 0.52$)].

Conclusions: (i) Compared with our in-house data from 2001 the isolation rate of ESBL producers at the four institutions has increased over the past 2 years, due to improved screening procedures. (ii) Prevalence of ESBL producing organisms in our area is similar to that observed in other areas of Switzerland. (iii) Based on detection algorithms other than the phenotypic screening method recommended by the NCCLS (automated systems such as VITEK2), the number of ESBL producers may be underestimated, in particular, in areas with a low prevalence.

R1979 Antimicrobial resistance in high complexity hospitals in Bogotá, Colombia, 2001–2002

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Background: Resistance to antimicrobials has increased during the last decade around the world. Few data on resistance trends in Colombia has been published.

Objective: To establish a surveillance programme of antimicrobial resistance in high-complexity hospitals in Bogotá (Colombia, South America), evaluating the trend in 2001–2002.

Methods: A cross-sectional study was performed of microbial isolates obtained in microbiology laboratories from 13 high-complexity hospitals in Bogotá. Isolate identification and antimicrobial susceptibility testing were performed using standard manual and automated systems and NCCLS criteria. Microbiological data from 2001 and 2002 was transferred into WHONET (ver. 5.2, World Health Organisation).

Results: Between 2001 and 2002, 65 520 microorganisms were isolated. *E. coli* was the most frequent isolate (13 162). According to NCCLS criteria, 15% of *E. coli* isolates in intensive care units (ICU) were suspected of extended spectrum β-lactamase (ESBL) production, and 12% of those found in non-ICU wards. 43% of *K. pneumoniae* isolates in ICU and 32% of those in non-ICU wards were suspected of ESBL production. 13% of *K. pneumoniae* isolates were resistant to ciprofloxacin. Methicillin-resistant *S. aureus* (MRSA) was more frequently found in the UCI (71%) than in other wards (47%). Isolates of *P. aeruginosa* resistant to imipenem and ciprofloxacin were 43 and 69% of isolates in ICU, and 27 and 46% of those in no-ICU wards, respectively.

Conclusions: In hospitals of high complexity in Bogotá, there is growing number of resistant isolates. MRSA, ESBL-producing *K. pneumoniae* and multiresistant *P. aeruginosa* are the most important and serious microorganims related to this problem. The data presented here is the base for the formulation of policies in infection control for high complexity institutions in the country.

R1980 Antimicrobial susceptibility patterns of Gram-negative bacteria in Swedish intensive care units involved in the MYSTIC study (2001–2003)

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Objective: To investigate the antibiotic susceptibility amongst Gram-negative isolates in Swedish intensive care units (ICUs).

Methods: Four Swedish hospitals participating in the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme collected 1178 initial isolates of Gram-negative bacteria during 2001–2003. Minimal inhibitory concentration (MIC) of ceftazidime (CTZ), ciprofloxacin (CIP), tobramycin (TOB), imipenem (IPM), meropenem (MEM) and piperacillin/tazobactam (PTZ) were determined. The breakpoints of the Swedish Reference Group for Antibiotics (SRGA) were used.

Results: The most commonly isolated bacteria were *Escherichia coli* (24%), *Pseudomonas aeruginosa* (17%), *Enterobacter aerogenes* (10%), *Klebsiella pneumoniae* (10%) and *Proteus mirabilis* (6%). The number of antibiotics to which >90% of isolates were susceptible was one for *P. aeruginosa* (TOB), two for *P. mirabilis* (CTZ, PTZ) and *E. aerogenes* (TOB, MEM/IMP) and four for *E. coli*/*K. pneumoniae*/*K. oxytoca* (TOB, MEM/IPM, CTZ, PTZ). MEM was more active than IMP against *P. mirabilis* and *P. aeruginosa*. A low frequency of ESBLs amongst *E. coli* (1.1%) and *K. pneumoniae* (1.7%) was seen. No such isolates of *P. mirabilis* were found.

Conclusions: CIP showed lowest activity of the agents tested for the most frequently isolated species highlighting the escalating problem with quinolone resistance. The overall susceptibility based on SRGA breakpoints for the different antibiotics were CTZ (93%), MEM (92%), TOB (92%), IMP (90%), PTZ (90%) and CIP (82%).

R1981 *Streptococcus pneumoniae* infections in a general hospital: characteristics and resistance profiles

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Marbella, E

Objectives: To know the characteristics of the patient with *S. pneumoniae* infection and the antibiotic susceptibility.
Methods: We have studied the retrospective cases of *S. pneumoniae* infections with isolated in sputum, blood, pleural effusion and cerebrospinal fluid in our hospital from April 2002 to December 2003. Sputum were considered valid only with less than five cells and more than 25 leucocytes
Results: We found 57 patients, 39 males (68%) and 12 females (21%). Mean-age was 63 years. 15 were suffering from chronic bronchitis or airway obstruction. In this group 73% had other chronic illness. Seven had HIV infection and in this group 57% had other chronic illness. Other 14 patients (24%) had a chronic illness. Eleven had not pathological conditions. Isolated were 56% from sputum, 33% blood cultures, 7% cerebrospinal fluid and 4% pleural effusion. Diagnosis were 23 pneumonia, two empyema, 18 respiratory infection without pneumonia, seven with sepsis criteria (respiratory origin), four meningitis and one peritonitis. The susceptibility (S) was defined by IMC < 1, intermediate susceptibility (I) IMC: 1–2, resistance (R) IMC > 2. The patters of susceptibility were: penicillin 67% S and 3% R, clavulanic-amoxicilin 96% S, cefotaxime 100% S, eritromicin 75% S and 25% R, clindamicin 77% S and 23% R, cloramfenicol 98% S and vancomicin 100% S
Conclusions: In our centre the neumococal infection profile is a patient with a chronic health problem suffering from a respiratory tract infections (pneumonia or infection without pneumonia) originated by a *S. pneumoniae* susceptible to be treated with high doses of penicillin, clavulanic–amoxicilin, cefotaxime, cloramfenicol and vancomicin.

R1982 *In vitro* activity of telithromycin against 1891 *S. pneumoniae* isolated in Europe, Africa, Middle East and Asia (e-BASKETT 2002–2003)

H.B. Drugeon, M.E. Juvin for the e-Baskett 2 Study Group

Objective: E-BASKETT is an international prospective survey (23 countries) initiated in 2001 to identify trends in the development of antibacterial resistance to *Streptococcus pneumoniae* isolated from community-acquired respiratory tract infections and to determine the activity of a new ketolide, telithromycin (TEL). From the first year, marked geographical differences were observed in the prevalence of penicillin (PEN) and macrolide resistance. Overall, TEL was highly active in the different geographical areas studied. We present here the results from the second year.
Methods: From September 2002 to June 2003, 1891 clinical isolates of *S. pneumoniae*, collected from 23 countries, were tested at a central laboratory. PEN, erythromycin (ERY) and TEL MICs were determined by broth dilution method following NCCLS guidelines.
Results: The prevalence of PEN (PEN-I, MICs: 0.125–1 mg/L, PEN-R, MICs $\geq$ 2 mg/L), ERY (MICs $\geq$ 1 mg/L), and TEL (MICs $\geq$ 4 mg/L) resistance was shown in the table.

Overall, 99.8% of all isolates of *S. pneumoniae* were inhibited by TEL MIC $\leq$ 1 mg/L.
Conclusion: (i) E-BASKETT 2 confirmed large differences in rates of PEN and ERY resistance among *S. pneumoniae* isolates in different countries. An extremely high incidence of PEN and ERY resistance was observed in some parts of Asia with no discernible trend up or down compared with resistance rates reported in E-BASKETT 1. (ii) TEL was highly active against *S. pneumoniae* clinical isolates including PEN- and ERY-resistant strains. No resistance to TEL was reported.

Countries (*n*)	PEN-I	PEN-R	ERY-R	TEL-R
Greece (107)	12	48	47	0
Netherlands (64)	2	0	5	0
Spain (153)	21	15	34	0
Switzerland (70)	9	3	7	0
Hungary (103)	36	24	43	0
Poland (96)	6	8	8	0
Russis (85)	5	1	6	0
Slovaquia (49)	24	26	33	0
Slovenia (97)	9	7	4	0
Turkey (260)	23	11	17	0
Egypt (14)	36	21	21	0
Emirates (56)	31	14	25	13
Saudi arabia (119)	41	22	30	0
Tunisia (5)	20	40	40	0
Ivory Coast (50)	24	0	0	0
Senegal (5)	0	0	0	0
Hong-Kong (89)	20	35	52	0
Korea (50)	18	64	92	0
Malaysia (10)	20	10	10	0
Pakistan (50)	24	0	24	0
Singapore (20)	20	20	45	0
Taiwan (308)	20	50	90	0
Thailand (31)	23	29	45	0

R1983 Increasing antimicrobial resistance of *Pseudomonas aeruginosa* in an intensive care unit in Italy

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Verona, I

Objective: To evaluate the patterns of antimicrobial resistance (AMR) of *Pseudomonas aeruginosa* strains isolated in patients admitted in an intensive care unit (ICU) during 2 years of study.
Methods: During two different periods (period A: August 1999–July 2000; period B: April 2001–March 2002) we conducted a prospective survey on AMR of principal microorganisms isolated in patients admitted for more than 48 h to ICU. We present data about AMR in *P. aeruginosa* isolates. Susceptibility tests were performed by Kirby–Bauer method (Becton Dickinson). Data refer only to strains with different antibiotype in case of repeated isolations in the same patient. We classified isolates as multidrug-resistant (MDR) when resistance to piperacillin, imipenem, ceftazidime and gentamicin was detected and as PAN resistant (PAN-R) when the isolate was resistant to all tested antibiotics.
Results: In period A and B respectively, 397 and 400 patients were evaluated. During period A, among 138 isolates of *P. aeruginosa*, 18 (13.0%) were MDR, of which, two PAN-R. During period B (183 isolates), among 27 (14.7%) MDR, PAN-R strains increased to 14 ($P = 0.02$). The proportion of ICU-acquired infec-

Antibiotic	Period A (138 isolates) R%	Period B (183 isolates) R%	*P*
Gentamicin	58.0	51.9	NS
Imipenem	33.3	57.4	<0.01
Piperacillin/Tazobactam	47.1	43.2	NS
Amikacin	15.2	33.3	<0.01
Ceftazidime	38.4	39.3	NS
Cefepime	23.8	37.2	.040
Azthreonam	48.5	53.0	NS
Ciprofloaxin	49.3	56.3	NS

tions caused by *P. aeruginosa* increased from 18.3 (24/131 episodes) to 30.3% (30/99) ($P = 0.04$). Resistance patterns of *P. aeruginosa* isolates against the principal antibiotics are represented in Table.
Conclusions: Compared with period A, ICU-acquired infections caused by *P. aeruginosa* and isolation of PAN-R strains significantly increased during period B. AMR against the principal available antibiotic reached very high levels, significantly increasing for imipenem, amikacin and cefepime. Since new anti-pseudomonas drugs are not expected to be soon available, current possible solutions are the targeted choice of the best antibiotic associations based on synergy assays and the implementation of antibiotic rotation after periodically evaluating the local AMR patterns.

R1984 Antibiotic susceptibility of urinary tract isolates of *Escherichia coli* from outpatient women in Kosovo: multicentre study

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Pristina, CS

Objective: Urinary-tract infections are amongst most common infections caused by pathogens with increasing resistance to antimicrobials. The vast majority of these infections arise in female outpatients with *E. coli* as primary aetiologic agent. The objective of this study was to assess antimicrobial susceptibility pattern of *Escherichia coli* among urinary isolates in oupatient women in Kosova.
Methods: Retrospective study was carried out of urine samples presented growth of *Escherichia coli* from outpatient women from January 2, 2002 to December 1, 2003. Only one positive isolate of *E. coli* per patient was included in the study. Bacterial susceptibility testing was performed by disc-diffusion method. The study was conducted in five regional centres of Public Health of Kosova: Prishtina, Gjilan, Mitrovica, Peja and Ferizaj.
Results: Results were available for 3720 urinary isolates of *E. coli*. Of these isolates 65.1% were resistant to ampicillin, and 46.3% were resistant to trimethoprim–sulphamethoxasole. Nitrofurantoin, ceftriaxone and ciprofloxacin expressed the highest susceptibility among these isolates. Their sensitivity pattern was 96.9, 96.8, and 95.5%, respectively. Of all isolates, 8.7% (324) were resistant to three or more agents and considered multi-drug resistant.
Conclusions: Resistance rates indicates the need for re-evaluation of empiric treatment of urinary-tract infections caused by *E. coli* and for continuous surveillance and monitoring studies.

R1985 Antimicrobial susceptibility and serotype distribution of clinical isolated *Salmonella* spp. strains during 2000-2002 in a general hospital

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Objectives: The aim of this study was to determine the trend in the prevalence of *Salmonella* serotypes, to analyse their distribution and their antibiotic resistance patterns.
Methods: We studied the serotypes and we investigated the *in vitro* antibiotic susceptibility patterns of *Salmonella* spp. isolated from clinical specimens. The isolates were identified by conventional microbiological methods. Serotyping was carried out by the National School of Public Health of the University of Athens. Susceptibility tests to ampicillin (AMP), ciprofloxacine (CIP), trimethoprim/sulphamethoxazole (SXT) and ceftriaxone (CRO) were performed using the disc-diffusion method according to the NCCLS recommendations.
Results: During the 3-year study period (2000–2002) a total of 52 *Salmonella* spp. Strains were isolated: 47 from 465 stool specimens (10.1% positive for *Salmonella*) four from blood specimens and one from urine specimen. 14 different *Salmonella* serotypes were isolated. *S. enterica* serotype Enteritidis was the predominant isolate (21 strains, 40.4%), followed by *S. enterica* serotype Typhimurium (13.4%), *S. kottbus* (7.7%), *S. agona* (5.7%), *S. oranienburg* (3.8%) and other less-common serotypes (29%). The majority of the *Salmonella* strains (65%, 34 strains) were isolated from children (<14 years old) and the seasonal distribution revealed an increased number of isolates in the summer months. Thirty-nine (75%) of the *Salmonella* strains were susceptible to all antibiotics tested. eight strains (15.4%) were resistant to AMP and five strains (9.6%) to SXT.
Conclusion: *Salmonella enteritidis* continues to be the most common clinical isolate, with a decreasing frequency compared with our previous data. In our geographical area (South Greece) the resistance of *Salmonella* spp. to AMP and SXT remains at relatively low levels in a decreasing rate. Susceptibility to fluoroquinolones is still preserved. Though we did not observe any resistance to CIP and CRO, the universal resistance pattern is alerting for rational antibiotic use in salmonellosis and continuous surveillance for resistance changes.

R1986 Detection of genetic diversity of pneumococcal surface protein A in *Streptococcus pneumoniae* strains by PCR-RFLP

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Tehran, IR

Objective: *Streptococcus pneumoniae* is a formidable human pathogen responsible for a major cause of over 3 million deaths world wide. The high morbidity and mortality associated with Pneumococcal disease is also being exacerbated by the rate at which this microorganism is acquiring resistance to multiple antibiotics. Pneumococcal surface protein A (PSPA) is an important virulence factor of the pneumococcus that influences bacterium-host interotions through interference with the fixation of complement C3. Pneumococcal surface protein A (PSPA) is a serologically variable protein of *Streptococcus pneumoniae*.
Materials: We determined the degree of PSPA gene diversity among 50 isolates by PCR, restriction profiling.
Conclusions: Amplification of PSPA by polymerase chain reaction and restriction endonuclease digestion showed that there is high variation among these strains. However, isolates with identical resistance pattern and same serotype which has suggested a clonal origin in *S. pneumoniae*, now it reveals that this protein is genetically variant among these pneumococcal isolates. Variability at all PSPA can be exploited for purposes of strain identification in epidemiologic investigations.

R1987 *In vitro* activity of telithromycin against *Streptococcus pneumoniae* resistant to β-lactams and macrolides isolated from respiratory tract infections in France in 2002

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Objectives: The objective of this surveillance study was to monitor the *in vitro* activity of telithromycin (TEL) and other antibiotics against *Streptococcus pneumoniae* (SP) isolated from respiratory tract infections (RTI) in adult patients in France.
Methods: Strains were collected in 42 metropolitan French hospitals during 2002. MICs of penicillin (PEN), erythromycin (ERY), amoxicillin (AMX), cefuroxime (CFM), clarithromycin (CLA) and TEL were determined by broth microdilution. Susceptibility rates were calculated according to the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA SFM) recommendations. CA SFM TEL breakpoints are: susceptible (S) MIC $\leq$ 0.5 mg/L, intermediate (I) MIC 1–2 mg/L, resistant (R) MIC > 2 mg/L. *ErmB* and *mefA* genes were detected by PCR. Quality control

was performed with three SP strains (Susceptible- ATCC 49619, *ermB*- Drug 3031, *mefA*- Drug 4428).
Results: In total, 432 (44.8%) SP strains concomitantly nonsusceptible to ERY and PEN (ERY-IR/PEN-IR) were isolated among a total of 965 SP strains. ERY-IR/PEN-IR strains were isolated from patients aged 61.1 ± 18 years and 65.9% of them were male. The diagnoses associated with these isolated ERY-IR/PEN-IR strains were: pneumonia 62.7%, acute exacerbation of chronic bronchitis 16.9%, sinusitis 2.9%, others 17.6%. In total, 117 ERY-IR/PEN-IR strains (27.1%) were isolated from at least one blood culture. The MIC50/90 (mg/L) and% of S/I/R against ERY-IR/PEN-IR SP was the following: AMX: 1/2 – 28.2/66/5.8 CFM: 4/8 – 9.5/17.6/72.9 CLA: 32/32 – 4.2/8.7/87 TEL: 0.03/0.25 – 97/3/0 In total, 420 (97.2%) ERY-R strains contained the *ermB* gene, six (1.4%) the *mefA* gene and two (0.46%) contained both genes. The four (0.93%) remaining ERY-R strains were PCR-negative. No ERY-R strain was resistant to TEL.
Conclusion: This study demonstrated that TEL has high activity against SP resistant to β-lactams and macrolides, which represent approximately half of the strains currently isolated from RTI in adult patients in France.

R1988 The comparative *in vitro* activity of ciprofloxacin against urinary-tract pathogens isolated from Europe during 2003 from Libra Targeted Surveillance

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Objectives: To assess the prevalence of bacterial pathogens causing urinary tract infection (UTI) and their antimicrobial agent susceptibility within Europe.
Methods: A total of 15 centres in four countries (France, Germany, Italy and Spain) submitted a total of 529 consecutive isolates. Bacteria were reidentified and their susceptibility to the orally available agents ciprofloxacin (CIP), ampicillin (AMP), amoxicillin–clavulanate (AMC), nitrofurantoin (NIT), trimethoprim–sulphamethoxazole (SXT) and the parenteral agents gentamicin (GEN), ceftazidime (CTZ), piperacillin (PIP) and PIP-tazobactam (PTZ) was determined using the NCCLS broth microdilution method in a central laboratory.
Results: *E. coli* was the most prevalent pathogen (391 isolates, 60.3%), followed by *E. faecalis* (52, 9.8%), *K. pneumoniae* and *P. mirabilis* (31, 5.9% each). Overall per cent susceptibility for all pathogens and all *E. coli* collected, plus comparisons for all pathogens from each of the four countries surveyed are shown below:

% susceptible	CIP	AMP	AMC	NIT	SXT	GEN	CTZ	PIP	PIZ
All pathogens	81.9%	46.1%	73.0%	79.0%	64.8%	78.4%	84.9%	62.2%	90.9%
E. coli	84.6%	50.5%	76.5%	93.1%	75.5%	91.2%	98.7%	55.2%	92.8%
France	88.9%	52.0%	77.8%	87.7%	74.3%	83.6%	86.5%	64.3%	91.2%
Germany	78.8%	39.8%	69.9%	82.3%	57.5%	79.6%	85.8%	61.9%	88.5%
Italy	73.9%	52.2%	63.0%	73.9%	54.3%	58.7%	76.1%	67.4%	88.0%
Spain	81.0%	40.5%	75.8%	69.9%	66.0%	83.7%	87.6%	56.9%	94.1%

Conclusion: PTZ and CTZ were the most active agents overall, but these are not available orally. CIP was the most active oral agent overall, but NIT was the most active against *E. coli*. However, inactivity against *P. aeruginosa* and *Proteus* spp. reduced overall NIT activity and clinical utility. Irrespective of country examined, CIP remains the most widely active broad-spectrum oral antimicrobial agent against bacteria causing UTI in Europe. CIP has the additional advantage of also being available in a parenteral form.

R1989 *Helicobacter pylori* and its related risk factors

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Object and aims: Role of *Helicobacter pylori* (HP) in many gastrointestinal disorders such as peptic ulcer, gastritis, intestinal metaplasia and stomach cancer is established. In order to determine risk factors of its infection this study was done.
Material and methods: A total of 232 patients with gastrointestinal signs were studied. Endocopy was done by internist and from two to three biopsies from antrum was taken. Specimens were processed by Shandon tissue Processor (Citadel). Prepared slides were stained by Haematoxycillin and eosin and also giemsa. Two pathologists and one microbiologist studied the slides. Patients, blood groups were determined. Patients, data including age, sex, smoking, residential status, clinical signs, pathologic diagnosis, blood group were recorded and then analysed.
Results: Of 232 patients 130 (56%) were females and 102 (44%) were males. 222 patients (124 women and 96 men) showed HP infection . Frequency of infection in females was 95.4% and in males 94%. A total of 88 patients (38%) were in age group ≤30 and 144 (62%) in age group 31–80 years. HP was detected in 84 (95.4%) in first group and 136 (94.4%) in second group. 95.7% rural and 41.8% urban showed HP infection. The most frequent blood group was B (78%). All smoked patients and patients with history of smoking showed infection. HP infection in active gastritis was 98.3% and inactive gastritis with intestinal metaplasia was 64.2%. The most common symptom was epigastric pain and the least common was hiccup and vomiting.
Conclusion: Frequency of HP infection is high (95.7%). No relation is between age, sex, clinical signs and HP infection. Persons with blood group B are more predipose to HP and smokers are high risk for HP infection. No difference is between urban and rural.

R1990 Prevalence of erythromycin resistance among *Streptococcus pyogenes* isolated in Kingdom of Bahrain

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Objective: Numerous published studies have documented the rise in erythromycin resistance among *Streptococcus pyogenes*. The aim of our study is to determine the resistance to erythromycin among our isolates.
Methods: A total of 55 clinical strains of *S. pyogenes* were isolated from patients during the period from February to November 2003, the strains were isolated from throat swab (65.5%) wound swabs (9.1%) blood (3.6%) pus swabs (9.1%) sputum (3.6%) vaginal swabs (3.6%), ear swabs (1.9%) and skin (3.6%). Identification was performed by routine laboratory techniques including latex agglutination. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc-diffusion test. The minimal inhibitory concentration (MIC) of resistant isolate was determined by E-test methodology according to the recommendation of the manufacturer.
Results: All *S. pyogenes* strains were fully susceptible to erythromycin except one strain (1.9%) which was highly resistant, MIC > 256 mg/mL.
Conclusion: Erythromycin resistant among *S. pyogenes* isolated at Salmaniya Medical Complex in Kingdom of Bahrain is low (1.9%) compared with other similar studies elsewhere; Italy 24.5%, Portugal 23.8%, Spain 21.2%, France 12.9%, Turkey 2.9%.

R1991 Empiric treatment in urinary tract infections based on resistance patterns

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Plasencia, E

Background: Antibiotic resistance varies due to its irrational use. Urinary tract infections (UTI) are very common infectious disease and the clinicians need to know the epidemiology, and above all the frecuency of agents and its resistance patterns in order to manage UTI properly.
Objective: Stabilising an empiric treatment in our area depending on the uropathogens isolated and its susceptibility to the antimicrobial agents used in UTI.
Materials and methods: Uropathogens agents isolated, from urine cultures in our Microbiology laboratory, in 2002 were retrospectively studied. Specimens came from outpatients mainly. We consider 715 Gram-negative bacilli (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae*, *M. morganii*) and *E. faecalis*. Susceptibility strains was performed against 15 drugs determining MIC with microdilution NCCLS tests.
Results: We took into account the main microorganisms, above 2%; and focus on eight antimicrobial agents. The final drug effectiveness, considering the frequency and susceptibility of each microorganism, was ampicillin (40.96%), cotrimaxole (68.97), gentamicin (93.66), fosfomycin (90.43), ciprofloxacin (83.88), cefuroxime (87.58), amox-calvuanic acid (72.86), nalidixic acid (74.85). The most important pathogen was *E. coli* (80.4%) and then *K. pneumoniae* (6.6%).
Conclusions: The best choice in UTI without complications is fosfomycin, well tolerated and with few side-effects and no resistance involvement. Amoxicillin-clavulanic acid was the first choice drug in our area 5 years ago, and the overuse might increase its resistance rates. Cefuroxime or fluorquinolones, may be a good alternative and the other agents need a susceptibility test prior to treatment.

R1992 Penicillin-resistant pneumococci in a hospital in Saudi Arabia

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Objective: To study the pattern of antibiotic resistance among *S. pneumoniae* in Saudi Aramco Medical Services Organization.
Methods: This is a retrospective study of pneumococcal isolates. The isolates were tested with oxacillin diffusion disk and subsequently E-test was performed.
Results: A total of 162 isolates of *S. pneumoniae* were identified in the study period from January 1999 to December 2002. Thirty-five per cent of the isolates were obtained from the blood, 42.6% were respiratory isolates, 2.5% were obtained from the CSF and 19% were obtained from other sites. Of the total 162 isolates, 83 (51%) were penicillin susceptible and the remaining 79 (48.8%) were penicillin nonsusceptible. The prevalence of high level resistance was 19.8% and that of intermediately resistant was 29%. A total of 58 (35.8%) isolates were obtained from the blood. Of these isolates, 31 (53%) were penicillin susceptible, 22 (38%) were intermediately susceptible to penicillin and five (1.7%) were highly penicillin resistant. Of the total 69 respiratory isolates, 35 (51%) were penicillin susceptible, 15 (22%) were intermediately susceptible to penicillin and the remaining 19 (27%) were highly resistant to penicillin. Of the 134 isolates tested for ceftriaxone, the majority (95%) of the isolates were susceptible. Erythromycin resistance was present in 30 of 119 (25%) tested isolates. Twenty-nine per cent of the isolates were resistant to tetracycline and 42% were resistant to trimethoprim-sulphamethoxazole (TMP–SMX). However, none of the isolates was resistant to vancomycin. Penicillin-resistant isolates were more likely to be resistant to erythromycin (50% of high-penicillin resistant isolates, and 24 and 17% of intermediately resistant and susceptible *S. pneumoniae*, respectively ($P < 0.05$). Resistance to tetracycline was present in 18% of penicillin susceptible *S. pneumoniae*, and 65% of high penicillin resistant organisms, respectively ($P < 0.05$). TMP-SMX resistance also increased from 23% among penicillin susceptible *S. pneumoniae* to 78% among high penicillin resistant *S. pneumoniae* ($P < 0.05$) (Fig. 2). Resistance to >3 or more class of antibiotics is defined as multi-drug resistant (MDR) *S. pneumoniae*. The prevalence of MDR *S. pneumoniae* was between 9 and 12%.
Conclusion: High resistance of *S. pneumoniae* to antibiotics calls for more efforts to promote the use of antimicrobials more judiciously.

R1993 Prevalence and antimicrobial resistance of urinary bacterial isolates from pregnant women in rural Tanzania

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Objectives: Correct management of urinary-tract infections in pregnancy can reduce morbidity in mother and child, but antimicrobial resistance can impede effective chemotherapy. The objective of the study was to describe the prevalence and antimicrobial resistance of bacterial isolates causing urinary-tract infections in pregnant women in a rural area in Tanzania.
Methods: Urine specimens obtained from April 1995 to March 1996 from 5163 pregnant women in a rural area in Northern Tanzania were examined. Among a total of 543 women who had positive dip-slide tests, 102 randomly chosen dip-slides were investigated further with bacterial identification and susceptibility testing by the disc-diffusion method. The results were compared with data from studies from urban areas of Tanzania and elsewhere.
Results: A total of 116 bacterial isolates, 72 Gram-negative and 44 Gram-positve, were recovered from 102 positive dip slides. The most frequently recovered Gram-negative and Gram-positive isolates were *E. coli* ($n = 27$) and enterococci ($n = 18$), respectively. Isolates of *E. coli* showed low rates of resistance to ampicillin (17%), mecillinam (0%), cefalexin (0%), nitrofurantoin (4%), trimethoprim (0%) and sulphamethoxazole (0%). Isolates of Enterobacter ($n = 9$) and *Klebsiella* spp. ($n = 4$) displayed higher rates of resistance to these drugs, ampicillin (44 and 75%, respectively), mecillinam (33 and 25%, respectively), cefalexin (22 and 0%, respectively), nitrofurantoin (56 and 25%, respectively) trimethoprim (40 and 44%, respectively) and sulphamethoxazole (25 and 11%, respectively). All enterococcal isolates were sensitive to ampicillin and trimethoprim, and only two isolates were resistant to nitrofurantoin. The resistance rates in this study were low compared with data from urban areas of Tanzania.
Conclusion: Although still lower than in urban areas, antimicrobial resistance is emerging in these rural areas of Northern Tanzania. Irrational use and over-the-counter sale of antimicrobials and use of expired or poor quality drugs may have contributed to this development.

R1994 Prevalence of oral candidiasis and antifungal susceptibility of Mexican oral yeasts isolated from HIV-infected and noninfected patients

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Objective: To establish the prevalence of oral candidiasis and the *in vitro* antifungal susceptibility of Mexican oral yeast isolates from HIV-infected and noninfected patients.
Patients and methods: A prevalence study over a 3-year period was conducted on a consecutive series of HIV-infected and noninfected patients attending the General Hospital of Mexico, the 'Federico Gomez' Child Hospital and the Odontology Clinics at the Facultad de Odontologia (Universidad Nacional Autonoma de

Mexico, Mexico DF). Oral swabs from 161 adults (52 HIV-infected) and from 163 children (60 HIV-infected) were plated onto Sabouraud dextrose agar plates and 187 yeast isolates were recovered. Yeast isolates were identified by mycological methods, including germ tube and chlamydospore production, and carbohydrate assimilation profiles by using API ID 32C (Biomerieux, France). Antifungal susceptibility to 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, fluconazole and itraconazole was assessed with Fungitest (Bio-Rad, USA).
Results: Yeasts were isolated from 179 of 324 patients (55.2%); of them 51 patients (45 HIV-infected) presented oral candidiasis. *Candida albicans* was the most frequent species (117 of 187 isolates, 62%, of them 94 were serotype A. Non- *Candida albicans* species were isolated from 52 patients: *Candida glabrata* from 35 patients (31 from nonHIV-infected patients), *Candida tropicalis* from 12, *Saccharomyces cerevisiae* from three and *Candida parapsilosis* from two. *Candida dubliniensis* was not isolated in the oral specimens studied. There were six episodes of infection or colonisation by at least two different yeast species. Overall 18.7% of the yeasts were resistant at least to one or more azole antifungal agents, being ketoconazole and itraconazole the less active. Most resistant isolates were *Candida glabrata* from patients without HIV infection or *Candida albicans* from HIV infected patients.
Conclusions: This is one of the first studies providing baseline data on oral *Candida* colonisation and infection in Mexican patients. Azole resistance was present in <20% of oral isolates. Many resistant isolates, mostly *Candida glabrata*, were from patients without candidiasis. This work was financed in part by grants 1/UPV 00093.327-E-14645/2002 from the Universidad del Pais Vasco and PAPIIT 214300 DGAPA-UNAM.

R1995 A 2-year survey of *K. pneumoniae* isolates at a new teaching hospital in Turkey

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Zonguldak, TR

Objective: To determine the antibiotic susceptibilities and the prevalence of ESBL production in *K. pneumoniae* isolates 2 year.
Methods: A total of 207 isolates (107 urine, 36 wound, 21 genital, eight blood, 14 sputum, 11 drain and 10 others) were investigated. Isolates were identified with conventional methods. Antibiotic susceptibility was determined by Kirby–Bauer disc-diffusion method according to NCCLS. ESBL production was determined by double disc synergy test (DDS) and confirmed by E-test (AB BIODISK, Sweden).
Results: Ratio of ESBL producing strains was 23/207 (21.7%). Among ESBL producing strains 75.5% of the isolates were recovered from inpatients. Susceptibility to cephalosporins were 0–37% in ESBL-producing strains and 82–94% in non-ESBL producing strains. ESBL-producing strains were 20% more resistant to ciprofloxacin. Isepamicine was the most effective (100%) aminoglycoside antibiotic against ESBL-producing strains. Karbapenem resistance was not observed.
Conclusion: Depending on the high prevalence of ESBL-producing strains of *K. pneumoniae* in nosocomial infections, clinical microbiology laboratories must effectively detect and report the presence of ESBL routinely. DDS is reliable, cheap and not much time consuming test for detecting ESBL production in routine use.

R1996 *Acinetobacter baumanii*: epidemiology and phenotypes of resistance to antibiotics in a Tunisian hospital

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Tunis, TN

A. baumanii is recognised by his nosocomial character. Our study was justified by several aspects : the increase in the isolation of the strains these three last years, their multiresistance to antibiotics which becomes usual, the exposed patients who are immunocompromised and finally, epidemic character of the infections. This study related to 499 strains of *A. baumanii* isolated from 65 852 pathological specimens in 3 years (2001–2003). The identification was made using the morphology (Gram) and biochemical characters: negative oxidase and rapid identification (Api GN - biorad). The antibiogram was carried out according to CA-SFM standards and the interpretation was done by the software 'SIR-i2a'. The strains supposed at the origin of epidemics were analysed by AP-PCR after extraction of their DNA by the kit 'QIAmp DNA minikit-Quiagen'. *A. baumanii* was isolated with a prevalence from 0.75% primarily from blood, punctures and katheters, especially in the medical intensive care and surgery units. Concerning β-lactams, 70% of the strains had a penicillinase and a cephalosporinase, concerning in particular, ceftazidim, cefsulodin, cefepim and tazobactam. A low sensitivity or a resistance to imipenem was noted in 40% of cases, to fluoroquinolones in 80% and to cotrimoxazole in 51%, to phosphomycin in 60% of cases. Concerning aminosides, we noted the phenotype KGA in 38% of cases, KGTA in 20%. A sensitivity to colistin was noted in 100% of cases. Two epidemics were noted with the medical intensive care unit (in September 2002 and from September to November 2003). All these data show that *A. baumanii* gives a problem with surgery and intensive care units in our hospital, with possibilities of antibiotic treatment often limited to imipenem and tobramycin. The epidemic strains were often most resistant to antibiotics.

R1997 Resistance patterns of *Streptococcus pneumoniae* strains isolated from infected individuals in 2001–2003 from Western Pomeranian region of Poland

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Szczecin, PL

Introduction: The spread of pneumococcal infections caused by multidrug-resistant strains is recently observed in the community.
Objectives: We analysed the antibiotic resistance patterns of *S. pneumoniae* strains isolated from respiratory-tract infections in years 2001–2003 in region.
Methods: The collection of 84 multidrug-resistant strains isolated from nasopharyngeal swabs (78), BAL (two), eye (one) was established using disk diffusion method according to NCCLS for the following antibiotics: erythromycin (E), clindamycin (L), tetracycline (T), cotrimoxazole (S) and oxacillin (P). Sensitivity of multidrug (five and four) resistance strains (33%) was verified with Etests.
Results: Initially determined resistance degree of *S. pneumoniae* was 60% for P, 40% E, 38% L, 50% T and 79% S. MIC results confirmed resistance of *S. pneumoniae* for E (>256 μg/mL), L (>256 μg/mL) and T (8 to >32 μg/mL). E-test verification for P revealed that among examined strains with zone diameter <20 mm for oxacillin, only 4% could be classified as PRSP (MIC 2 μg/mL), 32% as PISP (MIC 0.125–0.75 μg/mL), 64% was sensitive (MIC 0.023–0.064 μg/mL). For S 30% initially resistant strain showed intermediate susceptibility (MIC 1.5–32 μg/mL). These results found that real resistance degree to P is 29% and to S 69%. In the years 2001–2003 the resistance for E increased from 22 to 50% and for L from 22 to 45%, and reduced for T from 67 to 43% and S from 74 to 64%. We also noticed increase of percentage four-drug (7–12%) and three drug resistant strains (30–36%) and reduce two-drug (33–29%) and one-drug resistant strains (30–24%). Finally we observed the following resistance patterns: ELTS 8%, PELT 2%, ELT 18%, ELS 7%, PTS 5%, PEL 1%, PES 1%, PS 18%, TS 10%, PE 1%, EL 1%, S 20%, T 7%.
Conclusions: (i) We observed the increase of resistance for E and L, and the decrease for T and S and the growth of multidrug (four and three-drug)-resistant strains during last 3 years. (ii). E-test verification is necessary for determination of *S. pneumoniae* sensitivity to P and S.

R1998 Typing of *S. aureus* and *S. epidermidis* ocular isolates by different methods

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Catania, Messina, I

Objectives: The aim of this study, was to determine the number of different strains among 52 staphylococci ocular isolates (33 *S. epidermidis* and 19 *S. aureus*) using four typing methods as alternative to the pulsed-field gel electrophoresis (PFGE).
Methods: The typing methods used were: (i) analysis of plasmid profile, (ii) RAPD (random amplified polymorphic DNA analysis), (iii) PCR-ribotyping and (iv) PCR analysis of the Inter-IS256 spacer length polymorphysims.
Results: As for the 19 *S. aureus* isolates we used the first three methods the results of which show a certain accordance resulting in the typing of six, and seven different strains respectively. As regards the 33 *S. epidermidis* isolates we used (i), (ii) and (iv) of the typing methods listed above. The PCR analysis of the Inter-IS256 Spacer Length Polymorphysms and plasmid profile seem to be the most efficient method, both discriminating 23 different strains; however RAPD analysis carried out with primers 1 and 6 also has a good discriminatory power, identifying 12 and 10 strains, respectively.
Conclusion: Genotyping techniques are valuable tools for the epidemiologic study of bacterial infections. Pulsed-field gel electrophoresis (PFGE) is the current method of choice for typing of staphylococci but it requires expensive equipment. The use of these alternative methods proved to be able to monitor, with a certain degree of significance, changes within the bacterial genomes.

R1999 Childhood *Haemophilus influenzae* type B meningitis in Ural

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Yekaterinburg, Kamensk-Uralsky, Asbest, RUS

Bacterial meningitis in children are one of the life-threatening infections. However, the vaccination against the main causes of bacterial infections such as *S. pneumoniae* and *H. influenzae* is not part of the childhood immunisation programme in the Ural.
Objectives: The aim of this research was to investigate aetiology of bacterial meningitis in children of the Ural region during period from 1995 to 2003. Study was carried out in such towns as Ekaterinburg, Asbest, Kamensk-Uralskiy, Kachkanar. From 150 to 200 children with bacterial meningitis were included in the study annually.
Methods: The diagnosis was confirmed by the identification of the responsible pathogen from CSF culture and/or detection of soluble antigens in CSF (Slidex Meningitis Kit, Directigen Meningitis combo Test). For investigation of aetiology were used 'Haemoline'. Identification was carried out by routine methods API NH, JD srtep, JD g- or API NG. Biotyping of *H. influenzae* was followed out according Kilian.
Results: Quantity of the bacterial meningitis increases during actual period from 0.21 per mile in 1995 to 0.36 per mile in 2002. Contribution of *H. influenzae* type b as casual agent to the general quantity of bacterial meningitis varied from 3.5 to 10.1% in different years. Hib-induced meningitis were the most frequently (10.1%) in 1997 and in 2002. Other serotypes of *H. influenzae* was not found. The *H. influenzae* biotype 1 was in the major cases. The study group was from 1 till 5. One patient with Hib-meningitis was 3 months old (1996). Unlike meningococcus-induced meningitis in children there was no any season fluctuations of epidemiology of Hib-meningitis. We tested a total of 64 cultures for antibiotic resistance by ATB NH or ATB Haemo using automatic tester ATB-Expression. 95.2% of cultures were sensitive to: amoxicillin, cefaclor, cefataxim, spektimycin, tetracycline, pefloksacin, rifampicin, trimetaprim clotrimaxazom but they was resistant to gentamycin and had intermediate susceptibility to erythromycin. Intermediate susceptibility to kanamycin was found in two cultures and to amoxicillin – in one culture. All cultures were sensitive to chloramphenicol.
Conclusion: Hib-induced meningitis in children of our region are relatively constant (from 3 to 10%) and take second or third place after meningitis induced by *N. meningitidis* or *S. pneumoniae*.

R2000 Phenotypic and molecular identification of *β*-lactamase genes in Gram-negative bacteria isolated from a university children's hospital

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Objectives: To examine the presence and distribution of *β*-lactamase (bla) genes in clinical isolates at Children's Hospital of University (CHU), Riga, Latvia.
Methods: A total of 215 isolates collected from inpatients of CHU in the period May–November 2003 were investigated. Bacterial isolates were identified by BBL Crystal. Antimicrobial susceptibility were determined by disc diffusion method; extended spectrum *β*-lactamase production (ESBL) in *E. coli* and *Klebsiella* spp. – by disc opposition and double disc methods. Typing of bla alleles was performed by PCR amplification using primers specific for blaSHV, blaTEM, blaCTX-M, blaOXA and blaIMP.
Results: The biggest number of isolates belonged to *E. coli*, *K. pneumoniae* and *K. oxytoca* – 52, 34 and 11, respectively. More than 30% of these isolates were ESBL producers. From all tested isolates >40% (93 isolates) contained at least one bla allele while majority of bla positive strains harboured two or three alleles. Prevalent were blaTEM (in 72% of bla positive strains) and blaSHV (55%) types but none of the tested isolates carried blaIMP allele.

R2001 Bacteriocin production and inmunity diversity among enterococci from different origins

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Material and methods: The diversity of bacteriocins in *Enterococcus* was investigated in a collection of 100 broad-range bacteriocin producers isolated from faecal samples [healthy volunteers (33), food-handlers (19), animals (24)], clinical samples (20) and sewage (four) selected from a total of 720 isolates. Clonal relationships among strains were studied by PFGE- *Sma*I, using the Phoretrix 5.0 software. A chessboard strategy was used to classify groups of production/susceptibility, using all strains either as producers or indicators. Factorial correspondence analysis combining qualitative and quantitative variables was used to establish the cluster analysis, and Dice's coefficient was calculated for both production and immunity.
Results: Considering all the isolates, members of the species *E. faecalis* (57%) were more frequently bacteriocin producers than members of *E. faecium* (50%) or *Enterococcus* spp. (8%). Nevertheless these frequencies may be altered when a particular origin is considered. Among isolates with different production patterns, similar immunity patterns tend to occur in isolates from each particular origin suggesting the interchange of immunity genes as mechanism of resistance. Percentage of total inertia (32.76%) in the factorial analysis of correspondence can be explained by three first axes and suggests a single phenotypic evolutionary structure both in production and in immunity. Several pulsotypes, bacteriocin and immunity types were indeed shared by strains from different groups.
Conclusions: Bacteriocin production in enterococci is common between different origins, and although differences can be observed, this phenomenon could be part of a single structure with ecosystem specialisation's, but no foreign elements have been acquired. Separated transference of immunity or production

genes could be taken in account, especially between strains from the same origin.

R2002 Antibiotic susceptibility pattern and population structure of *Streptococcus pneumoniae* isolated from cystic fibrosis patients

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Background: *Pseudomonas aeruginosa* is the most common cause of chronic colonisation in cystic fibrosis (CF), but other bacteria as *S. pneumoniae* may play a significant role.
Material and methods: A total of 47 *S. pneumoniae* isolates were obtained from deep-sputum samples of 26 CF patients, collected along 1995–2003. Susceptibility testing to 19 antibiotics was performed by the microdilution method (Wider system, Fco. Soria Melguizo, Madrid, Spain). PFGE- *Sma*I was applied to analyse genetic relatedness. Mutation frequencies were assessed using 5% sheep blood agar plates containing 2 μg/mL of rifampin.
Results: The most representative serotypes were: 23F (16 isolates) and 19F (eight isolates). High percentages of resistance (intermediate plus resistant) were observed for penicillin (75%), cefotaxime (33%), erythromycin (42%), tetracycline (58%), SxT (67%) and chloramphenicol (48%). All isolates remained susceptible to ciprofloxacin, levofloxacin, and vancomycin. Co-resistance to four or more antibiotics was found in 56% of the isolates. Thirty-one different pulsotypes were identified among the 47 strains (from A to AD). Twenty-nine pulsotypes were represented by a single isolate each; two isolates of serotype 37 belonged to the clone AB, whereas clone A involved 16 isolates (all corresponding to the 23F serotype) with five sub-pulsotypes (A1–A5). This clone (A) was present in nine of 26 patients, being isolated along the 7-year-period of the study. Significantly, the same clone A was recovered in two patients after several years without *S. pneumoniae* detection. This clone displayed penicillin MIC of 2 μg/mL and cefotaxime MIC of 1 μg/mL and was resistant to macrolides, tetracycline, SxT, and chloramphenicol. The most representative patterns of colonisation were as follows: *S. pneumoniae* as sole isolate (18%), and *S. pneumoniae* with *Haemophilus influenzae* (18%). Considering all isolates, mutation frequencies to rifampin ranged from 7×10^{-10} to 1×10^{-7}, 32% being considered as mutator (mutation frequency range 1–5 $\times 10^{-7}$).
Conclusions: CF- *S. pneumoniae* isolates displayed a higher percentage of mutators and antibiotic resistance if compared with non-CF patients of the same age, being co-resistance a frequent feature. This fact may be related with antibiotic selection in these patients, persistence, and cross-colonisation of multi-resistant clones.

R2003 Fluoroquinolones in Gram-negative bacilli meningitis

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Iasi, RO

Objective: To study the effectiveness and tolerance of fluoroquinolones in the treatment of Gram-negative bacilli meningitis
Material and methods: During 1997–2003 we conducted a retrospective study, about 19 patients (15 males and four females, aged from 9 month to 73 years) with Gram-negative bacilli meningitis, treated with fluoroquinolones.
Results: The meningitis was postneurosurgery in nine cases, posttraumatic in three cases and othogenic in two cases. The germs (*Escherichia coli*, five cases; *Proteus mirabilis*, two cases; *Klebsiella pneumoniae*, six cases; *Acinetobacter baumanii*, three cases; *Salmonella*, one case) were multiresistants to antibiotics, but susceptible to fluoroquinolones. The fluoroquinolones (pefloxacine, 13 cases, ciprofloxacine, six cases) was administered by first intention in 16 cases, with synergic antibiotics association, for 13–30 days period. The clinical parameters were normalized after 7–10 days and laboratory parameters after 2 weeks of antibiotic therapy. The evolution was favourable in 13 cases, with complications in six cases (cerebral abcess, one case; subdural empiema, one case; hydrocephalus, three cases, deft, one case and two deceased. We registered four side-effects (increase TGP, one case; digestive, two cases; rash, one case).
Conclusions: Fluoroquinolones are effectiveness and well tolerated in the treatment of Gram-negative bacilli meningitis in associations with synergic antibiotics.

R2004 Resistance to imipenem in *Pseudomonas*

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Beirut, LBN

Introduction: *Pseudomonas aeruginosa* is a common nosocomial pathogen particularly isolated among immuno-compromised patients. In the year 2000, 14% of the Saint George Hospital isolates of *Pseudomonas* species were resistant to imipenem. Resistance of *Pseudomonas* to carbapenems is increasing and noted worldwide. The potency of β-lactams is limited by a rather impermeable outer membrane, which works in synergy with Inducible β-lactamase (plasmidic or nucleoidic). The aim of this study was to determine the susceptibility of the isolates of *Pseudomonas* spp (including *P. aeruginosa*) and to analyse their resistance to Imipenem.
Material and methods: This is a descriptive prospective study extending from 1 Janvuary 2000 to 1 August 2002. All the isolates of *Pseudomonas aeruginosa* and other *Pseudomonas* species were collected and antibacterial susceptibility testing (AST) was performed. The results were compiled and statistically analysed. Bacterial identification and antibacterial susceptibility testing were done according to the National Committee for Clinical Laboratory Standards (NCCLS).
Results: Table 1 shows some characteristics of *Pseudomonas* isolates where 68.4% of the patients were hospitalised and 95.3% consisted of the species *P. aeruginosa*. Table 2 illustrates the different susceptibility patterns of the *Pseudomonas* isolates including those that are resistant to imipenem (IRP). Table 2-IRP: Imipenem-resistant *Pseudomonas* (*aeruginosa* and other species). IP- Imipenem, PIP- Piperacillin, TAZ- Tazob/pip, CFZ- Ceftazidim, CFP- Cefepim, AZT- Aztreonam, GN- Gentamycin, TB- Tobramycin, AK- Amikacin, OF- Ofloxacin, CP- Ciprofloxacin.
Discussion: *P. aeruginosa* was more isolated than *Pseudomonas* spp regardless of the susceptibility patterns. The percentage of resistance to Imipenem was higher among the hospital isolates in comparison with isolates coming from outpatients. The Imipenem-resistant strains show higher level of resistance than those who

Table 1.

	All *Pseudomonas* Frequency (%)	IRP-Frequency (%)
In	491.0 (68.4)	126.0 (83.5)
Out	226.0 (31.6)	25.0 (16.5)
P. aeruginosa	683.0 (95.3)	146.0 (96.7)
Pseudo. species	34.0 (04.7)	05.0 (03.3)
Total	717.0 (100.0)	151.0 (100.0)

Table 2.

	IP	PIP	TAZ	CFZ	CFP	AZT	GN	TB	AK	OF	CP
IRP Frequency (%)	–	66 (43.7)	70 (46.4)	86 (57.0)	61 (40.4)	53 (35.1)	34 (22.5)	46 (30.5)	57 (37.8)	31 (20.5)	43 (28.5)
All *Pseudomonas* Frequency (%)	541 (75.4)	533 (74.3)	548 (78.5)	580 (81.0)	523 (73.0)	483 (67.5)	415 (58.0)	475 (66.4)	499 (69.7)	393 (54.9)	452 (63.5)

are susceptible to Imipenem (average of 32.9% of decrease in susceptibility). The average of susceptibility of IRP to antibiotics other than Imipenem is estimated to 36.2 compared with 69.1 for the susceptible strains. The highest percentage of susceptibility was observed with Ceftazidim in both cases and the lowest percentage was observed with Ofloxacin.

R2005 Consumption of antibiotics and antifungals for dermatological disorders

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Objective: To define and analyse the use of drugs for skin disorders in Zagreb based on ATC (anatomical- therapeutic-chemical) drug clasification system recommended by WHO.
Methods: The data obtained from 31 pharmacies units of Zagreb Pharmacies Group were collected. The number and size of packages together with financial indicators for each individual drug and for each ATC classification level were extrapolated on the total number of pharmacies in Zagreb in 2001.
Results: The main anatomical group D for dermatological disorders ranks eighth, or 4, 10% of all prescribtion drugs used in Zagreb in 2001. The corticosteroids (D07) are on the top of the rank, accounting 37.85% of group D drug use. They are followed by antimycotics (D01), which makes 26.99% of the use. Antibiotics and chemotherapeutics (D06) agents occupy the third position in the rank and they account 21.77% of group D drug use. The three major therapeutic group s of the drugs i.e. the D01, D06, D07 account for 86.61% of the overall use of drugs pertaining to the ATC group D.
Conclusion: Comparing our data with those obtained for Finland, it may be concluded that the structure of drug consumption is similar in Zagreb and Finland for the main therapeutic group comprising antimycotics. The use of antibiotics is twice smaller in Finland than in Zagreb, whereas the use of preparations for anti acne preparations is three times in Zagreb than in Finland.

R2006 Resistance of *Mycobacterium tuberculosis* to the primer antituberculous agents in Greek, foreigner, and repatriated Greek patients during the 10-year period 1993–2002

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Objective: We investigated the resistance to Isoniazid (INH) and Rifampicin (RIF) and the multidrug resistance (INH and RIF), of 4108 *M. tuberculosis* strains recovered at the National *Mycobacterium* Reference Centre of Athens, during the 10-year period, 1993–2002.
Methods: *M. tuberculosis* strains were recovered from 3368 Greek patients and from 730 immigrant patients. Among the immigrants, 189 were repatriated Greeks from Eastern European countries and 551 were foreigners from other countries. All specimens were stained by the Ziehl-Neelsen method and cultured on Löwenstein–Jensen medium or on the Bactec MGIT 960 or 460TB systems. The susceptibility testing of strains was performed by the method of proportion on Löwenstein–Jensen medium, or by using the MGIT 960 or the radiometric Bactec 460TB system.
Results: During the study period, the rates of newly diagnosed tuberculosis have shown a decreasing trend in Greek and repatriated Greek patients, whereas, in the foreigners they were increasing. The rates of resistance to INH and RIF as well as the multidrug resistance were respectively 6.6, 2.9 and 2.5 for Greeks; 29, 10.6 and 10% for repatriated Greeks and 11.4, 6 and 5.4% for foreigners. A twofold and a fourfold increase in the INH-resistance rates was detected in the Greeks and repatriated Greeks, respectively, whereas in the foreigners there was not any significantly difference detected. Concerning RIF-resistance, there was a sevenfold, a threefold and a 12-fold increase in Greeks, foreigners and repatriated Greek patients, respectively, while concerning multidrug-resistance there was an eightfold, twofold and 12-fold increase in Greeks, foreigners and repatriated Greek patients respectively.
Conclusions: The rates of the newly diagnosed tuberculosis show a decreasing trend, both in Greeks and repatriated Greek patients, probably due to an improvement of the standards of living and of health care services. Our results, although may not represent the mycobacterial resistance of the entire country, they give a good information about the problem. The increasing rates to the major of the primer antituberculous drugs in all three groups of the patients studied, suggest the need of a continuous monitoring *M. tuberculosis* drug resistance.

R2007 Sepsis in an infectology centre, Latvia: epidemiological and microbiological aspects

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Objectives: (i) To carry out the epidemiological investigation of patients with sepsis treated at the Infectology Centre of Latvia and (ii) to estimate the main pathogens and their antimicrobial susceptibility.
Methods: During the years 1993–2002 the case reports of 591 patients discharged from the Infectology Center of Latvia with diagnosis of sepsis were evaluated with regard to patients sex, age, duration of treatment, antimicrobial susceptibility. The American College of Chest Physicians/Society of Critical Care Medicine consensus conference (1992) definitions of sepsis were applied.
Results: A total of 591 patients with sepsis make 1.42% of all patients admitted to hospital during 10-year study period. Mortality rate was variable during this period, ranging from 0.92 to 20% of all sepsis patients. 357 patients (60.4%) had positive blood cultures and 234 – negative (39.6%). Predominant pathogens were Gram-positive bacteria, ranging from 55 to 80%. *Staphylococcus aureus*, coagulase-negative staphylococci, α- and β-haemolytic streptococci were mostly detected Gram-positive pathogens. The most frequent Gram-negative pathogens were *E. coli* and *Klebsiella* spp. In terms of antimicrobial resistance the methicillin-resistant *S. aureus* (MRSA) was documented overall in 18.7% of cases. During the study period 28.5–66.6% of coagulase-negative staphylococci were identified as methicillin-resistant. Only in 4.49% of cases Gram-negative bacteria were nonsusceptible to ciprofloxacin and more frequently (6.74%) resistance to ceftriaxon was registered.
Conclusions: Patients predisposed to sepsis were the elderly and patients with comorbid conditions. As sepsis is a significant health care burden and is associated with the use of extensive healthcare resources our findings may prove useful for the making of infection control policy and correct treatment decisions in the Infectology Centre of Latvia – tertiary care hospital in Latvia.

R2008 Carriage and acquisition of extended-spectrum β-lactamases in Lebanese hospitals

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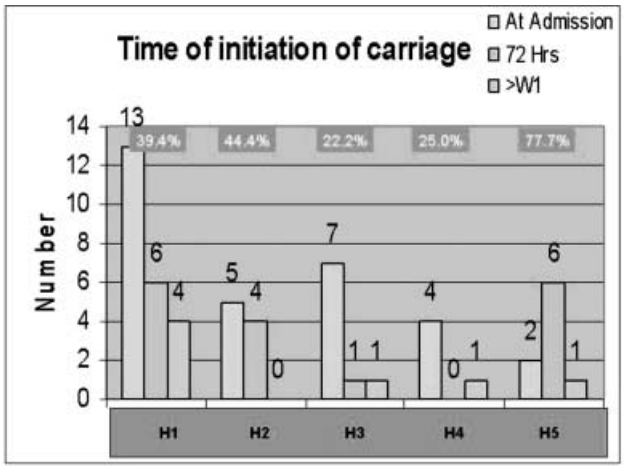
Introduction: Outbreaks of nosocomial infections due to extended-spectrum β-lactamase (ESBL)-producing strains are being increasingly reported in Lebanese health care facilities. The reservoir for these strains appears to be the gastrointestinal tract of patients, ESBL production being found mainly in *Klebsiella pneumoniae* and *Escherichia coli* and also in other Enterobacteriaceae including, *Proteus mirabilis*, *Salmonella*, *Enterobacter*, *Citrobacter* and *Serratia*.
Material and methods: A prospective multicentric point-prevalence study extending over a 12-months period was performed in the

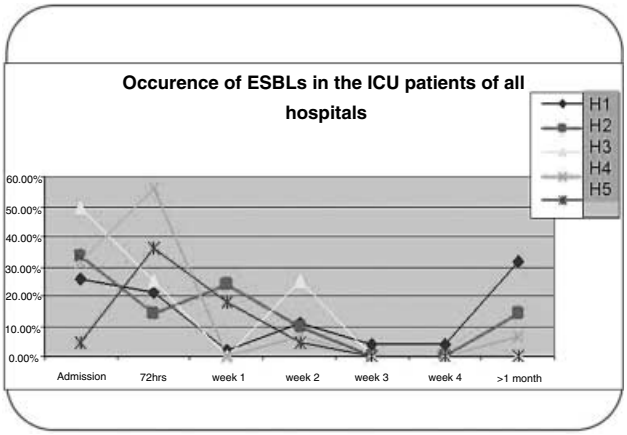
intensive care units of the five Lebanese hospitals located in different areas of Lebanon (Saint George Hospital, Rizk Hospital, Saint Joseph Hospital, Nini Hospital, and Arz Hospital) in order to evaluate the epidemiology of ESBL-producing strains in the country. The target population included the patients and the hospital staff of these hospitals. A total number of 1500 faecal samples were collected overall the five hospitals. Patients were checked for ESBL-producing strains upon admission, 72 h later and then at weekly intervals over a 3–month period. Health workers were checked for ESBL-producing strains once at the beginning of the study and then monthly.

Results: Results are shown in the tables.

Results- Isolated bacteria

	Percentage of occurrence
E coli	80%
Klebsiella	12%
Enterobacter cloacae	8%





Phenotypes of Resistance

E coli- ESBL (+)	*Kleb* ESBL (+)	CAZ	CFT	
31%	61%	R	R	High prelavence
58%	37%	R	S	SHV & TEM
11%	2%	S	R	CTX-M

Conclusion: The identification of the strains in patients and health workers by means of surveillance cultures was helpful to assess the prevalence of colonisation with this type of organisms, analyse the endemic situation, identify predisposing conditions, and evaluate -lactamase characteristics.

R2009 Resistance pattern in strains of *Staphylococcus aureus*, isolated in a Spanish military hospital

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Madrid, E

Objective: The knowledge of our epidemiological status, related to *S. aureus* at our hospital (Hospital Central de la Defensa, Military Hospital of 600 beds, in Madrid, Spain), specially referred to it's oxacillin resistance (MRSA, meticillin resistant *Staphylococcus aureus*) and the resistance pattern associated to it.

Methods: *Staphylococcus aureus* from clinical samples received in our laboratory during the last year were isolated and identified by latex agglutination (Pastorex Staph-Plus, BIORAD) to detect the clumping-factor of the A-protein and the polysaccharides of the capsule, confirming with the automatic system VITEK2 (Bio-Mérieux), which also permits antibiotyping, with a later confirming using a modified Kirby-Bauer (adding a supplement of NaCl-2%, as NCCLS recommends).

Results: A total of 209 samples from 194 patients were selected, 32.5% MRSA. Resistance patterns more observed were OEC (36.51%), OECCL (22.22%), OC (12.69%), OECCLG (11.11%) and OECG (6.65%), being O = oxacillin resistance, E = erythromicin resistance, C = ciprofloxacin, CL = clindamicin and G = gentamitamicin. Individual resistance was outlined, qualified by oxacillin susceptibility, as follows: for MRSA (E = 83.6%, C = 93.5%, CL = 36.5%, G = 28.3%, V = 0%) and for MSSA (methicillin-susceptible *Staphylococcus aureus*, E = 15.7%, C = 9.2%, CL = 3%, G = 6.1%, V = 0%, P = 85.3%), being V = vancomicin and P = penicillin G.

Conclusions: The surveillance of MRSA (32.5%) in our hospital is very similar to that observed in other Spanish hospitals, considered by Oteo as 33.6 in big hospitals (500–1000 beds) of our country. Resistance pattern for Spain is more frequently OECG (40.32%), while ours is only 6.35%, although our first pattern is OEC (36.51%), so it means that resistance to gentamicin is not so usual in our population. But our hospital patterns seems to be similar to the French ones. The total strains isolated were susceptible to vancomicin. It might be due to the change of our soldiers profile: many of our new professionals are proceeding from northern countries of African Centre or South America. In France, the immigration from these countries is also important. The patterns, obviously are linked to them too.

R2010 *In vitro* antibiotic resistance of *E. coli* in urinary tract infections during the last 2 years

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Objectives: To estimate the resistance of *E. coli* strains that isolated from urinary-tract infections in our laboratory during the last 2 years (October 2001–September 2003).
Methods: During the last 2 years we isolated 1819 (57.6%) *E. coli* strains from 3157 positive urine cultures. The identification and the susceptibility testing were carried out either by the breakpoint system mini API or the Vitek system (Biomerieux).
Results: Sixty-three per cent (1144/1819) of *E. coli* strains were isolated from outpatients' urine cultures and 37% (675/1819) *E. coli* strains were isolated from inpatients' urine cultures. 79% (904/1144) from outpatients and 69% (466/675) from inpatients were women. The resistance of *E. coli* strains to antibiotics for outpatients/inpatients respectively was: ampicillin 32.8/35.8%, piperacillin 10/9.6%, amoxicillin/clavulanic acid 5/6%, Cefalothine 11.6/12.2%, cefotaxime 1/1.5%, Imipenem 0/0.7%, Tobramycin 1.7/2.2%, Amikacin 0.9/1.5%, Netilmicin 1/1.2%, Gentamicin 2.1/2.5%, Trimethoprim/Sulphamethoxazole 15.8/19.3%, Nitrofurantoin 2.4/3.6%, Nalidixic acid 8.6/9.2%, Ciprofloxacin 7/7.4%.
Conclusions: Women were the majority of the patients especially in outpatients. The outpatients' *E. coli* strains had lower *in vitro* resistance than inpatients'. The differences in resistance in the majority of antibiotics were at the most 1.2% except ampicillin and trimethoprim/sulphamethoxazole where the differences were 3 and 3.5%, respectively. Ampicillin and trimethoprim/sulphamethoxazole found to have the less *in vitro* activity against *E. coli* strains.

R2011 Antimicrobial resistance of *Salmonella enterica* isolates from diarrhoeal patients in Crete, Greece

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Objective: To determine the antimicrobial drug resistance and the prevalence of *Salmonella enterica* serotypes in isolates from diarrhoeal patients in Crete, Greece.
Methods: A total of 176 *Salmonella enterica* strains isolated from faecal samples were studied. Identification was made according to standard microbiological procedures and serotyping was performed with commercial antisera by the slide agglutination method. Antimicrobial susceptibility testing was checked by the disc-diffusion method following the recommendations of the NCCLS. The following antibiotics were tested: ampicillin, cefotaxime, ceftriaxone, gentamicin, chloramphenicol, tetracycline, trimethoprim/sulphamethoxazole, nalidixic acid and ciprofloxacin.
Results: A total of 176 *Salmonella enterica* strains were isolated, corresponding to 13 serotypes. These strains were more frequently isolated from children (70%). *Salmonella enterica* serotype enteritidis was the predominant serotype (135 strains), accounting for 81.6% of all salmonellae isolated. *Salmonella enterica* serotype Typhimurium (21 strains) constituted 11.9%, while the remaining 11 serotypes were rare, constituting 11.5% of the strains. Resistance to ampicillin, tetracycline and nalidixic acid was observed in 4, 3.4, and 13% of all the isolates, respectively. Only one isolate was resistant to gentamicin, one to chloramphenicol and one to trimethoprim/sulphamethoxazole. All isolates were susceptible to third-generation cephalosporins tested and ciprofloxacin.
Conclusions: (i) *Salmonella enterica* serotype enteritidis predominates among other serotypes. (ii) resistance to commonly prescribed antibiotics in *Salmonella enterica* is rare in our region.

R2012 Low nasopharyngeal carriage and high ampicillin resistance of *Haemophilus influenzae* type B among children in Taiwan

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Objectives: In clinically, *Haemophilus influenzae* cause meningitis, sepsis, pneumonia, epiglotitis, cellulitis and arthritis in neonate and young children. The annual incidence of invasive *H. influenzae* type b (Hib) disease in Taiwan was estimated to be 1.5–2 cases/100 000 children >5 years of age/year, that was relatively low compared with western countries. Because of there is no any report of the Hib nasopharyngeal carriage rate in Taiwan. This study is aimed to define the overall carriage rate of Hib among healthy children in the community. In addition, antibiotic susceptibility, serotype and the clonal relationship of these isolates will be done.
Methods: (i) Survey of nasopharyngeal carriage of *H. influenzae*. From January to December 2003, nasopharyngeal specimens was obtained from healthy children, then identify isolated strains as *H. influenzae*. (ii) antibiotic susceptibility test. The MICs of antibiotics were determined using Etest. (iii) serotype of isolates. Using Bacto *H. influenzae* antisera seperate typable from nontypable isolates. Typable isolates were further serotyped by the slide agglutination test and (iv) molecular typing of isolates. Using PFGE method.
Results: Thirty-two *H. influenzae* strains isolated from nasopharyngeal of 642 healthy children. Among these strains, four isolates belong to type b; others are nontypable, the carriage rate of Hib is 0.6%. Antibiotic susceptibility test revealed ampicillin resistant rate is 62.5%, cotrimoxazole resistant rate is 46.9% and resistance to azithromycin, chloramphenicol, tetracycline are 6.3, 28.1, 28.1% respectively, All isolates sensitive to the second and the third cephalosporins. The PFGE of isolates showed diversity of genotype.
Conclusion: According to the results of this study, the nasopharynx carriage rate of Hib is very low in Taiwan, and association of high ampicillin resistance that may be due to antibiotic overuse, these two factors may contributed the low incidence of invasive Hib diseases in Taiwan.

R2013 Antimicrobial susceptibility of *Escherichia coli* isolated from humans and retail meat

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Freiburg, D

Background: Multi-drug resistant food-borne pathogens have increased markedly in recent years. The need for an integrated surveillance system between human and veterinary microbiology laboratories has been emphasized by the World Health Organisation.
Methods: *Escherichia coli* strains were isolated from human samples ($n = 653$) in South Germany, and from retail poultry, pork and beef ($n = 113$) grown in the same area. Identical isolation and identification methods were used for both human and food samples. Antimicrobial susceptibility testing was performed for all isolates using the VITEK system (biomérieux).
Results: All the human and animal strains were 100% susceptible to imipenem. Most isolates were susceptible to cefuroxime (94 and 98%, respectively) and gentamicin (97 and 95%, respectively). Susceptibility to ofloxacin was 90 and 93%, respectively; to piperacillin 66 vs. 79%, to ampicillin 60 vs. 65% and to cotrimoxazole 73 vs. 70%. Resistance among clinical isolates to tetracyclines was 34%, but was very high (60%) among food-borne isolates, which may reflect the frequent use of this substance group in animals husbandry.
Conclusions: *Escherichia coli* isolates from meat tend to be similarly resistant to commonly used antibiotics as isolates from humans. Further investigation is necessary to establish whether the source of the resistance in food isolates is directly related to antibiotic use in animal farming.

R2014 *A. baumanii* isolated from blood cultures during a 3-year period

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Athens, GR

Objectives: The purpose of this study was to determine the antimicrobial susceptibility of *A. baumanii* isolated from blood cultures during a 3-year period.
Methods and material: Blood cultures were performed by the Bactec system (Becton Dickinson), the identification of *A. baumanii* was carried out by the Vitek system and the susceptibility testing by disc diffusion.
Results: During a 3-year period, 52 strains of *A. baumanii* were isolated from blood cultures in our hospital. All isolated strains were resistant to cefoxitin, cefotaxime, ceftazidime, ceftriaxone, ofloxacin, pefloxacin, ciprofloxacin. Frequency of resistance to gentamicin, tobramycin and netilmicin was 57, 36 and 36%, respectively. All strains were sensitive to colistin.
Conclusion: Our study showed a high rate of multidrug-resistant *A. baumanii* isolated from blood cultures in our hospital. We should re-examine our policy on restrictions on the use of antibiotics and warn clinicians of the emerging problem.

R2015 Compliance and impact of an ID physician's advisory consult on restricted antibiotic usage

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Objectives: This study was to evaluate compliance and short-term impact of targeted antibiotic advisory consult implemented since October 2002 in Dongsan Medical Center.
Methods: A total of 339 prescriptions in 187 patients were retrospectively reviewed and antimicrobial utilisation density was compared quarterly from January 2002 through June 2003.
Results: Overall 85.6% of prescriptions were inappropriate, with 73.7% inappropriate for therapeutic regimens and for 100% of prophylactic regimens. Overall compliance antibiotic advisory consult was 46.5%. The compliance was higher in the medical services than in the surgical services (64.2% vs. 43.1%, $P = 0.005$) and for therapeutic use and prophylactic use (54.7% vs. 36.5%, $P = 0.001$). From 1Q 2002 to 2Q 2003, use of aminoglycosides was reduced from 269.1 DDD/1000 patient days to 171.8 DDD/1000 patient days, and that of antipseudomonal cephalosporins from 112.1 DDD/1000 patient days to 64.5 DDD/1000 patient days. Overall inpatient use of parenteral antibiotics was decreased from 770.8 DDD/1000 patient days to 626.8 DDD/1000 patient days.
Conclusion: Targeted antibiotic advisory consult resulted in substantial reduction in antibiotic use despite relatively poor compliance. This type of antibiotic control programme can be an alternative for hospitals with limited human resources trying to implement an antibiotic policy.

R2016 Epidemiological analysis of carbapenem-sensitive and resistant *Pseudomonas aeruginosa* within a single hospital in Scotland

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Objectives: One ICU within the Royal Infirmary of Edinburgh in 2002 showed that a few patients were consistently colonised by *P. aeruginosa* strains with reduced carbapenem susceptibility. This study set out to identify whether these bacteria were genotypically or phenotypically related to similar strains elsewhere in the hospital.
Methods: The genotype of seven *P. aeruginosa* strains with decreased meropenem susceptibility isolated from the original unit in 2002 was compared with 104 *P. aeruginosa* strains collected in a prospective study from throughout Edinburgh in early 2003. These isolates were analysed by pulsed-field gel electrophoresis (PFGE) using Bionumerics computer software to determine the level of homogeneity and heterogeneity within these two groups. The susceptibilities of both groups of isolates were investigated to imipenem, meropenem, ceftazidime, piperacillin/tazobactam and ciprofloxacin.
Results: Five of the seven 2002 ICU isolates were identical when measured by PFGE, one isolate was 98% similar and the other was 85% similar. However, the 104 isolates were far more heterogeneous and, except for one strain, showed <82% similarity to the 2002 ICU isolates. In addition there was no clear clonality amongst the *P. aeruginosa* strains in this prospective study, as clusters of strains showing >90% similarity always comprised greater than of equal to four strains. There was just one isolate from the prospective 2003 study that showed 90% similarity with the 2002 ICU strains. Interestingly, amongst the 2003 isolates, strains that had identical resistance patterns often were unrelated genotypically and strains from patients in close proximity were usually unrelated.
Conclusion: In 2002, there was a small outbreak of closely related meropenem-resistant strains in 1 ICU. This outbreak was contained and did not spread into the hospital as a whole. Within the prospective study the *P. aeruginosa* strains are very heterogenous and almost always carbapenem sensitive.

Community-acquired infections

R2017 ASSURE rapid test for diagnosis of *Helicobacter pylori* infection: a new proposal test?

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Izmir, TR

Objectives: *Helicobacter pylori*, plays an important role in the pathogenesis of some several extra or intragastroduodenal diseases. The aim of this study was to evaluate the seropositivity of adult dyspeptic patients who were referred to upper gastrointestinal endoscopy following rapid urease test and histopathologic examination. Serological status was determined by IgG ELISA and ASSURE *H. pylori* Rapid Test with current infection marker for diagnosis of acute *H. pylori* infection.
Methods: During upper gastrointestinal endoscopy, biopsies were taken two from gastric antrum and two from corpus was performed in 54 patients (mean age 47 years) with dyspeptic complaints. *Helicobacter pylori* was assessed by rapid urease test and by histopathological examination in these biopsy specimens. Patients' sera were tested with two serological tests: Anti-*H. pylori* IgG ELISA (EUROIMMUN Medizinische Labordiagnostika, Lübeck) and ASSURE *H. pylori* Rapid Test (Genelabs Diagnostics, Singapore).
Results: A total of 54 patients were evaluated and *H. pylori* infection was diagnosed in 46 (85%) patients by rapid urease test and/or histopathology. Of the 46 patients, 34 (74%) were positive for both ELISA and ASSURE, seven (15.2%) were positive by ELISA, three (6.5%) were positive by ASSURE, two (4.3%) were both ELISA and ASSURE negative. Rapid urease test and histopathology negative eight patients sera showed three positivity by ELISA and ASSURE, two were positive by ELISA, one was positive by ASSSURE and remaining two patients were negative by both these tests.
Conclusion: Nowadays, there is a common effort to find a correct, reliable and simple *H. pylori* test. Among these noninvasive tests are more prominent in the available tests of the dyspeptic patients. That is why serology is widely used, cheap and noninvasive method to detect *H. pylori* infection but it has some limitations because of the not able to distinguish between active

infection and a previous 'contact'. It seems that ASSURE *H. pylori* rapid test with current infection marker provides us rapid diagnosis of *H. pylori* infection than ELISA.

R2018 The correlation between emergence of the troponin T in the blood from patients with tonsillitis with immunological and haemodynamic changes in early and late convalescence periods

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Objective: Explore changes of the immunity and haemodynamics in patients with tonsillitis (PT) depending on emergence of the troponin T (TnT) in the blood.
Methods: Bacteriological analysis of the microflora of the tonsil lacunas, immune marker analysis of the lymphocytes, trials of the IgA, -M, -G and circulating immune complexes (CIC). Two-dimensional Doppler echocardiography, trials of the TnT by the electrochemiluminescent method on the analyzer 'Elexis'. A total of 138 PT (mean age 19.8 ± 0.5 years) were surveyed during acute period (AP) of the tonsillitis (2–4 days from the beginning of the illness), the early (7–9 days) and late (20–25 days) convalescence (EC and LC). 20 healthy humans were the control group.
Results: 'Nonrheumatogene' species of the *Streptococcus* (*Streptococcus viridans*, etc.) were in 87% pts. Increasing level of the TnT (TnT>0.01 ng/ml) was discovered in the 15.2% PT during EC and LS periods – TnT (+) group of the pts. The other 84.8% pts – TnT (−) group. Quantity of immune competent cells with cytotoxic function (CD8, CD16) have increased during AP for pts from TnT (+) group more than for pts from TnT (−) group ($P < 0.005$). Quantity of CD16 was more in TnT (+) group then in TnT (−) group at EC and LC periods ($P < 0.05$). Quantity of IgA, -M, -G and CIC in TnT (+) group remains high during LC, but at the same time this quantity are normal in TnT (−) group. CIC = 0.07 ± 0.04 units of optical density (UOD) in TnT (+) group, 0.04 ± 0 01 UOD in TnT (−) group ($P < 0.01$). Cardiac index (CI) was less in TnT (+) group than in TnT (−) group at LC period (2.58 ± 0.25 L/min/m^2 and 3.29 ± 0.21 L/min/m^2, $P < 0.05$). Peak E/peak A was 2.07 ± 0.04 in TnT (+) group, and 1.87 ± 0.04 in TnT (−) group ($P < 0.001$) in LC period.
Conclusion: More 'bad showings' as cellular and humoral immunities, so central and intraheart haemodynamics were in PT increasing TnT in the blood by comparison with the PT without increasing TnT. Determination of the TnT at PT is significant because its increasing points to myocard's damage.

R2019 Transferrable beta-lactamase with extended-spectrum produced by multi-resistant Gram-negative bacilli

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A transferable extended-spectrum produced by a multidrug-resistant clinical isolate of Gram-negative bacilli isolates from patients with diarrhoea was studied. The gene was carried by a large plasmid were transferable to recipient strains of *Escherichia coli* MM294. The transfer of gene determinant from donor strains was demonstrated by the analysis of resistance spectrum in transconjugat clones of recipient strain by the method of indirect selection. The ability of production of extended-spectrum beta-lactamase (ESBL) was demonstrated by the double disc diffusion test. It is concluded that pencillinase and the resistance markers for pencillin was encoded by 4.2 kb plasmid, while that cephalosporinase and the resistance markers for cefotaxime and cephaloxin were encoded by 3.2 kb plasmid transferred by conjugation into *Escherichia coli* MM294.

R2020 *Neisseria meningitidis* bacteraemia: 95 episodes

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Objective: To assess the epidemiological and clinical features of *N meningitidis* (Nm) bacteriaemia in our hospital. Nm serogroup B was traditionally more frequent than serogroup C in our country. In the last years the serogroup C became more prevalent. In 1997 children and teenagers aged between 18 months and 20 years were vaccinated with the polysaccharide vaccine A + C (415 000 people 88.6% coverage). In 1999–2000 after a decrease in the incidence of disease by Nm C a new cluster of cases was noticed. Children fewer than 7 years were vaccinated against *N. meningitidis* C with the new conjugated vaccine. This vaccine is administered now to children at 2–4 and 6 months.
Methods: Prospective study of all cases of positive blood cultures with *N. meningitidis*. An infection control nurse visited every patient and filled a previously defined form including demographic data, underlying illnesses, clinical presentation, and analytical data: microbiology WBC, coagulation and outcome. Study period: December 1993 to December 2002. Blood culture method: Bactec 9240 (Becton-Dickinson). Identification: Biochemical test API NH (Biomerieux) and Serogroup (Difco). Serotyping was performed at the Hospital Donostia.
Results: A total of 95 patients had *N. meningitidis* bacteraemia during the study period. Sex: 51.58% men. Age: 2 months to 1 year: 16 cases, 2–10 years: 36 cases, 11–20 years: 14 cases, 21–60 years: 11 cases and >60 years: 18 cases. 20% were admitted to the ICU because of the severity of the disease. Serogroups: C 52 (54.7%), B 39 (41%), Y 1, and three nongroupable. Serotype (50 cases studied): 2a/subtype 5: 16; 2b-8 and serotype 4: 11 cases were the most frequents. Concomitant meningitis: 26 cases, 33% of cases of *N. meningitidis* C and in 20% of cases of serogroup B. Crude mortality: 11.57%. Eight of 11 (72.72%) died the same - day of admission. Two clusters of cases were identified during 1997 and 2002 with 16 cases diagnosed each year. Cumulated epidemic index 2.4 and 6.9 cases/100 000 inhabitants were reached in our community in the period January to June 2002. During the last cluster there have been three young women with symptoms mimicking pielonefritis and two aged patients without symptoms of sepsis.
Conclusions: *N. meningitidis* is the aetiologic agent of 1.8% of bacteraemia in our hospital. *N. meningitidis* serogroup C related to clusters is now more frequent in our hospital than the endemic serogroup B. The clinical diagnosis of some cases can be difficult. Mortality was higher in serogroup B (15.38%) than in C (9.61%).

R2021 Application of SIR (susceptible infectious recovery) epidemiological model in determination of measles epidemics

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Introduction: In the nineteenth century recurrent epidemics of cholera and influenza and decreasing cases of diseases in the later years caused that produce the question why communicable disease would suddenly produce epidemics and disappears then. Designing one model bases mathematical such as SIR (susceptible infectious recovery) model have been perfomed for the question response the aim of research survey model on the measles in Iran and determination of outbreaks of measles at 1996 and determine necessary least vaccine covering for prediction of measles epidemics in the future.
Methods and materials: With collection of data from disease control centre, the number of suffering to measles, age mean of measles and life expanses calculated and the number of suffering to measles, age mean of measles and life expanses calculated and the amount of R0 (Basic Reproduction Rate) resulted.
Results: Calculating R0 amount at range 5.5–7.49 have been resulted that for the reason R0 amounts greater than one the measles

epidemic has occurred in our country and least effective vaccine covering has been 81.8–86.65%.
Discussion: Calculating R0 and necessary least vaccine covering for prediction measles epidemics determine that for epidemic control least vaccine covering, must more than 82.65%. Decreasing herd immunity and increasing measles age mean under conditions due to another epidemic in the 2005.

R2022 Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections considering demographic and clinical data

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Móstoles Madrid, E

Objetives: In a population-based study we assessed the antibiotic resistance of *E. coli* from community-acquired ITU considering demographic and clinical data.
Methods: This prospective study considered 164 isolates of *E. coli* (one per patient) from urine samples of patients with community-acquired UTI diagnosed in the Emergency Service of our hospital. The antibiotic susceptibility was determined for ampicillin, gentamicin, fosfomycin, nitrofurantoin, cefazolin, nalidixic acid, norfloxacin, ciprofloxacin and cotrimoxazole. The clinical records of the 164 patients were reviewed and a protocol was filled out for each one.
Results: The 164 *E. coli* isolated from urine proceeded from 112 women and 52 males with a mean age of 54.12 years (SD = 21.15 years). After analysing the clinical records it was noted that 82 (50%) proceeded from uncomplicated UTI and 82 (50%) from complicated UTI (52 males and 34 females). Globally, 57.3% of the strains were resistant to ampicillin, 25.0% to cotrimoxazole, 20.1% to nalidixic acid, 14% to norfloxacin and ciprofloxacin, and 0% to fosfomycin and nitrofurantoin. Of the 82 strains proceeding from complicated UTI, 16 (19.5%) were resistant to norfloxacin and ciprofloxacin whereas only seven (8.5%) from uncomplicated UTI were resistant ($P = 0.043$). Sig-Significant differences for the quinolones were found when the resistance of isolates from patients $\geq$50 years were compared with those from patients <50 years: 28% (25/90) vs. 11% (8/74) ($P = 0.007$) for nalidixic acid, and 20% (18/90) vs. 6.7% (5/74) ($P = 0.015$) for the fluoroquinolones tested. Comparison of the resistance by sex showed statistically significant differences in cefazolin, 11.5% (6/52) in males compared with 1.7% (2/112) in women ($P = 0.013$); and in fluoroquinolones where the resistance was 25% (13/52) in males and 9% (10/112) in females ($P = 0.006$).
Conclusions: In acute uncomplicated UTI fosfomycin, nitrofurantoin, and the fluoroquinolones show adequate rates of susceptibility for empirical use in our area. For optimal interpretation of susceptibility in cumulative data from the Primary Health setting it is necessary to take into account the type of UTI (uncomplicated vs. complicated), the sex and age of each patient.

R2023 *Salmonella* and *Campylobacter* food-borne infections in the Czech Republic in 1984–2002

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Objectives: Acute enteric infections caused by *Salmonella* and *Campylobacter* are still important issues in recent era of mass industrial production and processing of food. Our aim was to provide concise overview of main epidemiological features of these food-borne infections.
Methods: Salmonelloses and campylobacterioses are notifiable diseases on obligatory basis. Morbidity data on salmonelloses are notifiable and available since 1951. *Campylobacter* infections are notified since 1984. Data on both entities were collected by computerised 'Information System of Communicable Diseases' the ISPO. Newer system of notification, the EPIDAT, was implemented in 1993. The core data structure remained the same and it allowed preserving the continuity of both notification systems
Results: Incidence of salmonellosis was growing during the period from 1981 and got the plateau in late 1980s. The brake was in 1989 when incidence reached three times higher levels than in previous years. The highest incidence rates were notified in 1995. Since 1998 the rates are steadily dropping down. Salmonelloses are unevenly distributed in our country. The highest rates are notified in agricultural districts in the east. Imperfect production of milk for nursed children was the most important cause in children under 1 year of age, the mostly hit age group until 1989. After 1989, quality of food products for babies improved. Less attention is paid to thermic processing of poultry and eggs and they became predominant risk food. *Salmonella enteritidis* is the prevalent serotype (95% of all cases) in recent years. *Campylobacter* is routinely diagnosed only in recent years and we observe typical seasonal variation in its incidence. The increasing trend in incidence was partly due to spread of diagnostic in all country. Campylobacterioses have importance comparable with salmonelloses. The highest increase in morbidity is recorded for the lowest age groups that are indicative of worsening conditions in food processing (particularly in households). Almost three-fourths of cases were infected via poultry products. Incidence of salmonelloses was 271/100 000 and campylobacterioses 225/100 000 inhabitants.
Conclusions: *Salmonella* and *Campylobacter* infections are important food borne diseases. In recent years they reached equal levels. The preventive potential is in improvement of food processing and storage.

R2024 Lyme borreliosis morbidity/incidence rate among the inhabitants of Podlasie region, 1996–2002

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Objectives: The purpose of the study was to evaluate Lyme borreliosis morbidity/ incidence rate among inhabitants of Podlaskie voivodship in the years 1996–2002.
Material and methods: The notifiability of Lyme borreliosis cases from Podlasie region in 1996–2002 was analysed.
Results: In Poland, 9437 cases of borreliosis were registered in years 1996–2002 including 2195 (23.26%) in Podlasie region. The morbidity rate of Podlasie region was: in 1996 – 15.05%; in 1997 – 30.79%; in 1998 – 46.56%; in 1999 – 33.96%, in 2000 – 21.29%, 2001 – 18.57% and in 2002 – 17.66% of all cases registered in the country. The incidence rate per 100 000 inhabitants in Podlasie region was higher than in Poland and equaled in 1996 to 9.09; in 1997 – 16.25; in 1998 – 29.37; in 1999 – 24.78; in 2000 – 32.2; in 2001 – 37.6 and 2002 – 29.4. In 2002 year, the incidence rate of Podlasie region exceeded 5.59-fold the incidence rate of the whole country. The highest morbidity rate was reported in the east and centre of the region (76.95%) abundant in large forests. A significantly lower rate was registered in the area of Augustowskie and Suwalskie Lake Districts (14.62%) and the lowest rate in the west of the region (6.7%). The incidence of the disease was analysed according to age of patients showing decrease in its incidence among patients over 60 years old. The highest number of cases was observed among patients aged 30–49, so Lyme borreliosis affects mainly professionally active people.
Conclusion: The results of our studies indicate that Lyme borreliosis is a serious health problem among the inhabitants of Podlasie region, the area abundant in big forests being an endemic area of a main Lyme disease vector – *Ixodes ricinus* ticks infected with *Borrelia burgdorferi* spirochete.

R2025 Epidemiology of infective endocarditis in Hungary: a pilot study

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Objectives: In Hungary, the epidemiology of infective endocarditis is unknown. For the establishment of Hungarian Infective Endocarditis Registry a prospective pilot study was conducted.
Methods: Between October 1, 2002 and September 30, 2003 data of 37 consecutive cases of infective endocarditis (IE) were collected. Patients were either hospitalised in our Institution or were encountered through infectious disease consultations. The diagnosis of IE was validated by using the modified Duke Criteria.
Results: Pathogens: The following organisms were isolated: *Staphylococcus aureus* in nine cases (24.32%), enterococci in nine cases (24.32%), [*E. faecalis* eight (21.62%), *E. faecium* 1 (2.70%)], *Streptococcus* (S.) *bovis* three (8.10%), viridans streptococci three (8.10%) (*S. mitis* 1, *S. oralis* 1, *S. salivarius* 1)], other pathogens seven (18.91%). Six cases were blood culture negative. Predisposing factors were valvulare disease: 11 cases (29.79%), prosthetic valve: 12 (32.43%), pacemaker five (13.51%). Affected valves were mitral: 15 (40.54%), aortic: eight cases (21.62%), tricuspidal two (5.40%), aortic and mitral: 10 (37.0%), aortic, mitral, tricuspidal: 1 (2.70%), pacemaker was affected in one case. No IV drug abusers or HIV positive patient were identified. The mean time to establish the diagnosis of IE from the first symptoms was 6.2 weeks (standard deviations: 5.23). Vascular phenomena were observed in 15 cases (nine cerebral, seven Janeway lesions, two splenic, two pulmonary, two arterial embolisation). Surgery was needed in 17 cases (45.9%). In-hospital mortality was 29.73%.
Conclusion: The results of this pilot study suggest the increasing role of enterococci in IE. Further investigations are needed to establish real epidemiology of IE in Hungary.

R2026 Epidemiological features in 469 adult cases of brucellosis in Babol, north of Iran

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Objectives: Brucellosis still remains an important public health problem in Mediterranean countries including Iran. The purpose of this study was to evaluate the epidemiological features in adult cases of brucellosis in Iran.
Methods: This study was conducted on 469 adult cases of brucellosis who were attended to the department of infectious diseases, Babol Medical University from January 1997 to January 2003. Epidemiological data in all cases were recorded.
Results: A total of 469 cases of brucellosis [267 (56.9%) males and 202 (43.1%) females with the mean age of 31.99 $\pm$ 17.10 years, ranged 16–90 years] were studied. A total of 184 cases (39.2%) were from urban and 285 (60.8%) were from rural areas. With regard to seasonal distribution, the disease was seen in 118 (25.2%) cases in spring, 188 (40.1%) in summer, 103 (22%) in autumn and 60 (12.8%) in winter. Risk factors of the disease were seen in 203 (43.3%) of the patients. Brucellosis was also seen in family members of 45 (9.6%) cases. The disease was acute in 255 (54.4%), subacute in 179 (38.2%) and chronic in 35 (7.4%) cases. The distribution of these findings between sexes was equal ($P < 0.05$).
Conclusion: Lack of risk factors of brucellosis in more than 50% of our patients and involvement of more than 65% of cases in spring and summer, show that the usage of unsafe dairy products are the main source of infection.

R2027 Comparison of community-acquired pneumonia clinic in young patients from different social groups

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Object: To compare the features of community-acquired pneumonia CAP clinic at young soldiers (YS), at young intravenous drug users (IVDU) and young adults (YA) with common life.
Methods: By means of the specially developed form, in which demographic, anamnesis, objective and laboratory-instrumental data were analysed, were clinically inspected 100 YS, 99 YA and 30 IVDU without HIV-infection. Age of patients was 15–30 years. Statistic analysis was conducted using chi-square statistics and *H*-test Kruskal–Wallis.
Results: The severity CAP was observed at 80% of IVDU, at 9% of YS and at 14.1% of YA ($P < 0.05$). At the admit to hospital 26.8% of IVDU had altered mental status from the dormancy to the coma, 53.2% of patients of this group were excited and aggressive. YS and YA didn't have same violations. Haemophtysis was observed at 1% of YS, at 2% of YA and at 33.3% of IVDU($P < 0.05$). Chest pain disquieted 47.5% of YA, 72% of YS and 73.3% of IVDU($P < 0.05$). The dyspnoea was at 21% of YA, at 30% of YS and at 70% of IVDU($P < 0.05$). Rheumatic paint, myalgias and digestive dyspepsia were observed at 5% of YA, at 3% of YS and at 30% of IVDU ($P < 0.05$). Objectively, except for the CAP signs, there were the cardiovascular changes: tachycardia was at 27.3% of YA, at 15% of YS and 86.7% of IVDU, hypotonia – at 34.3% of YA, at 33% of YS and at 60% of IVDU ($P < 0.05$). The hepatolienal syndrome was observed at 3% of YA, at 4% of YS and at 50% of IVDU ($P < 0.001$). Radiographic pattern of CAP was presented small nodal or segmental infiltration at 81% of YS and at 85.9% of YA. Lobe pulmonary consolidation or nonintensive, many focal, confluent infiltrates were present at 70% of IVDU ($P < 0.001$). The thin-walled multiple cavities without an air-fluid level and with poor inflammatory board were formed at 30%. The pathogen of CAP was established at 29% of YS 29%, at 18.2% of YA and at 53.3% of IVDU. There was *S. pneumoniae* at 31.4% of YS and 56.7% of YA. *S. aureus* was a main pathogen at IVDU(62.5%). The complicated disease was observed at 9% of YS, at 30.3% of YA and at 86.7% of IVDU ($P < 0.001$). At IVDU sepsis was developed at 50%, septic endocarditis with affect the right side of the heart was at 20%. The 13.3% of this group had infectious-toxic shock. The 13.3% of patients died. Infectious-toxic shock dominated at YS (4%) and pleural effusion dominated at YA (8.1%).
Conclusions: CAP at IVDU unlike YA and YS is characterised by the severe, complicated clinic and unfavourable prognosis.

R2028 Orchiepididymitis: a manifestation of brucellosis

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Objectives: To estimate the characteristics of primary orchiepididymitis of *Brucella melitensis* in the region of the mountainous region of Achaia.
Methods: This is a retrospective study and included 127 patients with orchiepididymitis that admitted our hospital during 1995–2003. The mean age of patients was 43.75 $\pm$ 9.38 years and the mean duration of hospitalisation was 7.11 $\pm$ 4.18 days. The seroreaction Wright was performed by the Difco *Brucella* antigen and the blood culture were processed and cultured by the Bactec 9120 (Becton Dickinson). The laboratory analysis also included blood examination and ultrasound.
Results: 10 (7.9%) of the 127 patients with mean age 38.65 $\pm$ 8.75 appeared with unilateral orchiepididymitis due to *Brucella melitensis*. All the patients were farmer–breeders. All the patients had a painful inflation of one of the testicles and fever >38°C. nine

(90%) patients had redness of the same order of semiscrotum and five (50%) patients had gentle dysuria. All patients had positive the seroreaction Wright and eight (80%) had positive blood culture for *Brucella melitensis*. All the patients had leukocytosis with a slight lymphocytosis and their urine culture was negative. The ultrasound showed inflation of testis and epididymis. All patients were treated with Doxycycline 200 mg and Rifampicin 900 mg daily p.os for 6 weeks. The patients were also treated with anti-inflammatory drugs and their testicles were suspensioned by little pillows. The majority of patients showed a significant remission in the sixth to eigth day of treatment. The patients were followed up in 3, 6 and 12 months and found to have negative blood culture, a normal ultrasound, a normal spermodiagram and a low title of seroreaction Wright.

Conclusions: Orchiepididymitis as the main manifestation of brucellosis was not rare during the last years in the region of Achaia where the disease is endemic. The mean duration of hospitalisation has no considerable difference than orchiepididymitis due to other causes. The disease goes on to cure with the proper treatment. In patients with orchiepididymitis that have come from mountainous region of Achaia we have always to consider of *Brucella melitensis* as a cause.

R2029 Induced sputum cell count at different infectional aetiology of COPD exacerbation

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Objectives: To reveal mean cytological markers at different infectional aetiology of COPD exacerbations.

Methods: Clinical inspection of patients, Gram bacterioscopy of sputum smears, quantitative bacteriological sputum research with definition of germ concentration in 1 mL of sputum, definition of *Chlamidia pneumoniae*, *Mycoplasma pneumoniae* antigens in sputum via PCR method, IgG, IgM levels to them by immuno-assay method, induced sputum (IS) cytological research were made. Kruskell–Wallis analysis of variation was used. The COPD exacerbations caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamidia pneumoniae*, *Mycoplasma pneumoniae*, *Moraxella catarrhalis* were analysed.

Results: Common cell count in 1 ml of IS at COPD exacerbations caused by *Mycoplasma pneumoniae* was reliable most low ($1.87 \pm 0.16 \times 106$/mL, $P < 0.05$), but at other aetiology of COPD exacerbations it was comparing and was defined from $2.46 \pm 0.12 \times 106$/mL to $2.74 \pm 0.32 \times 106$/mL. Percentage of macrophages in *Mycoplasma pneumoniae* COPD exacerbation was reliable most high ($31.91 \pm 3.99\%$), but in *Moraxella catarrhalis* and *Haemophilus influenzae* COPD exacerbations it was reliable most low ($16.92 \pm 1.57\%$, $17.58 \pm 3.97\%$ accordingly, $P < 0.05$). Their absolute count in the group was comparing. Percentage and absolute count of neutrophiles in *Haemophilus influenzae* COPD exacerbation were reliable most high ($70.35 \pm 3.72\%$, $1.83 \pm 0.13 \times 106$/mL), and in *Mycoplasma pneumoniae* COPD exacerbation it was reliable most low ($48.70 \pm 5.42\%$, $P < 0.05$, $0.969 \pm 0.146 \times 106$/mL, $P < 0.05$ accordingly). Percentage and absolute count of eosinophiles in *Moraxella catarrhalis* and *Streptococcus pneumoniae* COPD exacerbations were reliable most high ($3.09 \pm 0.64\%$, $0.061 \pm 0.015 \times 106$/mL, $2.82 \pm 0.35\%$, $0.061 \pm 0.008 \times 106$/mL accordingly), but they were most reliable low in *Haemophilus influenzae* COPD exacerbation ($1.35 \pm 0.46\%$, $P < 0.05$, $0.036 \pm 0.013 \times 106$/mL, $P < 0.05$ accordingly). Percentage of lymphocytes in *Mycoplasma pneumoniae* COPD exacerbation was reliable most high ($17.70 \pm 4.37\%$), in *Haemophilus influenzae* COPD exacerbation they was reliable most low ($9.13 \pm 1.47\%$ $P < 0.05$). Their absolute count in the groups was comparing.

Conclusion: Thus there are pathogenesis features of inflammation at COPD exacerbation in dependence on aetiology of infectious process that is reflected on induced sputum cell count.

R2030 Infectious diseases as a major cause for hospitalisation in internal medicine wards

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Introduction: Infectious diseases (I.Ds) remain one of the most common causes for hospitalisation in general and in Internal Medicine Wards (IMW) in particular.

Objectives: To evaluate the percentage of patients hospitalised in I.M.W due to I.Ds and to compare demographic and clinical characteristics with patients hospitalised due to cardiovascular diseases.

Materials and methods: The study was conducted during the - months February, May, August and October 2002 in the Internal Medicine Ward B of Ha'Emek Medical Center, Afula, Israel. During this period, 792 persons were hospitalised; 181 (22.9%) due to I.Ds, and 324 (40.9%) due to cardio-vascular diseases (C.V.Ds). The patients with I.Ds were significantly younger (62.6 vs. 66.3 years, $P < 0.0001$); length of hospitalisation was longer (5.78 vs. 4.6 days, $P = 0.001$) and mortality rate was twice higher than of the C.V.Ds patients (7.7% vs. 2.2%, $P = 0.006$). As expected, respiratory tract infection was the main reason for hospitalisation (48.6%), while 28.2% were hospitalised due to pneumonia. Urinary Tract Infections were the second leading reason, 18.8%. Regarding underlying diseases, 6.2% of the I.Ds patients had underlying diseases vs 13.8% of the C.V.Ds patients ($P = 0.001$). The frequency of diabetes was similar in both groups (27.1% vs. 36.1%). 138 of 181 patients received antibiotics (76.2%); beta-lactams - penicillins 42% and cephalosporins 13.8% were the most common antibiotics prescribed, followed by quinolones (12.7%).

Conclusions: I.Ds represent one of the most frequent reasons for hospitalisation in Internal Medicine Wards, creating a heavy burden regarding beds occupancy and financial expenses. Therefore, intensive programs of diagnosis and treatment of infectious diseases in the community should be implemented, in order to decrease hospitalisation rates and accompanied expenses.

R2031 Complications of *Brucella* infection among adults: a 16-year retrospective evaluation

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Objective: Brucellosis is a systemic infection in which any organ or system of the body can be involved. We evaluate here complications involvement of *Brucella* infection in Erzurum, Turkey.

Methods: We analysed retrospectively 216 adult (89 female and 127 male) cases of brucellosis, which were diagnosed and treated in Infectious Disease Clinic in Ataturk University Research Hospi-

Table 1.

Complications	N%
Skeletal	147 (68.1)
Spondylitis	63 (27.9)
Sacroileitis	118 (56.2)
Peripheral monoarthritis	19 (8.8)
Peripheral poliarthritis	46 (21.3)
Nervous system	9 (4.2)
Cutaneous	9 (4.2)
Endocarditis	5 (2.3)
Orchitis	7 (5.5)
Thrombophlebitis	2 (0.9)
Hematologic	6 (2.8)
Thrombocytopenia	4 (1.9)
Pancytopenia	2 (0.9)
Gastrointestinal system	
Hepatitis	7 (3.2)

tal from 1985 to 2002. The diagnosis was based on clinical findings and serologic results and/or blood culture confirmations.
Results: Complications in 216 patients with brucellosis are shown in Table 1.
Conclusion: The most common complication of brucellosis was skeletal involvement, followed by orchitis, nervous, cutaneous and other complications. Primary health care physicians should be alerted regarding the clinical and laboratory findings of *Brucella* complications.

R2032 A retrospective analysis of the clinical and microbiological characteristics of acute bacterial meningitis in Crete, Greece during a 9-year period

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Objectives: Acute bacterial meningitis is a life-threatening infection. The aim of this study was to evaluate the clinical and laboratory characteristics of the disease in the area of Heraklion of the island of Crete, Greece.
Methods: Over a period of 9 years (1994–2002) 48 patients with clinical and laboratory diagnosis of bacterial meningitis cured for in the Department of Medicine of the University Hospital of Heraklion, Crete were retrospectively reviewed.
Results: Twenty five men (55%) and 23 (45%) women with acute bacterial meningitis were studied. The median age of the patients was 25 (1–77) years. Sepsis criteria of systemic inflammatory response syndrome (SIRS) were present in 41 (85%) patients. The most frequent clinical manifestations were fever in 40 (83%), headache in 36 (75%) and vomiting in 29 (60%). Skin rash was present in 12 (16%) patients. Clinical signs of meningeal inflammation such as nuchal rigidity, and Brudzinsky's and Kerning's signs were present in 34 (71%), 10 (21%) and eight (17%) patients respectively. Photophobia was observed in 30 (63%) patients while changes in mental status were noted in 10 (21%). Microorganisms were isolated in the cerebrospinal fluid (CSF) cultures of 23 (48%) patients. The most common microorganism isolated was *Streptococcus pneumoniae* in nine (39%) of 23. No strains of *S. pneumoniae* resistant to penicillin were detected. Other responsible microorganism identified were *N. meningitidis* in seven (30%), *Listeria monocytogenes* in three (13%), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Streptococcus suis*, and *Brucella* spp. were isolated in one patient each (4%). Antibiotic treatment was given in all patients on empirical basis on admission. The regimens were proven appropriate based on results of the CSF cultures, in all patients with identified bacteria (48%). Forty-six of the 48 patients (96%) had a favourable outcome. The reason of failure was delay in the initiation of the appropriate antibiotic in one patient. The other died despite early initiation of appropriate treatment because meningitis was fulminant.
Conclusions: Acute bacterial meningitis remains a serious infection. However, early diagnosis and treatment may reduce fatal outcome and improve the severity of the disease. In the present series the causative organisms of community-acquired meningitis were mainly *S. pneumoniae* and *N. meningitidis*. No strains of *S. pneumoniae* resistant to penicillin were detected.

R2033 Vaginal exudates. Aetiology and epidemiology in outpatients

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Objectives: To study the incidence of pathogens resposible of vaginal infection in outpatients of the Centre Area of Madrid (Spain).
Methods: 1530 patients were studied between November 2002 and April 2003. The patients were asked about: age, nationality and symptoms. The samples were studied by microscope and cultured in blood agar, Sabouraud agar and chocolate-agar when clue cells were viewed. In pregnant women with more than 20 weeks, the samples were inoculated in Todd medium for isolating *S. agalactiae*. The identification and sensibility were performed by the MicroScan System (DADE-Behring), according to NCCLS criteria.
Results: The patients' ages varied from 3 to 85 years old. The symptoms were: pregnancy 637 (41.6%), itch 392 (25.6%), flow 142(9.3%), post-treatment 108 (7.1%), IUD change 50(3.3%), pain 45 (2.9%), inflammation 10 (0.7%), discomfort 46 (3.0%), sting 40 (2.6%), aontrol 57 (3.7%) and bleeding three (0.2%). Among 1530 patients,752 (49.2%) were from Spain and 770 (50.8%) from foreign countries:603 (39.4%) South America, 68 (4.4%) Europa, 56 (3.7%) Asia, 49 (3.2%) Africa and 2 (0.1%) North America.

The positives samples were: 491 (32.1%). The microorganisms isolated were: *Candida* sp. 389 (79.22%), *G. vaginalis* 40 (8.14%), *S. agalactiae* 33 (6.72%), *E. coli* 19(3.86%) *T. vaginalis* 12 (2.44%), *H. influenzae* three (0.6%), *S. pyogenes* 2(0.4%), *S. aureus* 2 (0.4%) and other three (0.6%). The samples with two or more pathogens were 13 (2.6%). The positives samples were isolated 238 (48.5%) in Spanish women and 253 (51.5%) in foreign women.

The number of pregnant women was: 637 (41.6%); 266 (41.8%) Spanish and 371 (58.2%) foreigner.

The percentage with *S. agalactiae* in pregnant women was: 15 (2.4%).
Conclusions: The number of foreign patients was higher than Spanish patients. *Candida* sp. was the pathogen more frequent. *G. vaginalis* was more frequent in South American women. The positive samples were more frequent in foreign women. The percentage of *S. agalactiae* isolations in pregnancy was lower than other published works.

R2034 Typical and atypical pathogens in lower respiratory tract infections: a 2-year serological survey in a general hospital, Perugia

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Objectives: The 'atypical' pathogens are important agents of lower respiratory tract infection, causing mild to life-threatening diseases. The most common atypical pathogens are *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Others include *Legionella* ssp., influenza, parainfluenza, respiratory syncytial virus and adenovirus. Infection rates for these agents are difficult to determine because many clinicians do not routinely test for them. The aim of this study was to evaluate the serological results obtained during a 2-year survey in patients affected by lower respiratory tract infections.
Methods: Nine hundred and sixty-eight patients were examined from October 2001 to September 2003. Patients were classified according to age, clinical symptoms and suspected diagnosis. Two serum samples were collected from each patient in the acute and convalescent periods. The following serological tests were used: automated complement fixation (CF) for detection of total Ig to adenovirus, A and B influenza virus, parainfluenza virus and respiratory syncytial virus; CF for detection of total Ig and enzyme immunoassay for IgM against *M. pneumoniae*. Specific IgG and IgM antibodies to *C. pneumoniae* were determined by a microimmunofluorescence test (MIF). Total Ig to *Legionella pneumophila* was detected by indirect immunofluorescence assay (IFA). An acute infection was defined by seroconversion or IgM positivity.
Results: Forty-one per cent of patients were over 60 years old. Pneumonia was the suspected clinical diagnosis in 47.2% of patients. The others manifested fever (12.8%), acute exacerbation of chronic bronchitis (7.6%) and cough (4.3%). In 28.1% cases the clinical manifestations were not related to respiratory disease or

not reported. Acute infection by *C. pneumoniae*, *M. pneumoniae* and influenza B virus was ascertained in 7.9, 4.0 and 0.4% of patients with pneumonia, respectively. Acute infection by *C. pneumoniae* was also demonstrated in 11.0% of patients with acute exacerbation of chronic bronchitis, in 3.2% of patients with fever and in 4.8% of patients with cough. Furthermore, acute infection by influenza B virus was observed in 5.4% of patients with acute exacerbation of chronic bronchitis.
Conclusion: The study confirms the relevance of atypical pathogens as aetiological agents of pneumonia and acute exacerbation of chronic bronchitis, and underlines the need to routinely test for them in case of lower respiratory tract infections.

R2035 Mortality risk factors in community-acquired pneumococcal disease

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Objective: To describe the risk factors associated to mortality from invasive pneumococcal disease in a university hospital during an 11-year period.
Material and methods: We performed a retrospective review of case histories from invasive pneumococcal disease patients attending the emergency department of the Fundacion Jiménez Díaz between January 1993 and August 2003. We registered mortality, medical- microbiological evolution and disease relapses.
Results: A total of 263 cases of *S. pneumoniae* bacteraemia and invasive disease (pneumonia, meningitis, sepsis, bacteraemia without focus and arthritis) were assessed. The mortality was 12.5 %. In the logistical regression analysis the variables associated with mortality from bacteraemia and invasive pneumococcal disease were the absence of leucocytosis ($P = 0.04$), acid blood pH ($P < 0.01$), respiratory symptoms or signs (chest pain, wheezing and high respiratory rate) ($P = 0.02$) and neurological manifestations (confusion) ($P < 0.01$).
Conclusion: In our hospital, patients critically ill without leucocitosis, but with serious respiratory or neurological manifestations and undergoing invasive procedures such as mechanical ventilation or tracheotostomy were prone to death from invasive pneumococcal disease.

R2036 Treatment of *Chlamydia pneumoniae* infection in asthmatic patients

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Objective: Chronic *Chlamydia pneumoniae* infection has been suggested, as a cause for adult onset of asthma. The purpose of this study was to determine weather anti-chlamydial treatment with azithromycin would reduce asthma symptoms and improve lung function in asthmatic patients.
Methods: The patients with moderate to moderately severe asthma were included in the study. Enrolled patients were treated with 1000 mg azithromycin once weekly for 6 weeks. Post-treatment lung function and symptom score were compared with baseline values.
Results: Thirty patients (mean age 35.5 years), with moderate to moderately severe asthma, with proved *Chlamydia* infection, detected by specific IgA 1: 40 and specific IgG 1:256 against *Chlamydia pneumoniae*, received 1000 mg azithromycin once weekly for 6 weeks. Six weeks after azithromycin treatment the significant reduction in symptom score ($P < 0.01$) and significant improvement in lung function FEV1 ($P < 0.01$) were observed.
Conclusions: Treatment with azithromycin significantly improved asthma symptoms and lung function indicating that *Chlamydia pneumoniae* may play an important role in enhancing the inflammatory processes in lower airways.

R2037 Survey of Legionnaires' disease agents in treatment devices and drinking water reservoirs in Iran

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Objectives: *Legionella* species are ubiquitous in both, natural and man-made water systems, which spread by the inhalation, or aspiration of aerosolised organisms rose from water. Survey of Legionnaires' disease agents in treatment devices and drinking water reservoirs in Iran, was the objective of this study.
Methods: In a 9-month study, 210 samples collected from different water sources, such as: neonatal incubators, dental units, water baths, cooling towers, drinking water reservoirs, hot water taps or showers and little pools in parks, were examined. Sediment of water samples were inoculated onto selective and nonselective media such as : BCYE, BMPA and MWY, before and after acidic treatment with KCl–Hcl buffer. Isolated colonies, which were suspected to be *Legionella*, were examined by tests such as : fluorescence of colonies under UV lights, no growing on ordinary media (as EMB, McConkey or blood agar) and standard biochemical tests.
Results: This study reSsulted in detection of 14 isolates (6.6%) of *Legionella*, which were distinguished to nine isolates(64.3%) of *Legionella pneumophila* and five isolates 35.7%) of *Legionella* spp. Most of the contaminated sources were dental units (with 19%) and the lowest of them (with 2.9 %) belonged to hot water taps and showers. Two isolates of *Legionella* were identified from each of examined sources, such as dental units, water baths and cooling towers.
Conclusion: The results of this study showed that treatment equipments and different water sources, as drinking water reservoirs, were contaminated by *Legionella pneumophila* and so, regulatory disinfection of these sources is necessary for prevention of aerosols transmission which are contaminated to Legionnaires' disease agents.

R2038 *Flavimonas oryzihabitans* peritonitis in an adult patient on continuous ambulatory peritoneal dialysis

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Objectives: To report a case of peritonitis caused by *F. oryzihabitans* in a patient on continuous ambulatory peritoneal dialysis (CAPD).
Methods: Peritoneal effluent specimens were examined for cells count and after centrifugation were cultured onto appropriate media and inoculated in brain heart infusion broth. Ten millilitres of the samples were also inoculated in blood culture bottles (Bactec Plus, Becton Dickinson Sparks, Maryland, USA). The identification of the microorganism was performed by standard methods, colonies morphology and API ID32 system (bioMerieux). Susceptibility testing was performed by disc diffusion method according to the NCCLS performance standard.
Case report: A male patient aged 87 years on CAPD with end stage renal failure (nephroangiosclerosis), was admitted to the hospital because of turbidity of peritoneal effluent. He started CAPD five months ago. He was hypertensive and suffering from coronary artery disease. This was his 3-day peritonitis episode (previous two 30 and 45 days ago, the first due to *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and the second to *Staphylococcus aureus* CNS. For their treatment tobramycin I.P. and cefazolin I.P. were administered, respectively. On the day of admission, WBCs count of peritoneal fluid was 300/ml (82% neutrophils) and was increased to 1000/l (88% neutrophils) the next day. From three peritoneal effluent cultures *F. oryzihabitans* was isolated. The strain was resistant to first and second generation of cephalosporines, to trimethoprime/sulfamethoxazole, to aztreonam, to ampicillin and susceptible to third generation of cephalosporines, to aminoglycosides, to amoxicillin/clavulanic acid, to ciprofloxacin

and to imipenem. The patient was treated with tobramycin 20 mg × 4 I.P. per day for 3 weeks and he recovered completely.
Conclusions: *F. oryzihabitans* is an infrequent cause of opportunistic infections, especially in patients with the presence of a foreign material or catheters (peritoneal, central venus). This is the first case report of peritonitis with this agent in a CAPD patient in Greece and the ninth worldwide. *F. oryzihabitans* should be included in the potential pathogens that cause peritonitis in patients on CAPD.

R2039 Peritonitis in patients on continuous ambulatory peritoneal dialysis

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Objectives: The study of peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD) during a 2-year period (2002–2003).
Methods: 250 peritoneal fluid specimens from 25 adults patients on CAPD, aged 31–86 years (median 65 years) were examined during the study period for WBC count and detection of microorganisms by Gram stained smears. They were cultured on appropriate media for aerobic and anaerobic microorganism after centrifugation and were also inoculated in Bactec culture vials (Becton Dickinson). The identification of microorganisms was performed by standard methods and API systems (bioMerieux). The susceptibility testing was carried out by disc diffusion method and the susceptibility testing of yeasts by microdilution method and by E-test (AB biodisk, Solna, Sweden). Also in special cases the specimens were examinated of the presence Mycobacterium spp.
Results: Peritonitis developed 11 patients (44%) in overall 27 episodes. Peritonitis rate was one episode per 18.3 patient-month. From the patients 3/11 had one episode, 4/11 two, 2/11 three, 1/11 four and 1/11 six. In 29.6% of the peritonitis cases were polymicrobial. No episodes with amicrobial peritonitis were noticed. The most frequent isolated bacteria were CNS (37%), *Streptococcus* spp. (11%) and *Enterococcus faecalis* (7.9%). Fungi were isolated in three patients, one of them died from this cause. It is interest the isolation of very rarely reported microbial species, as *Streptococcus vestibularis*, *Alcaligenes faecalis*, *Corynebacterium jejunem*, *Aeromonas hydrophila*, and *Flavomonas oryzihabitans*.
Conclusions: Peritonitis was frequent in the CAPD population studied. The most frequent causative agents were CNS and *Streptococcus* spp. Unusual causative agents were also isolated. The fungus peritonitis were treated with appropriate antifungal administration, catheter removal and replacement after 6 weeks.

R2040 Lyme neuroborreliosis, viral co-infections and collateral conditions – a case series in a teaching hospital of infectious diseases

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Objectives: To present diagnostic difficulties and treatment hints in neuroborreliosis.
Methods: Chronic Lyme disease was diagnosed based upon the known criteria (duration – more than 1 year, persistent neurological involvement, active infection with *Borrrelia burgdorferi*) and meeting the surveillance CDC ranking for Lyme borreliosis highly likely of more than seven points. Between 2000 and 2003 we studied five patients (age range 22–57 years) in the Cluj-Napoca Teaching Hospital of Infectious Diseases presenting evidence of immunity to *Borrelia burgdorferi*: immunoflurescence, IgM antibodies (ELISA) and polymerase chain reaction and chronic neurological manifestations with no other identifiable cause. Neurological involvement was clinically evaluated and pathological findings were completed through analysis of cerebrospinal fluid, electroencephalograms, electromyographies and magnetic resonance tomographies. A great number of other laboratory tests were performed including evaluation of endocrine abnormalities, viral co-infections and autoimmune disorders.
Results: The neurological manifestations were represented by leucoencephalitis with epileptic manifestations, encephalomyelitis with symmetric spastic tetraplegia and in the remaining three cases polyneuropathies with cranial nerves involvement. All cases experienced fatigue, headache, poor stamina, myalgia, low-grade fever and mild psychiatric disorders. In two cases active cytomegalovirus infection coincident with reactivation of neuroborreliosis was found and in another case hepatitis C virus infection with vasculitis was detected. Endocrine abnormalities were demonstrated in two cases in the presence of clinical hypothyroidism. Electrolytes disorders were refractary to therapy in one case. Treatment and retreatment with intravenous ceftriaxone 2 g/day, 3–4 weeks was performed in all cases. In one case Jarish-Herxheimer reaction was observed after 3 days of treatment involving short time relapse in neurological manifestations. Combination therapy with macrolides and ciprofloxacin was considered in one case. Orally ganciclovir was administered if primary cytomegalovirus coinfection was present. Improvement occurred during the first treatment cycle, all cases were considered cured after 2–4 treatment cycles, other therapies and rehabilitation were also performed.
Conclusion: Diagnosis and treatment of late Lyme neuroborreliosis are difficult, antibiotic treatment greatly improves the prognosis.

R2041 Evaluation of brain-stem auditory potential in brucellosis patients with and without neurobrucellosis

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Objectives: Brucellosis may present with a wide variety of manifestations but involvement of the nervous system is rarely seen. Brain-stem auditory evoked potentials are generated by the auditory nerve and the brain-stem in response to a click stimulus. Involvement of the auditory pathway within the brain-stem in patients with brucellosis has been reported in a few studies. In this study, we investigated the changes in brain-stem auditory evoked potentials in patients with brucellosis.
Methods: Twenty-nine patients older than 14 years diagnosed with brucellosis in the Department of Infectious Disease and Clinical Microbiology of Ankara Training and Resarch Hospital were included. The diagnosis of brucellosis was based on the following criteria: (1). Clinical picture compatible with brucellosis and (2). detection of specific antibodies by standard tube agglutination testing at 1/160 titres or a fourfold or greater rise in antibody titer in serum specimens drawn 2–3 weeks apart or (3). positive blood or bone marrow culture. Seven of 29 patients were neurobrucellosis. No patient had a symptom of hearing loss. Brain-stem auditory evoked potentials were evaluated in all patients before treatment.
Results: Demographic characteristics of brucellosis patients with and without neurobrucellosis were compared and were not found significant difference. Patients who had neurologic involvement didn't show any abnormalities in their brainstem auditory evoked potentials recording. Only one brucellosis patient without neurobrucellosis showed prolonged I–III interpeak latencies but this finding was not statistically significant.
Conclusions: In this study, we suggest that brucellosis patients who were in acute phase of the illness with or without neurobrucellosis and had no hearing problem, may have normal brain-stem auditory potentials. The recording of brain-stem auditory evoked potentials may not be a sensitive method for involvement of the nervous system in brucellosis.

R2042 Coronary artery mycotic aneurysm due to *Salmonella enteritidis* presenting eith pericardial effusion: a case report

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A 55-year-old man was hospitalised because of dyspnea and large pericardial effusion detected on echocardiography. *Salmonella enterica* subspecies enterica serotype enteritidis was isolated from the culture of haemorrhagic pericardial effusion. Computed tomography and cardiac magnetic resonance imaging detected an aneurysm in right coronary artery which was subsequently confirmed by coronary angiography. Despite appropriate antibiotic treatment and emergency surgical intervention, the patient died due to rupture of the aneurysm. Pathological examination revealed mycotic coronary artery aneurysm and acute inflammatory involvement of pericardium. In this case mycotic coronary artery aneurysm due to *Salmonella* infection occured in the absence of infective endocarditis and a definite reason for bacteraemia. However, this is the second case of pericarditis caused by *S. enteriditis* in Turkey.

R2043 Eleven years of bacteraemia caused by *Brucella* spp. in a Madrid hospital

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Leganés, E

Objectives: Description of the epidemiology and clinical characteristics of *Brucella* spp. bacteraemia detected in a 450 bed community-based Hospital during eleven years (1990–2001).
Methods: Retrospective study on the clinical records of patients with *Brucella* spp. bacteraemia. For isolation of *Brucella* in blood cultures Bactec 660 was used from year 1990 up to the end of 1994 and therefore was changed to Bactec 9240 allowing a reduction of the isolation time.
Results: During the 11-year period included in the study, 41 strains were isolated (0.9% of all relevant bacteraemias). At the moment of obtaining blood cultures, 37% of patients with *Brucella* bacteraemia were outpatients and 63% were admitted in hospital. The median age of patients was 36 years (range 6–90) and 62% were male. Nineteen patients (46%) consulted for fever and malaise, 34% had fever and arthralgia without patent arthritis, 8% had orchiepididymitis, 8% showed arthritis, and 5% had a previous diagnosis of brucellosis. An epidemiology background compatible with brucellosis (raw milk or cheese compsumtion, or laboral exposure) was found in 81% of the cases. In 54% of the cases, paired acute and convalescence sera were tested (Rose Bengal and COOMBS tests); in 31% only one sample of the acute stage could be tested: in 8% of the patients no serologic tests were made. Concomitant clinical and serologic relapse was found in 8% of cases. A 15% of patients gave up the treatment prematurely. Sacroileitis was the most frequent complication (observed in 19% of our patients).
Conclusion: Bacteraemia caused by *Brucella* spp. is still relativelly frequent in our environment, and should be still suspected in young adults with fever, malaise, arthralgia, and/or orchiepididymitis. Serological tests are a useful tool for the follow up of patients.

R2044 3-Day azithromycin course with and without ambroxol: clinical comparative trial of efficacy and safety in the treatment of community-acquired pneumonia in adults

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Objective: To assess the clinical efficacy and tolerability azithromycin treatment with and without of mucolytic agent – ambroxol in the adult patients with CAP.
Methods: The patients with uncomplicated CAP, hospitalised in the Main military hospital of the ministry of defense, Kiev, Ukraine, form January to April 2003, were included in open randomised study. Diagnosis of CAP was established according to clinical data (fever, cough, general symptoms) and chest radiography demonstrating the presence of a new infiltrate. The patients were randomly assigned to treatment with azithromycin (SumamedR PLIVA. Croatia) administered orally 500 mg once daily for 3 days (group I) or equal dosage of azithromycin in combination with ambroxol (LasolvanR Boehringer Ingelheim. Germany) 30 mg three times/day for 14 days (group II). Cure was defined as resolution of fever within 96 h after initiation of treatment with disappearance of other signs and symptoms of pneumonia, and infiltrate regression within 2 weeks. Treatment efficacy was also evaluated by duration of fever after treatment initiation.
Results: Thirty-one male adult patients were enrolled in the study. Among them 15 patients were randomly assigned to treatment with azithromycin alone (group I) and 16 patients (group II) in combination with amoroso. Two treatment groups were demographically well matched. At the end of therapy clinical success was achieved in 14/15 patients (93%) in the group I and in 14/16 of patients (88%) in the group II. The results showed almost equal efficacy in both treatment groups ($P > 0.05$). The resolution of the signs and symptoms of pneumonia was similar in both groups. Both treatment regimens were well tolerated.
Conclusion: Azithromycin was effective and well tolerated in all patients with CAP. No statistical difference was found in clinical efficacy of the azithromycin treatment if it used alone or with mucolytic agent.

R2045 Specific antibodies IgG-IgM for cytomegalovirus detection in women of reproductive age between the second semester of 2000 and first semester of 2003

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Heraklion, GR

Objective: Measurement of specific antibodies IgG–IgM for the detection of CMV virus is useful in the diagnosis of early CMV infection. In this study we present our experience resulting from testing, in IgM-positive individuals in early diagnosis.
Materials and methods: During the 2000–2003 period 2293 women were tested. Prenatal screening was performed on those women during their pregnancy. CMV detection was made using ELISA-Cobas Core method (Roche Diagnostics). In addition, the confirmation of all positive samples was performed using ELISA (Biomerieux) on Vidas and Avidity test (Biomerieux).
Results: The following data represent our findings:
IgG(−)–IgM(−): 420 (18.31%) IgG(+)–IgM(−):1839 (80.2%) IgG(+)–IgM(+):24(1.04%) IgG(−)–IgM(+):10 (0.44%).
Conclusions: The present results show that 80.2% of the female population that was under study had been infected from the CMV virus. Much lower was the percentage (1.48%) of those cases that have recently been infected, while for the percentage of those women that have not been infected at all, a retesting is recommended throughout their pregnancy. Depending on the case, and after CMV virus diagnosis, the patient will be advised with special information in order to deal properly with the disease. Therefore, the early diagnosis of the infection is of great importance for the pregnant. Concerning the prenatal screening, it seems that its significance is based on the prevention and avoidance of unwanted dangers just in case of pregnancy.

R2046 Serological testing for rubella on women in reproductive age between 2000 (2nd semester) – 2003 (1st semester)

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Objective: Evaluation of immunity against rubella on women in reproductive age in terms of prenatal screening and during pregnancy.
Materials and methods: Between 2000 and 2003, 2135 women were tested in the hospital's microbiology laboratory. The prenatal serological screening included the IgG and IgM antibody testing for rubella. The diagnostic test was performed using ELISA method on Cobas Core (Roche Diagnostics).The confirmation of the positive samples was performed using ELISA method on Vidas and also Avidity test (Biomerieux).
Results: From a total of 2135 women tested the outcome was: IgG(−)IgM(−): 305 (14.28%) IgG(+)IgM(−):1816 (85.06%) IgG(+) IgM(+):14 (0.66%) IgG(−)IgM(+):0 (0%).
Conclusions: It is obvious from the above percentages that there is a number of seronegative women which strongly suggests the prenatal screening of all women in reproductive age in order to avoid the unwanted consequences of a first infection during pregnancy. It would be better for women to be tested for rubella antibodies before pregnancy.

R2047 Moxifloxacin therapy in high-risk patients with community-acquired lower respiratory tract infections or sinusitis

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Objectives: Oral therapy with moxifloxacin is indicated for mild/medium severe community-acquired pneumonia (CAP) and sinusitis. However, the biodisponibility is more than 90% and we evaluated the efficacy of oral therapy in patients with risk for severe respiratory/ENT infections.
Methods: Retrospective analysis of patients admitted in 'Matei Bals' Institute how received moxifloxacin between November 2002 – October 2003 for CAP, COPD exacerbation and sinusitis. We divided the patient in two groups: healthy immunocompetent adults (group A) and patients with comorbidities: COPD, congestive heart failure, diabetes, HIV (group B).
Results: 30 patients were included in group A and 31 in group B. Clinical success rate was similar in both groups: 93.33 and 93.54%; the failures were caused by misleading diagnosis of tuberculosis (group A), respectively recurrence of infection (group B). The type of infection and the regimen rank (first regimen or after failure) were similar in both groups; no differences were noted regarding mean duration of fever in cases with favorable response: 1.44 days (group A) and 1.72 days (group B), and for mean duration of therapy in sinusitis. The only difference was in mean duration of CAP therapy; it was longer in group B: 9.1 days than in group A 6.6 days. The side effects were registered in 14.5% of patients, lesser in group B.
Conclusion: Moxifloxacin administered in oral therapy is a valuable alternative for patients with respiratory tract/sinusitis, even in high-risk groups; the fast induction of apyrexia remains an important advantage of moxifloxacin.

R2048 Bacterial brain abscess: risk factors for unfavourable outcome

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Objectives: Despite the improvement of brain abscess management, the mortality rate is still high: 5–20%. The descriptions of involved factors are controversial.
Methods: 'Matei Bals' Infectious Diseases Institute database were utilised to identify all patients who had diagnosis of brain abscess during December 2001 to November 2003 period. We excluded the cases with definite or possible parasitosis (toxoplasmosis, cryptococcosis or cysticercosis). Subsequently, we evaluated the parameters correlated with mortality.
Results: Ten patients (six females, four males) fulfilled the criteria. The mean age was 19.3 years, with 8/10 patients less than 30 years old. In a half of cases the aetiology was identified, and in 6/10 patients an ENT infection was the primary source of germs. The mean diagnosis delay was 10.5 days for ENT-related BA and 28.75 days for other cases ($P = 0.02$). Nine of 10 patients associated a meningitis and six of 10 fulfilled the criteria for sepsis. Three patients died: two in the first week of hospitalisation, and one after 25 days with no initial improvement. Neither age, clinical manifestations, initial adecvate antimicrobial regimen nor abscess dimensions were related to unfavorable outcome. All the ENT-related BA patients survived and only one from other four patients ($P = 0.03$); all patients with only one abscess survieved vs. 2/5 with multiple abscesses ($P = 0.09$).
Conclusion: We identified two patterns in BA: the ENT-related are faster diagnosticated and the survival seems to be better. Multiple abscesses tend to be associated to a higher mortality.

R2049 Incidence of acute Q fever in patients with community-acquired pneumonia

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Objective: To determinate incidence of the acute Q fever in hospitalised adult patients with low respitratory tract infection.
Methods: A total of 72 hospitalised patients with CAP within 1 year period (2002/2003) were included in our study. The mean age was 45 years (range: 18–74), distributed by gender: 47 (65%) male and 25 (35%) female. Diagnosis of Q fever was established in a group of 10 patients by serological confirmation by detection of IgM, IgG or total antibody against *Coxiella burnetti* phase II Ag. with Pneumoslide M (Vircell) IF test and/or *Coxiella burnetti* IgM, IgG (Vircell) ELISA test.
Results: Acute Q infection as a cause of CAP in 72 hospitalised patient was in 10 (13.9%) patients. The patients with acute Q infection were with mean age 45 and distributed by gender: males six (60%), females four (40%). There was no significant discrepancy, from urban or rural distribution of these patients. In 1 of 10 patients with Q fever, coinfection with *Streptoccocus pneumoniae* was detected. Dominant clinical manifestation was febrile syndrome with acute onset. Altered liver function was noted in eight patients (80%), in two of them transferases were five times higher than the upper limit. Chest radiographic abnormalities were alveolar, lobar or segment infiltration in four (40%) cases, diffuse interstitial infiltrate in three (30%) cases, alveointestitial in the remaining ones.
Conclusions: Acute Q fever takes significant place among low respiratory infection. Evidence of high incidence of Q fever has created a ground for prospective study comprising several years period, which will help us to determine prevalence and epidemiological model in this region. The possibility of chronicity, points the necessity of adequate diagnosis and successful treatment.

R2050 Q fever: retrospective analysis of 25 patients (1985–2003)

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Objectives: Epidemiological, clinical, laboratorial and therapeutics characterisation of Q fever clinical cases admitted to our Infectious Diseases' Department from 1985 to 2003.

Methods: Clinical files' review of male subjects admitted to our Department with the diagnosis of Q fever, with positive *Coxiella burnetti* serologies (complemment fixation).
Results: Twenty-five clinical cases were reviewed, all caucasian, median-age of 37.6 years old (16–85 years). The main clinical features detected were fever (100% of cases), headache (80%) and myalgia (56%). Cough was present in 28% of cases and sputum in 24%. Gastrointestinal symptoms were detected in 16% of clinical cases. A diagnosis of atypical pneumonia was established in 28% of patients. In three cases (12%), a clinical evolution compatible with Chronic Q fever was seen (two cases with chronic hepatitis and one with endocarditis). Epidemiological query disclosed 50% of cases with exposure to sheep and goats. The laboratory study revealed leucocytosis in 16% of cases, leucopenia and thrombocytopenia, both in 20% of cases; in 76% of patients, an two- to threefold elevation of liver enzymes. No renal failure was detected. Monotherapy with doxycycline was used in 32% of patients, 12% was treated with an association of doxycycline and rifampicin, cloranphenicol and cotrimoxazol were used, each, in 8% of the patients. A favourable clinical evolution was seen in the majority of cases. The patients in whom Chronic Q fever was diagnosed were managed with doxycicline, rifampicine and ciprofloxacin.
Conclusion: A positive relationship of animal exposure and Q fever was established. In most of our patients Q fever presented as an auto-limited disease. Mild hepatitis was diagnosed in 76% of patients and atypical pneumonia in 28%. Three patients had a chronic evolution – 2 as a chronic hepatitis and 1 with endocarditis. Doxycicline was the main treatment option chosen, according to literature.

Nosocomial infections

R2051 Brucellosis complicating arthroplasty: two case reports

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Motril, E

Infection after arthroplasty is a serious complication associated with a high incidence of morbidity. Infection of prosthesis with *Brucella* is extremely rare.
Objectives: Presentation of two cases of brucellar infections complicating arthroplasty.
Methods: Case 1 – Men of 45 years of age with total hip arthroplasty by Perthes' illness in infancy, that presented a prosthetic slackening. In the culture of intraoperative exudate of replacement grew *Brucella melitensis*. Case 2 – Men of 69 years of age with total knee arthroplasty by gonarthrosis. Five months later, the patient developed a fistule with chocolate exudate where we isolated *Brucella melitensis*.
Results: After 2–3 days, nonhaemolytic colonies of oxidase-positive, Gram-negative coccobacilli and obligate aerobes were identified as *Brucella melitensis* for agglutination with *Brucella* antiserum. Both patient specimens were cultured in solid media (Columbia blood agar and chocolate agar) and enrichment broth. Their agglutination *Brucella* titres were 1/80 and 1/40 and Coombs test were 1/10 240 and 1/20 480, respectively.
Conclusions: Countries where brucellosis is still endemic it is recommended to evaluate all patients before arthroplasty. Screening for brucellosis should also be included if the patient has pain in their replaced joints at the follow-up examination.

R2052 The methods of postsurgical wound infection prophylaxis in patients with acute appendicitis

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Introduction: Surgical intervention for acute appendicitis takes one of the leading positions in the structure of urgent pathology in surgical department of common profile. Septic and purulent infections (SPI) developing after surgical interventions for acute appendicitis complicate the main disease, prolong the term of treatment and cause significant economic loss.
Materials and methods: The retrospective analysis of 1425 acute appendicitis cases (catharal – 309; destructive – 1126) surgically treated in third Minsk clinic during 1992–2002 was performed. Treatment of these patients included complex methods of wound purulent-septic complications prophylaxis based on protection of wound edges by antibacterial surgical serviettes (52 patients); decontamination of wound by CO_2 laser irradiation or by mechanical stream washing with isotonic solution (89 patients). Two components (rational empiric antibiotic therapy + stream washing with isotonic solution, 351 patients) and three component (rational empiric antibiotic therapy + protection of wound edges + stream washing of wound, 64 patients) prophylactic methods were also analysed. 869 conventionally treated patients formed a control group. Groups analysed were comparable for appendicitis form, surgical intervention, age and sexual structure.
Results and discussion: The research conducted allowed to decrease the rate of septic-purulent complications and to improve the outcome of treatment of patients with acute appendicitis. Application of isolating serviettes decreased the rate of septic-purulent complications (3.84 + 2.65) compared with control group (10.13 + 1.02%), $P < 0.05$. Stream washing of wound with isotonic solution reduced complications insignificantly (6.74 + 2 65%), $P > 0.05$. Two and three component complex methods decreased the complications rate to 5.69 + 1.26% and 3.12 + 2.18, respectively ($P < 0.01$). The microbiological monitoring of aetiological structure of postsurgical septic-purulent infection-causative agents and determination of their resistance to 26 antibiotics and eight antiseptics were also conducted. The main pathogens were enterobacteria, nonfermenting Gram-negative bacteria especially *P. aeruginosa* and *E. faecium* and *S. epidermidis*.
Conclusion: The worked out complex of measures for wound septic-purulent infection prophylaxis in acute appendicitis ensures the decrease of complication rate (to 3.12 + 2.18%) and severity and reduces the term of treatment.

R2053 Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) as nosocomial pathogen in a clinical centre, Serbia

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial agents. The spread of methicillin-resistant staphylococci has become an alarming problem throughout the world. The object of this study was to determine the frequency of MRSA, as a nosocomial agent, in different hospitals of the Clinical Center Serbia.
Methods: All clinical strains of *S. aureus*, isolated from June 1, 2001 to April 30, 2002, in patients hospitalised in five different hospitals of the Clinical Center Serbia, were included in the study. Antimicrobial susceptibility testing was performed by agar dilution method. The susceptibility testing and interpretation of the results were carried out according to the Torlak (Institute for Immunology and Virology) guidelines.
Results: A total of 389 SA strains in hospitalised patients, isolated from skin and soft tissue, respiratory samples, body fluids, urine and blood, obtained from June 1, 2001 to April 30, 2002, was tested. 363 (93.3%) MRSA and 26 (6.7%) MSSA were found. The prevalence of MRSA did not vary significantly between different hospitals. The highest rates of resistance to methicillin were found in the Urgent Surgery (98%), the institutes for urology (94.4%), abdominal (93.6%), cardiovascular surgery (93.3%) and nephrology (85.1%). Most of these methicillin-resistant isolates were susceptible

only to vancogal. In the Urgent Surgery, almost all isolates were found in patients who were treated in the intensive Care Unit.
Conclusion: Our study indicated that the rate of MRSA as a nosocomial agent was high and that the rate of resistance to other antibiotics was on the increase, which might be a serious problem for the treatment of hospitalised patients. Good infection control measures for reduction of the incidence of MRSA transmission is indicated.

R2054 Recurrent necrotising fasciitis caused by methicillin-resistant *Staphylococcus aureus*

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Objective: Colonisation with methicillin-resistant *Staphylococcus aureus* (MRSA) with subsequent recurrent soft-tissue infection is well documented. Recurrent necrotising fasciitis due to MRSA however has not been previously noted.
Methods: A 57-year-old female, with significant co-morbidities of diabetes and long-term steroid ingestion, was admitted for right thigh necrotising fasciitis. She was successfully treated and was discharged after an appropriate course of antibiotics. Tissue culture isolated MRSA. Nasal swabs taken were negative for MRSA carriage. Three months later, she was readmitted for left hand necrotising fasciitis. Tissue and blood cultures again isolated MRSA. She was successfully treated with early aggressive operative débridement. Again nasal swabs were negative for MRSA and abdominal CT and heart echocardiography were negative for occult sites of infection.
Results: The recurrence of necrotising fasciitis caused by the same bacteria has not been previously documented. The MRSA isolated had identical antibiogram. However, genomic fingerprinting was not performed for the MRSA isolates as the specimen from the first admission were unavailable for analysis. The identical antibiotic susceptibility pattern and close temporal relationship between the two episodes of necrotising fasciitis suggested that these infections were caused by the same MRSA bacterial strain. While the nasal swabs were negative on both admissions, other potential sites of colonisation and carriage such as axilla and the groin were not routinely investigated.
Conclusion: Recurrent MRSA necrotising fasciitis should be actively excluded in patients with previous episode of fasciitis. Patients who developed MRSA necrotising fasciitis may imply an underlying susceptibility and possibly colonisation with virulent strains of MRSA. Recurrent soft-tissue infections in a patient with previous MRSA necrotising fasciitis should be treated with a high index of suspicion. We report a first case of recurrent necrotising fasciitis, possibly due to the same bacterial strain carried by the patient and highlight the danger the recurrence in susceptible patients.

R2055 Nosocomial infections in a medical faculty hospital: results, 2002

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Objective: The aim of the study was to determine the prevalence of nosocomial infections among patients in our hospital; the most frequently reported infections, causative agents and their antimicrobial susceptibility.
Method: The study was performed prospectively on 34 404 patients, hospitalised at Atatürk University Hospitals. An infection disease specialist and infection control nurse analysed the patients by active surveillance method based on laboratory and patient data. The diagnosis of nosocomial infections was established according to the 'Centers for Disease Control and Prevention (CDC)' criteria.
Results: The rate of nosocomial infections was detected to be 4% in Atatürk University Hospitals. Catheter infections were detected as the most common infection with 38.9% ratio followed by urinary tract infections with 24.6% ratio and surgical site infections with 19.8%. The highest infection rates were detected in intensive care unit (34.1%) and burn unit (29.3%). The most frequently isolated micro-organisms were *Staphylococcus aureus*, *Escherichia coli*, CNS and *Pseudomonas aeruginosa*.
Conclusion: The study of prevalence pointed out the importance of epidemiological surveillance in hospital infections.

R2056 Surveillance of nosocomial infections and antibiotic consumption in a Latvian university hospital

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Stradins University Hospital with 967 beds is the largest teaching hospital in Latvia. Traditionally, infectious diseases specialists in Latvia are employed only in specialised infectious diseases hospitals and wards. Because of the mounting problem of antimicrobial resistance and nosocomial infection a qualified infectious diseases specialist and infection control nurse (first in the country) were employed in 2000 and 2002 respectively to improve treatment, surveillance and education activities. Three prevalence surveys on antibiotic consumption and nosocomial infection were performed in May 2001, 2002 and 2003. Standardised point prevalence methodology was used. Prevalence of NI was found to be 4.3% in 2001, 3.7% in 2002 and 3.3% in 2003. Prevalence of antibiotic use was 29% in 2001, 24% in 2002 and 25.9% in 2003. Four most commonly used antibiotics in 2001 were cefazolin 48%, gentamicin18%, ampicillin/amoxicillin 13%,and metronidazole 11.2%. In 2002: cefazolin 28%, ampicillin/amoxicillin 13%, metronidazole 12%, and gentamicin 8%. In 2003: cefazolin 26.4%, ciprofloxacin 17.2%, ampicillin/amoxicillin 11.9%, and metronidazole 10.9%. A prospective study was conducted in the multidisciplinary ICU during a 2-year period (May 2001–May 2003) to determine the incidence and main characteristics of ventilator-associated pneumonia (VAP). Internationally accepted diagnostic criteria were used. Among 512 mechanically ventilated patients 37 (7.2%) developed VAP. The estimated incidence of VAP was 13.6 per 1000 ventilator days. Incidence was notably higher in summer months. Seventy per cent of patients were males. The crude mortality in patients with VAP was 29.7%. In half of the patients BAL isolated more than one pathogen. *Pseudomonas aeruginosa* was isolated from 19 (51%), *Acinetobacter baumanii* in 11 (30%) *Klebsiella pneumoniae* in 13 (23%) and *Staphylococcus aureus* in four (11%) patients. Nosocomial urinary tract infections in urology ward were surveyed in the year 2002 using CDC definitions. The incidence of NUI was 7.7/1000 catheter days. Prevalent micro-organisms were *Pseudomonas aeruginosa* (47%) and *Klebsiella pneumoniae* (11%).
Conclusion: Introduction of infectious diseases specialist and infection control nurse in the multidisciplinary hospital was associated with decrease in prevalence of nosocomial infections and changes in antibiotic prescription. In addition, surveillance activities were started.

R2057 Clinical and epidemiological characteristics of first cases of MRSA infection in a Latvian university hospital

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Methicillin-resistant *S. aureus* (MRSA) is internationally well-known pathogen, which can cause severe nosocomial infections. There is no countrywide surveillance system for this pathogen in Latvia. Stradins University Hospital in the largest teaching hospital in Latvia and referral centre for cardio-thoracic and neurosurgery, transplantation and other highly specific manipulations. First confirmed case of MRSA was registered at the ICU in March, 2003. Patient was transferred from other hospital to perform haemofiltration. Since the vigorous surveillance and prevention strategies were introduced in the hospital. Molecular epidemiological methods are being used to characterise the outbreaks. In a period from 01.03.03 to 15.12.03, 38 patients had isolated MRSA.

Mean age of the patients was 52 years and 78% of patients were male. Eight patients did not have signs of infection and were identified as carriers during the screening activities. The largest number of MRSA cases (19) was identified in ICU (including five colonised) due to outbreaks in July, 2003 (eight patients) and November, 2003 (nine patients). These outbreaks are confirmed by molecular epidemiological methods. Mortality in ICU infected patients was 64% (nine of 14). None of the five colonised patients died. Other MRSA cases were identified in abdominal surgery (8), neurosurgery (4 + 1 carrier), cardiology (2), nephrology (1), pulmonary diseases (1), cardio thoracic surgery (2) and gastroenterology (1). Ten (50%) patients have apparently acquired the infection in other hospitals because their cultures on admission were positive. Mortality in these patients was 20% (4/20).
Patients with MRSA infection had following risk factors: previous antibiotic use 92% (36/39), surgical intervention 72% (28/39), central venous catheter 62% (24/39), urine catheter 74% (29/39), mechanical ventilation 56% (22/39), and known diabetes 15% (6/39).
Conclusion: MRSA infection has become endemic in Latvia since many patients were admitted from other hospitals. Two local ICU outbreaks with secondary cases were registered and confirmed by molecular epidemiological methods. MRSA infection in ICU is associated with high mortality. Previous antibiotic use was the most common risk factor for MRSA infection or colonisation.

R2058 Levofloxacin in the treatment of nosocomial pulmonary complications after lung resection for cancer

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A 2-year multicentre study is reported on 38 complicated patients with tested lung infection and resected pulmonary for cancer after antibiotic prophylaxis with cephalosporin. Twenty-seven cases had chronic bronchopulmonary disease and bronchial asthma in four. Thirty-two lobectomies and six pneumonectomies were performed. The microbiological diagnosis of infection was achieved in the majority of the cases by bronchoalveolar lavage. The isolated pathogens were *Haemophilus influenzae* (15), *Streptococcus pneumoniae* (11), *Moraxella catarrhalis* (3), *Mycoplasma pneumoniae* (7), *Chlamydia pneumoniae* (1) and *Legionella pneumophila* (1). A quinolone, Levofloxacin, was administered at the dosage of 500 mg i.v. twice day for 10 days. Cough was the most frequent observed symptom (35/38 cases) between the third and the 10th day of therapy; thoracic pain was present in 32/38 cases between the third and the 10th day; dyspnoea was in 29/38 cases between the second and the 10th day. At the basal visit, fever values ranged between 39.5 and 37.8°C, on day 4 between 39.1 to 37.5°C, and at the end of treatment between 37.5 and 36.5°C. The basal sputum was positive in eight cases, and on fourth day of therapy; at the end of treatment, any presence of pathogens was detected in the sputum. In the other 30 patients, the isolation of pathogens was performed during the basal visit by bronchoalveolar lavage. On day 4, the tests were still positive, while at the end of treatment only in two cases a pathogen was isolated, but with reduction of the bacterial count. All the isolated pathogens were *in vitro* susceptible to levofloxacin. A clinical success was observed in 36/38 cases, with only cough in two cases. The eradication of the pathogen was obtained in 34 patients, and presumed in two ones. The other two cases reported one partial eradication and one colonisation. Remarkable adverse effects did not occur after administration of levofloxacin: five diarrhoea for 5 days, three reported headache for 2 days (with nausea in one) and one asthenia for 6 days. In conclusion, on the microbiological findings, on the good clinical efficacy of the drug in the acute exacerbations of the pulmonary infections, on the low resistance rate of causative pathogens to new quinolones and on the high penetration into respiratory tissues, levofloxacin allowed not only to reach the microbiological eradication, but also a clean resolution of the symptoms with a reduction of the postoperative hospitalisation period and a saving of the related costs.

R2059 Bacteraemia caused by *Rahnella aquatilis*

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Konya, TR

A 70-year old women was hospitalised for coronary angiography. In her history there were coronary artery disease, cerebrovascular attack and coronary by-pass surgery 5 years ago. On the fourth day after hospitalisation her fever increased and after taking blood cultures therapy started with 6 g/day ampicillin. Despite antibiotic therapy the fever did not decrease and the patient was transferred to the infectious diseases department. From separate two blood cultures *Rahnella aquatilis* was isolated. According to antibiotic sensitivity tests, levofloxacin 500 mg/day was added to the therapy. On the second day of the combination therapy the fever decreased. Control blood cultures were negative and the patient was discharged on the 14th day. In this article a bacteraemia case caused by *Rahnella aquatilis* is presented and the literature is reviewed.

R2060 *Stenotrophomonas maltophilia* infection in cancer patients

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Objectives: Frequent use of empiric broad-spectrum antibiotic therapy in cancer patients might result in the selection of some resistant organisms. The aim of this study was to compare two periods of survey for frequency of infection and the antimicrobial susceptibility of *Stenotrophomonas maltophilia* isolate from cancer patients.
Methods: A total of nine episodes of *S. maltophilia* infections in immunocompromised-cancer patients were detected in 2001 and six episodes in 1991. Strains from blood cultures were isolated through the semi-automated system Bact-Alert and identified with Api20 NE. Kirby–Bauer method was used for the examination of the susceptibility pattern.
Results: From the surveillance data there was no increasing occurrence of *S. maltophilia* infections found. Five of the isolated strains were derived from blood, five from urine, two from sputum, two from pus and one from cerebrospinal fluid. Regarding the antibiotic susceptibility 70% of the strains were susceptible to cotrimoxazol, 85% were susceptible to ceftazidime, and 85% were susceptible to ciprofloxacin while 60% were resistant to carbapenems, imipenem and meropenem. Mortality rate due to infection was significantly high in the *S. maltophilia* bacteraemia.
Conclusions: The frequency of *S. maltophilia* infection in cancer patients was not changed during the last decade. Antimicrobial susceptibility was variable with no single antimicrobial agent inhibiting more than 85% of isolates. Some isolates were multidrug resistant and might require high-dose combination therapy.

R2061 The incidence of nosocomial infections, a parameter for the comparison of surgical departments?

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Nis, CS

Objective: To determine the applicability of registered nosocomial infections as parameter for comparison of surgical departments.
Method and population: Between July 1, 2001 and 2002 postoperative complications occurring in patients admitted to the surgical department of a district hospital were registered. The nosocomial infections were determined according to the CDC standards. A total of 142 patients were admitted 153 times to the surgical wards.
Results: Postoperative course was complicated in 18 patients (12.12% of admission). Airway and wound infections were the most frequently registered complications (in 3.7 and 3.5% of the admissions respectively), 42% of the wound infections were

recorded after discharge. 88% fully recovered by conservative means. Two patients with airway infections died (1.4%).
Conclusions: The contribution of wound infections to overall postoperative morbidity and the severity of these infections are low and vary with the reason of admission. The morbidity and the severity of airway infections are more serious.

R2062 Bacterial contamination and drug resistance in isolated bacteria from intensive care units (ICU and NICU) in Hamadan hospitals, West Iran

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Objectives: Many reports from all over the world indicate that multi-drug resistance in nosocomial organisms are increasing. Bacterial contamination in intensive care units, surgery and burning rooms is also one of the major problems in hospitals. The aims of this study were determination of bacterial contamination and antibacterial drug resistance in isolated bacteria from intensive care units (ICU and NICU) in Hamadan hospitals (Mobasher Kashani and Fatemie).
Methods: In this study, 682 samples were randomly collected from different areas including devices and apparatus, physical surfaces and staff uniforms. The samples were inoculated in EMB and blood agar by wet swabs and transferred to medical laboratory for identification and antibiogram of strains. A total of 140 cases were selected and cultured on Muller–Hinton agar for antibiogram tests (Kirby–Bauer method). The antibiotics disks consisted of: erythromycin, vancomycin, ciprofloxacin, carbenicillin, penicillin G, tetracycline, chloramphenicol, ampicillin, gentamycin and ceftriaxone.
Results: The ICU of Mobasher Kashani hospital showed more bacterial contamination (57.4%) rather than the NICU of Fatemie hospital (48.3%). The most bacteria isolated from two hospitals were as follow: micrococci, *Staphylococcus epidemidis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *E.coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Acinetobacter* and *Streptococcus faecalis*. The most contaminated part (80.3%), were the sink and staff uniforms the ICU of Mobasher Kashani hospital. In this study, more than 80% of strains which isolated from the ICU of Mobasher Kashani hospital were resistant to: erythromycin, vancomycin, carbenicillin, tetracycline, chloramphenicol and ampicillin, whereas, the strains of the NICU of Fatemie hospital were resistant to penicillin, ampicillin, chloramphenicol and gentamycin.
Discussion: Our results showed that intensive cares of Hamadan hospitals both the ICU of Mobasher Kashani and the NICU of Fatemie were contaminated with routine nosocomial micro-organisms. There were also the high drug resistance (in vitro) in strains isolated from two hospitals.
Conclusions: It seems that sterilisation and disinfection methods in hospitals were not performed correctly and related authorities should control it regularly.

R2063 Colonisation of unusual strains of *Klebsiella pneumoniae* in tracheal tubes of hospitalised patients in intensive care units

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Objectives: To evaluate colonisation of unusual urease-negative strains of *K. pneumoniae* in tracheal tubes of patients hospitalised in Intensive Care Units (ICUs).
Methods: Tracheal tubes were cultured by culture of the lumen's catheter after 10 times dilution, and then organisms isolated were identified by routine bacteriological method. Isolated urea-negative strains of *K. pneumoniae* rechecked by a sensitive method for assay of a few amount of urease enzyme. We ruled out nonmotile *E. aerogenes* strains from these isolated strains, with ornithine decarboxylase test (isolated strains were ornithine negative). Also confirmatory test performed on *K. planticola*. Then the antimicrobial susceptibility test was compared with urea-positive strains of *K. pneumoniae*.
Results: During 2 years of study (2001–2003), in total 765 tracheal tubes were cultured. A total of 158 species of *Klebsiella* isolated in which 29 strains were urea-negative *K. pneumoniae* and eight species were *K. planticola*. More strains are resistant to all common antibiotics except imipenem. Nitrogen control of urease synthesis occurs in a number of bacterial species. In *K. pneumoniae* urease is not synthesised when the cells are grown in the presence of high-quality nitrogen sources such as ammonia. We guessed. Alkalosis in this patients is caused by unusual strains of colonisation. We are going to follow up these isolates, which grow in minimal medium containing a poor nitrogen source for urease enzyme activation.
Conclusion: It is concluded that, there are unusual urea-negative and *K. planticola* species isolated as emergent multiresistant nosocomial pathogens in our hospital. In spite of, urease activity plays a central role in the pathogenesis but isolation these strains in two cases as blood stream infection showed the role of pathogens.

R2064 Surveillance of the infection in coronary artery bypass graft surgery: an evaluation over 4 years

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Objectives: Surgical site infections (SSIs) in coronary artery bypass graft (CABG) surgery result in significant patient morbidity and mortality, as well as consumption of significant healthcare resources. The purpose of this study was to describe the surgical site infections in CABG surgery.
Methods: Surgical site infections after CABG surgery have been monitored at the Hospital Universitario de Canarias for 4 years (2 months in 2000, three in 2001, six in 2002 and six in 2003). A prospective study of 197 patients operated, all were followed up for thirty days, and stratified according to intrinsic risk factors of infection NNIS System (National Nosocomial Infection Surveillance, CDC Atlanta). All infections are categorised using standard Centers for Disease Control definitions.
Results: During this period, seven infections were documented, two organ-space infections, two deep incisional infections and three superficial incisional infections. All incisional infections were founded in the safenectomy. The wound infection rates (wound infection*100/no. surgical patient) were 0 in 2000, 11.7 in 2001, 1.6 in 2002 and 2.5 in 2003. Table 1 shows rates of SSI by index category. *National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 to June 2002, issued August 2002 [*Am J Infect Control* (2002) **30**, 458–475].
The aetiology of the seven infections was: *S. epidermidis* and *Enterobacter cloacae* in organ-space infections, two *Morganella morganii*

Table 1. SSI rates by risk index category 2000–2003, and NNIS* data (pooled mean rate).

	NNIS 0			NNIS 1			NNIS 2			NNIS 3		
	N	SSI	RATE	N	SSI	RATE	N	SSI	RATE	N	SSI	RATE
2000	0	0	0	15	0	0	5	0	0	2	0	0
2001	13	0	0	18	4	22,2	3	0	0	0	0	0
2002	27	1	4,5	38	0	0	1	0	0	0	0	0
2003	3	0	0	75	2	2,6	3	0	0	0	0	0
2000–03	43	1	2,3	146	6	5,1	12	0	0	2	0	0
NNIS*			1,28			3,51			5,62			5,62

N: operative procedures.
SSI: Surgical site infections.
RATE = SSI*100/N.

in two deep incisional infections and *Proteus vulgaris*, *Enterobacter cloacae* and *Morganella morganii* in three superficial incisional infections.
Conclusions: Comparing our data with those of the NNIS report, our hospital rate in NNIS 0 and NNIS 1 is lower than the percentile 90, and NNIS 2 y NNIS 3 is lower than the percentile 10. The safenectomy is an area of the risk for surgical wound infection following CABG surgery.

R2065 Bacterial infection of lower respiratory tract associated with positive blood culture in haematology patients

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Objectives: The purpose of this study was to compare pathogens isolated from lower respiratory specimens and blood cultures from patients hospitalised at Clinic of Haematology, Clinical center, Skopje, Macedonia, during two year period (September, 2000 – September, 2002).
Methods: 85 patients hospitalised at Clinic of Haematology were chosen for having positive microbiological findings both in sputum and in blood culture. Cultivation, isolation, identification and antimicrobial susceptibility testing were made using standard microbiological laboratory methods.
Results: Sputum isolates were: *Candida albicans* 25, *Streptococcus pneumoniae* 17, *Pseudomonas aeruginosa* 16, *Candida* spp. 11, *Klebsiella* spp. 11, *Staphylococcus aureus* 8, *E. coli* 7, *Acinetobacter* spp. 4, *Haemophilus influenzae* 4 and *Streptococcus pyogenes* 2. Blood isolates were: *Staphylococcus* coagulase-negative 30, *Staphylococcus aureus* 15, *E. coli* 10, *Pseudomonas aeruginosa* 9, *Enterococcus* 6, *Klebsiella* spp. 6, *Acinetobacter* spp .4, *Candida* spp. 2 and *Candida albicans* 1. Nineteen 19 patients had identical findings both in their sputa and blood cultures: *Pseudomonas aeruginosa* 5, *Staphylococcus aureus* 5, *Klebsiella* spp. 3, *Acinetobacter* spp. 2, *E. coli* 1 and *Candida albicans* 1. Their bacterial antimicrobial susceptibility testing showed similar resistance pattern.
Conclusion: *Candida* and Gram-negative bacilli were predominant pathogens from lower respiratory tract samples. Gram-positive cocci (*Staphylococcus*) were main pathogen in blood cultures. Identical findings together in sputa and blood cultures in 19 patients showing similar resistance pattern have same origin.

R2066 Comparative results of a 7-year surveillance programme for nosocomial Gram-negative pathogens prevalence in Russian ICUs

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Objectives: To evaluate the changes in prevalence of Gram-negative nosocomial pathogens in Russian ICUs in 1997–99 and 2002–03.
Methods: Gram-negative pathogens isolated from patients with documented nosocomial infections from ICUs in different Russian cities were included in the study. Isolates were identified in local laboratories using standard biochemical tests. Strains were transferred to central laboratory in Smolensk where re-identification of all pathogens was performed.
Results: Twenty-eight and 26 centres took part in the study during 1997–99 and 2002–03, respectively. A total of 2664 isolates were obtained in 1997–99 and 1813 in 2002–03. The comparison of data obtained in 1997–99 and 2002–03 are presented in the Table.
Conclusions: As in 1997–99 the most common nosocomial pathogens in 2002–03 were *P. aeruginosa*, *E. coli*, *K. pneumoniae*. There was found to be a significant increase in prevalence of *Acinetobacter* spp. and *S. maltophilia* in 2002–03 as compared with 1997–99 period. These data suggest the increasing role of nonfermenters as nosocomial pathogens.

Microorganisms	1997–99 (%)	2002–03 (%)
P. aeruginosa	30.0	33.4
E. coli	18.4	12.6
K. pneumoniae	14.6	14.7
Proteus spp.	10.0	5.4
Enterobacter spp.	7.6	4.9
Acinetobacter spp.	6.9	16.8
Serratia spp.	4.1	3.5
S. maltophilia	1.3	2.7
Citrobacter spp.	1.3	1.2
M. morganii	0.8	1.1
Others	5.0	3.7

R2067 *Acinetobacter baumannii* bacteraemia in a medical ICU: sensitivity pattern and outcome

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Objectives: To investigate the characteristics of *Acinetobacter baumannii* bloodstream infection and its impact on outcome of adult critically ill patients in a medical ICU.
Methods: Retrospective study of patients admitted to the ICU between January 2001 and December 2003 with at least one blood culture positive for *A. baumannii*. Antimicrobial treatment was considered adequate if at least one drug used had *in vitro* activity against the isolated strain.
Results: Among 429 admissions, 14 patients presented *A. baumannii* bacteremia (3.3%). Eight patients were male. Mean age was 62.1 ± 5 years and mean APACHE II score 14 ± 4. Length of ICU stay before the onset of bacteraemia ranged between 2 and 63 days. The majority of the bacteraemic events occurred during two distinct periods of time: five episodes between July and August 2002 and 4 between May and June 2003. The source of infection was a contaminated central venous catheter in four cases (29%), ventilator-associated pneumonia in two cases (14%) and unknown in eight cases (57%). Six strains (43%) were resistant to all antibiotics except polymyxin B (MDR). Three patients were receiving adequate antimicrobial treatment at the time of diagnosis and in six adequate treatment was started after the culture results. Attributable mortality was 14%. There was no difference in mortality rate between MDR and non-MDR strains and between patients who received adequate and inadequate antimicrobial treatment.
Conclusions: Bacteraemia due to *Acinetobacter baumannii* presented mainly with an epidemic pattern in our medical ICU. Attributable mortality was relatively low even for multi-drug resistant strains and inadequately treated patients.

R2068 Epidemiological study and drug sensitivity against clinical strains of *S. maltophilia* A 4-year review

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Objectives: *S. maltophilia* is an important nosocomial pathogen. The number of *S. maltophilia* infections has dramatically increased in recent years. Long-term hospitalisation, antimicrobial pressure and presence of induelling catheters are serious predisposing factors for the disease. Also most of these strains are inherently and highly resistant to antibiotics. We conducted a 4-year review of episodes of colonisation or infection due to *S. maltophilia* in order to study the epidemiology and resistance profile of the microorganism.
Methods: During a 4-year period (2000–2003) *S. maltophilia* was isolated from 82 clinical specimens from 70 patients in our hospital. All isolates were identified by API or VITEK-II system

(Biomerieux, France). Susceptibility testing was performed by disc-diffusion method according to NCCLS guidelines. Nineteen antibiotics of all groups were tested. Also in some cases medical records of patients were reviewed.
Results: Clinical samples were various and mostly of the respiratory tract. In descending order other samples were blood, wounds, catheters, urine, drainage fluids. Thirty-eight of 82 (31.1%) specimens, 32 patients, were from the ICU and concerned mostly elderly and intubated patients. Most of these isolates were from the respiratory tract and from a polymicrobial culture. The rest 44 of 82 (36%) specimens, 38 patients, were from different wards (36 of 38) and outpatients (two of 38). As to the susceptibility results to antimicrobial agents, all isolates were resistant to several antibiotics, thus multiresistant. The most active antibiotics against *S. maltophilia* were the newer fluoroquinolones ciprofloxacin and pefloxacin (71.5%). The combination of Ticarcillin–clavulanic acid was the second most active antibiotic tested (62.3%) followed by Trimethoprim–sulphomethoxazol (42.9%). Another important finding was that susceptibility to ceftazidime showed to decrease through the study period. In 2000 it was 34.6% and in 2003 decreased to 14.3%. All other isolates showed no or poor sensitivity. Also of importance was that one recently isolated clinical strain from an ICU patient was found to be resistant to all 19 antibiotics tested.
Conclusions: We demonstrated that fluoroquinolones are most active against clinical strains of *S. maltophilia* followed by Ticarcillin–clavulanic acid that showed good sensitivity. Also the emergence of a strain resistant to all antibiotics tested is a worrying event and has to be taken under concern.

R2069 Incidence, aetiology and focus of postoperative infections in cardiac surgery patients

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Objectives: To determine the incidence, pathogens, focus and outcome of postoperative infections in patients undergoing cardiac surgery. To assess their relationship with operative risk score and type of surgery.
Methods: We reviewed 122 charts of adult patients who underwent cardiac surgery with microbiological confirmed infection out of 814 operated during a 2-year period in a new Cardiac Surgery Unit of an university hospital. Euroscore was used to evaluate operation risk.
Results: The incidence of microbiological confirmed infection was 16.2%. Sepsis was the most frequent infection (30.3% of infected patients), followed by catheter (23.4%), sternal wound infection (SWI) (16.6%), UTI (16.6%) and respiratory tract infection (12.8%). Among sepsis, 35% were catheter related, followed by primary focus (22.5%) and SWI (12.5%). Seven patients developed early bloodstream infection (0.8%) predominantly related to Gram-negative bacteria. Four patients suffered endocarditis. SWI was superficial in 14 patients and eight evolute to deep infection. A decrease in the proportion of postoperative infections during the second year was observed in parallel with the increase in the number of operations performed (18.6% vs. 14,6%). Causative agents were Gram-positive in 79%, being the most frequent in SWI and catheter, and Gram-negative (47.8%) were mainly isolated in sepsis, UTI and respiratory tract infection. MRSA was isolated in five patients (4%) and multiresistant *Acinetobacter baumanii* was found in one patient. Postoperative infections were more common in patients with a higher Euroscore (64% of infected patients) and after combined artery bypass grafting and valve-related operations, followed by valve replacement and coronary bypass grafting. Infection related mortality was 2% of all patients who underwent cardiac surgery. Global mortality was 5.1% related to a 5.27 value of Euroscore.
Conclusions: The incidence of postoperative infections remains relevant after the second year of cardiac operations but with a decrease parallel with the increasing number of operations. Catheter-related sepsis is the main cause of infection, and *S. aureus* is the most frequently isolated pathogen. Postoperative infections are usually found in more complex surgery and in patients with a superior preoperative euroscore.

R2070 Lung tuberculosis and different radiographic appearance

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Postprimary lung tuberculosis in recent years manifested in other than usual radiographic findings. In previously period we find out, lung tuberculosis shadows localised in others than usually localisations, especially in lowers lobes. 35 (9.6%) patients, 21 M–14 F, average years 51.3 years, were hospitalised during 1 year on the Institute for lung disease. Dominant symptom was malaise in 29 (86.8%), who were previously hospitalised for 4—6 months. Cough were the main symptom in 4 (12.9%) patients. Radiographic manifestations in seven (20%) patients revealed micronodular shadows and spot shadows pericardially to the right, and in eight (20.2%) patients similar lesions were localised on the left side. In 17 (45%) patients spirometry verified mixed types of moderately severe ventilation disorders, in eight (20.2%) moderately severe obstruction and in 10 (27.5%) patients restriction. 2 (5.7%) patients had associated diabetes and rheumathoid arthritis. PPD test were positive in 12 (32.2%) patients. Sputum bacilli were verified in one patient. Bronchoscopies were done in 34 patients with specific findings and TBB were done in 27 (73%) patients. Atypical lung radiographic appearance had recently become a new differential diagnostic problem. Duration of treatment in these group patients and duration of treatment in patients with usual radiographic manifestations were very similar (no statistical differences were found).

R2071 Nosocomial infection types and isolated microorganisms in Baskent University, Adana Education and Research Medical Centre

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Purpose: In this study, we aimed to assess the surveillance study results of our hospital infection control committee done between January and November 2003 and to direct the nosocomial infection control strategies.
Method: Centres for Disease Control criteria was used to define intensive care units infections. The identification of the microorganisms have been performed by using conventional methods and Becton Dickinson, (BBL Crystal) system. The study has been planned prospectively with patient and laboratory depending on active surveillance.
Results: Nosocomial infection rate during the period was found 1.66%. Nosocomial infections were mostly encountered in the burn unit (16.43%), followed by the intensive care unit (3.49%) and the other inpatient wards (0.73%). The most frequent form of infections were bacteraemia (39.14%), followed by urinary tract infections (35.85%), surgical wound infections (9.21%), catheter infections (6.25%), respiratory tract infections (4.96%), burn injury tissue infections (4.66%), and soft tissue infections (0.98%). Respectively a total of 341 pathogenic microorganisms were identified and 35.77% ($n = 122$), of those were determinated as Gram-positive bacteria, 52.78% ($n = 180$) Gram-negative bacteria and 11.43% ($n = 39$) *Candida* spp. The distribution of the bacteria was as isolated follow; methicillin-resistant *Staphylococcus aureus* (MRSA) (14.07%), *Enterococcus* spp. (7.91%), methicillin-resistant coagulase-negative staphylococci (7.03%), methicillin-sensivite *Staphylococcus aureus* (MSSA) (2.34%), α-haemolytic streptococcus (0.87%), β-haemolytic streptococcus (0.29%) and the Gram-negative bacteria was *Pseudomonas aeruginosa* (15.27%), *Acinetobacter* spp. (9.38%), *Escherichia coli* (14.36%), *Klebsiella pneumonia*

(6.74%), *Enterobacter* spp. (3.22%), *Klebsiella oxytoca* (1.46%), *Citrobacter* spp. (1.46%), and *Proteus mirabilis* (0.87%).
Conclusion: Dissimilar to other studies, bacteraemia is considered to be the most seen type of nosocomial infection in our hospital that we believe its sourced from burn unit.

R2072 Emerging antimicrobial resistance in bacterial strains isolated from intensive care unit patients with bacteraemia

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Objectives: To define the incidence of bacteraemia in intensive care unit (ICU) patients, to study the isolation frequency of microbial pathogens and evaluate their resistance patterns.
Methods: During a 3-year period (1 July 2000 to 1 July 2003) 1408 sets of blood cultures were obtained from 345 ICU-patients. The cultures were incubated in BACTEC 9240 automated blood culture system (Becton and Dickinson). Gram stain and subcultures to the proper media were performed from the vials reported positive by the instrument. The identification of bacteria was based on API system (BioMerieux) and BBL Crystal GP (Becton and Dickinson). The antimicrobial susceptibility testing was carried out by MIC values determination using broth microdilution method (Sensititre, UK). The susceptibilities of enterococci were studied by the E-test method as well (AB Biodisk, Sweden).
Results: Bacteraemic episodes were observed in 124/345 patients (incidence = 35.9%). Of the 124 patients with positive cultures 275 microbial strains were recovered. Gram-positive bacteria (GP) prevailed: GP 51.5 % (142/275), Gram-negative (GN) 40% (109/275) and fungi 8.5 % (24/275). Multimicrobial bacteraemia was detected in 21 cultures (8.2%). The most prevalent bacteria were: *Staphylococcus epidermidis* (21.9%), *Staphylococcus haemolyticus* (12.2%), *Enterobacter* spp. (11.8%), *Enterococcus* spp (10.4%), *Pseudomonas* spp. (10%), *Acinetobacter* spp (7.2%), *Klebsiella pneumoniae* (5%), *Escherichia coli* (3.6%) and *Staphylococcus aureus* (3.5%). The antimicrobial resistance rates (%) for the most frequently isolated GN bacteria are presented in the table below: Resistance to methicillin was detected in all *S. aureus* strains and in 94.4% of coagulase-negative staphylococci (CNS) strains. 41.5% of enterococci were resistant to vancomycin.

Bacteria	Amicacin	Pip/Tazo	Cefotaxime	Ceftazidime	Cefipime	Cipro	Imipenem
Enterobacter	15.6	87.5	96.8	96.8	51.5	96.8	15.6
Pseudomonas	67.8	46.5	100	75	60.8	82.2	75
Acinetobacter	90	90	85	85	45	95	60

Conclusion: The high prevalence of multiresistant, nosocomial strains in ICU-patients with bacteraemia, limits our treatment choices and subsequently leads to a high mortality rate.

R2073 The influence of diabetes mellitus on the spectrum of uropathogens and their antibiotic resistance

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Objectives: To evaluate the effect of diabetes mellitus on the spectrum of uropathogens and their susceptibility to the antimicrobials in patients (pts) with urinary tract infection.
Methods: Urinary isolates and their patterns of susceptibility to the antimicrobials were evaluated in 346 diabetic pts (229 females and 117 males) and 975 nondiabetic pts (679 females and 296 males) with proven significant bacteriuria (>105 CFU/mL urine). Both diabetic and nondiabetic pts were more than 50 years old. The mean age of the two groups was similar and the male/female ratio was 0.33 and 0.30 respectively in diabetic and nondiabetic pts.
Results: The most frequent causative organisms of bacteriuria in diabetic and nondiabetic females were respectively: *E. coli* 54.1 and 58.2% (P = NS), *Enterococcus* spp. 8.3 and 6.5% (P = NS), *Pseudomonas* spp. 3.9 and 4.7% (P = NS). The most frequent organisms in diabetic and nondiabetic males were respectively, *E. coli* 32.5 and 31.4% (P = NS), *Enterococcus* spp. 9.4 and 14.5% (P = NS), *Pseudomonas* spp 8.5 and 17.2% (P = 0.02). The rates of uropathogens in diabetic and nondiabetic pts with indwelling bladder catheter were respectively *E. coli* 30.8 and 24.9% (P = NS), *Enterococcus* spp. 21.8 and 16.4% (P = NS), *Pseudomonas* spp 12.8 and 18.1% (P = NS). No significant differences in resistance rates to ampicillin, nitrofurantoin, cotrimoxazole and ciprofloxacin of *E. coli* and *Enterococcus* spp. were recognised between diabetic and nondiabetic pts.
Conclusions: Diabetes mellitus doesn't seem to have a relevant effect on the spectrum of uropathogens and their susceptibilty patterns to the antimicrobials in pts with urinary tract infection.

R2074 Epidemiological study of *Acinetobacter* spp. in ICU and plastic surgery department over a 4-year period

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Objective: The aim of this study was to analyse the epidemiology of *Acinetobacter* spp. between 1996 and 1999 in two departments in our hospital.
Methods: From May 1996 to January 1999 *Acinetobacter* spp. from clinical and environmental specimens were studied. The isolates were identified using the API 20NE and Vitek System (BioMerieux). Susceptibility testing was performed by disc-diffusion method according to the NCCLS standards. Molecular study was done using recA/RFLP, PCR-fingerprinting and ADSRRS-fingerprinting.
Results: Sixty isolates were included in the study. Forty-seven of them were from clinical specimens from patients hospitalised in ICU and Department of Plastic Surgery and 13 from hospital environment. Identification in API 20 NE and Vitek System was confirmed by recA/RFLP. Fifty-nine isolates were identified as genomic species 2 (*A. baumannii*) and one isolate as genomic species TU 13. Seventeen isolates were grouped into PCR-fingerprinting type A, 23 to type C, 18- type D, one into type E and TU13 was type H. Using ADSRRS-fingerprinting the following types were obtained: A′ (n = 46), B′ (n = 1), C′ (n = 12), D′ (n = 1). Using those two methods of fingerprinting we obtained eight genotypes: 2DA′– 17 isolates (28.3%), 2AA′ – 15 isolates (25%), 2CA′ – 14 isolates (23.3%), 2CC′– nine isolates (15%), 2AC′ – two isolates and one isolate from genotypes 2DC′, 2EB′, 13HD′. We did not confirm correlation between antibiotic susceptibility profile and genotype.
Conclusions: (i) *A. baumanii* became an endemic organism in our hospital. (ii) Three predominant genotypes prevailed during the whole 4-year period in ICU and in 1 year (1998) in Department of Plastic Surgery. (iii). Two methods gave comparable results with regard to typing.

R2075 Genotypes of methicillin-resistant *S. aureus* blood culture isolates in Slovenia

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Objectives: To determine the number of MRSA genotypes and resistance profiles from blood culture isolates in Slovenia.

Methods: A total of 108 of 131 MRSA blood culture primoisolates from 11 Slovene hospitals in the years 2000 and 2001 were typed by pulsed-field gel electrophoresis of chromosomal DNA and compared with most prevalent European MRSA clones. Antimicrobial susceptibility testing was performed by the disc-diffusion method.
Results: Seven different genotypes (A-G) were found among 108 MRSA isolates. 99 isolates belonged to genotype A (12 subtypes) which was present in all hospitals and showed more than 80 % similarity with South German clone. Three isolates belonged to genotype E (1 subtype) and two to genotype F. Genotypes B, C, D and G were found in one patients each. Genotypes A, F and G were susceptible to rifampin, chloramphenicol, trimethoprim-sulphamethoxazole, tetracycline, vancomycin and teicoplanin, but susceptibility of clones B, C, D and E was different.
Conclusions: Although seven genotypes were found among MRSA blood culture isolates, a single genotype (A) was predominant, but other genotypes (B-G) occurred only sporadically in Slovenia.

R2076 Susceptibility vancomycin-resistant *Enterococcus faecium* to linezolid

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Objectives: Enterococci are important nosocomial pathogens and their growing resistance to antimicrobial agents is even more important from therapeutical point of view. The aim was to determine the *in vitro* susceptibility of clinical isolates of vancomycin-resistant *Enterococcus faecium* (VREM), phenotype VanA, to linezolid. Linezolid, the first in a new class of antibacterials, the oxazolidinones, has activity against Gram-positive organisms, both susceptible and resistant.
Methods: Susceptibility of 37 clinical isolates of VREM to linezolid, vancomycin and teicoplanin we determined by the Kirby–Bauer method according to NCCLS recommendations. MICs for vancomycin and teicoplanin were determined by E-Test method. These isolates were isolated from different clinical materials over one year from patients of haematology unit.
Results: Among the 37 clinical isolates of VREM, phenotype VanA, 36 were susceptible to linezolid. One isolate was resistant.
Conclusion: The study revealed a very good activity of linezolid against VREM VanA. The antimicrobial agent may be useful in the treatment of serious infections caused by multiresistant *E. facium*. Linezolid resistant *E. faecium* was isolated for the first time in our hospital.

R2077 Postoperative meningitis following neurosurgery in critically ill patients

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Objectives: Meningitis is a serious complication following neurosurgery and is often caused by coagulase-negative staphylococci (CNS). *Staphylococcus aureus* (SA) and Gram-negative bacilli may also be of increasing importance. Data on such infections in the critically ill is limited. The purpose of the study was to review the microbiology of postoperative neurosurgical meningitis/ventriculitis (PONMV) and to compare antibiotic resistance rates with empirical intravenous ceftriaxone and/or intraventricular vancomycin given in the critical care unit (CCU) prior to the availability of culture results.
Methods: A retrospective analysis of the microbiology of all cerebrospinal fluid (CSF) samples taken from patients with PONMV managed in the CCU of a tertiary referral centre, 1996–2003.
Results: A total of 71 adult patients were identified from whom 208 CSF isolates were identified. 84 (40%) were 'first time' isolates: 66 were Gram-positive (43 CNS, eight *Streptococcus* sp, four SA, four *Corynebacteria* sp, three *Bacillus* sp, two MRSA, one *Gemella* sp.,), 16 Gram-negative (seven *Pseudomonas* sp., three 'coliform'', three *Enterobacter* sp., two *Klebsiella* sp., one *Acinetobacter* sp.) and two *Candida* sp. MRSA was only identified in the year 2003. All Gram-positive isolates were vancomycin-sensitive. 39 'first time' isolates (46%) were ceftriaxone resistant (28 Gram-positive, including 22 CNS isolates, and 11 Gram-negative). CSF white cell counts varied from <1 to 87, 500/mm^3.
Conclusions: Ceftriaxone may not be a suitable empirical agent for PONMV, particularly if CNS infection due to external ventricular drains is suspected. For Gram-negative infections, antibiotic treatment with an anti-pseudomonal antibiotic should be considered. The absence of a white cell count response in the CSF does not always exclude infection.

R2078 Patterns of antibiotic prescribing in Italian hospitals

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Background: Inappropriate use of antibiotics is associated with the emergence of resistant bacteria and increasing hospital expenditures. Aim of the present study was to investigate patterns of antibiotic prescribing in Italian hospitals.
Methods: The first of a serial one-day point prevalence survey for the INF-NOS project 2002–2004 was performed in 31 Italian hospitals during October–November 2002. Data on antimicrobial use, type and site of nosocomial infections (NI) were collected trough an electronic case report form. Descriptive statistical analysis was conducted.
Results: A total of 3120 patients were surveyed, 45.0% of them receiving at least one antibiotic on the day of the survey: 52.6% for treating active infection (of community or nosocomial origin), 21.9% for surgical prophylaxis, 19.8% for prophylaxis for other reasons and 5.7% for treatment of no more active infection. Among 760 infected patients treated with antibiotics, 58.3% received monotherapy and among 286 patients with other reason for prophylaxis 52.8% were exposed to at least one invasive procedure, mainly urinary catheter. Teicoplanin (9.9%), vancomicin (5.7%) and piperacillin/tazobactam (5.7%) were the more common prescriptions for therapy of NI, instead ceftriaxone (12.3%) was the most preferred option for surgical prophylaxis. No active infection was recorded for 2429 patients (75.7%), 28.0% of them receiving antibiotic treatment, in 41.9% of cases for prophylaxis for other reason and in 12.2% for therapy of no more active infection.
Conclusions: High rates of antibiotic usage were observed in Italian hospitals. Areas for improvement for the prescribing habits were identified. The 1 day prevalence survey of antimicrobial use may represent a tool of feedback to prescribers for more appropriate drug selection and consumptions.

R2079 A natural antimicrobial agent able to inhibit bacterial biofilm formation on indwelling medical devices

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Objectives: Strategies to prevent medical device-associated infections include coating of polymeric surfaces with antimicrobials that eluting from the device prevent microbial colonisation. Our aim was to assess the antimicrobial activity of usnic acid, a secondary lichen metabolite, against several planktonic bacteria known to form biofilm on implanted medical devices: *Staphylococcus epidermidis*, *S. aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*.
Methods: To evaluate the possible inhibiting effect, the most widely employed polymers in medical device production, polyurethanes, were loaded with usnic acid. Since usnic acid exhibit acidic properties, the surface of polyether urethane acid was specifically modified by introducing amino groups able to establish electrostatic interactions with the acidic groups displayed by usnic acid. These functionalised polymers were then incorporated in a

flowcell designed for growing biofilms under a wide range of hydrodynamic conditions, and subsequently analysed using confocal microscopy. The ability of usnic acid to control biofilm formation was assessed using the Gram-positive *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa*.
Results: Usnic acid-loaded polymers have been shown to be resistant to biofilm formation by *S. aureus*. In contrast, *P. aeruginosa* biofilm was formed on the surfaces of both the untreated and the usnic acid-loaded polymers. However, usnic acid affected the morphology of *P. aeruginosa* biofilm, possibly indicating that this drug may interfere with cell–cell communication by influencing cell-signalling pathways.
Conclusion: The assessed activity of usnic acid on *S. aureus* and presumably other Gram-positive bacterial species, opens new perspectives in the development of medical devices refractory to bacterial colonisation and biofilm formation.

R2080 Microbiology of meningitis in hospitalised patients of West Caracas, Venezuela: 10-year surveillance

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Objectives: To study microbiology of meningitis in hospitalised patients of West Caracas, Venezuela [West General Hospital (WGH) of Caracas] in a 10-year period (1989–1999).
Methods: CSF of meningitis suspected patients hospitalised at WGH were studied at Microbiology Laboratory. Complete cultures and biochemical identification as well API and automated media were used to classify isolated organisms (bacteria and fungi).
Results: In this period, 4060 samples of CSF were submitted to Microbiology Laboratory. From this total, only 140 (3.44%) had bacterial growth. Most isolated organisms were: *Haemophilus influenzae* (25.71%), *Streptococcus pneumoniae* (14.29%), *Staphylococcus aureus* (10.00%), *Neisseria meningitidis* (8.57%), *Klebsiella pneumoniae* (7.14%), *Pseudomonas aeruginosa* (5.71%), *Enterobacter cloacae* (5.71%), *Escherichia coli* (3.57%), *Acinetobacter lwoffi* (2.86%), *Candida albicans* (2.86%), *Klebsiella ozoaenae* (2.14%), *Acinetobacter baumannii* (2.14), among others.
Conclusions: *H. influenzae* isolation frequency is related to paediatric services (more than half of CSF samples), meanwhile Gram-negative bacilli isolation is due to a significant proportion of nosocomial infections, especially those acquired at services of intensive care. Surveillance studies are needed especially in those countries in the developing world.

R2081 Change in antimicrobial resistance pattern of *Pseudomonas aeruginosa* species isolated from wounds and abscesses over a 3-year period

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Objectives: *Pseudomonas aeruginosa* is one of the most commonly isolated microorganisms from hospital infections and the wide use of broad spectrum antibiotics causes increase in the ratio of its antibiotic resistance. In this study the antibiotic resistance ratio of *Pseudomonas aeruginosa* species isolated from wound and abscess samples that were sent to our Microbiology Laboratory between January-December 2002 were searched. It is aimed to determine the change in the resistance ratio of *Pseudomonas aeruginosa* species, isolated from the same group of samples that were searched in 1999 in our hospital, compared with our findings.
Methods: A total of 100 *Pseudomonas aeruginosa* species, isolated from wound and abscess samples that were sent from various clinics to the Microbiology Laboratory in Sisli Etfal Research and Training Hospital between January and December 2002, were identified by conventional methods and API 20 NE (Biomerioux). Their antibiotic sensitivities were searched by Kirby–Bauer disc diffusion method according to NCCLS recommendations.
Results: The antibiotic resistance ratio of the isolated *Pseudomonas aeruginosa* species were 45% for piperacillin, 50% for ceftazidime, 55% for cefepime, 40% for aztreonam, 45% for gentamicin, 35% for amikacin, 39% for tobramycin, 30% for netilmycin, 36% for ciprofloxacin, 48% for imipenem and 49% for meropenem. In our study the resistance to antibiotics in *Pseudomonas aeruginosa* species were found to be high. The highest resistance was against cefepim, ceftazidim and carbapenems while the lowest resistance was against netilmycin, amikacin, tobramycin and ciprofloxacin.
Conclusions: In our study a marked increase in resistance was seen when we compared the change of resistance ratio of the isolated *Pseudomonas aeruginosa* species in 1999 and 2002 ($P < 0.01$).

R2082 Impact of nosocomial infections on medical consumption in intensive care units of military hospital in Tunis

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Objectives: Nosocomial Infections (NI) represents a major problem of public health by their increasing frequency and their mortality rate, but also by their considerable cost. In a context of control of health expenses, the measure of the cost of the NI (direct and indirect cost) became necessary.
Methods: In our survey, we are interested to determine the direct costs generated by the NI: antibiotics and other medicines, complementary investigations, medical devices and supplementary days of hospitalisation. The survey took place in two departments of ICU of the Main Military hospital of instruction of Tunis (HMPIT), the adult ICU and the neonatology ICU.
Results: We recovered, on a period of 3 months spreading of the 12 March 2001 at 11 June 2001, a total cost of 28777 Euros for 31 NI recorded in adult ICU, and 15061 Euros for 14 NI in neonatology. The cost of a NI in adult ICU was therefore 928 euros. The one of a NI in neonatology was 1076 Euros. Components that have the more weighed in the total cost of the NI were antibiotics (62.5% of the total amount) follow-up by the biologic exams (15.7%), in adult ICU. In neonatology, it is the supplementary days of hospitalisation bound to the NI (47.2%) and the other medicines than antibiotics (21.06%) that were determinants in the total cost. In adult ICU, *Acinetobacter* was the more expensive germ, with a middle cost by NI of 1446 Euros. If we exclude nosocomial meningitis due to *Acinetobacter*, that cost 2867 Euros, the middle cost by the most elevated infection has been assigned to pneumonia (1882 Euros). In neonatology, *Serratia marcescens* detains the more important part in the total cost of the NI (73.01%), with a middle cost of 1571 Euros by infection. To the exception of one nosocomial pneumonia, all infections observed in neonatology were septicaemias of which one was complicated by a brain abscess. The middle cost of a septicaemia was 1107 Euros or 891 Euros, if we exclude the complicated septicaemia.
Conclusion: Our survey reveals that in relative difficulty spite to the calculation of the cost of the NI, notably with regard to the supplementary days of hospitalisation, the financial load is too much important to be disregarded. It's what can motivate the nursing staff to fight more again the NI and to incite administrators and decision-makers to finance programmes of struggle against these infections.

R2083 Bacterial profile and antimicrobial susceptibility pattern

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Objective: Aim of this study was to determine aetiology and the antimicrobial resistance rates of bacteria causing catheter-related infections in our hospital. After removal, semiquantitative culture of the external surface and the internal lumen quantitative catheter culture was performed.

Methods: Catheter colonisation was considered if >15 CFU in the roll procedure or >1000 CFU in the quantitative.
Results: A total of 175 catheter samples were analysed, bacterial growth was positive in 56 (32%) and negative in 119 (68%) samples. The most common isolate was *Pseudomonas aeruginosa* (21.4%), followed by coagulase-negative staphylococcus (CNS) (19.6%), *Acinetobacter* spp. (17.9%), *Staphylococcus aureus* (10.7%), *Stenotrophomonas maltophilia* (10.7%), *Klebsiella* spp. (5.4%), *Corynebacterium* spp. (3.6%), *Candida* spp. (3.6%), *Enterococcus* spp. (1.8%), *Escherichia coli* (1.8%), *Enterobacter* spp. (1.8%) and *Serratia marcescens* (1.8%). The prevalence of methicillin resistance was common in CNS (81.8%), compared with *S. aureus* (16.7%) isolates. *Enterococcus* spp. was not observed glycopeptide resistance. Carbapenems and aminoglycosides were the most effective antibiotics in Gram-negative bacteria.
Conclusion: A knowledge of the resident microbial flora and their antimicrobial susceptibility pattern is necessary for formulating a rational antibiotic policy in patients with catheter infection.

R2084 Risk factors for *Clostridium difficile* infection during an outbreak situation on a geriatric ward

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Objectives: *Clostridium difficile*- associated diarrhoea (CDAD) remains the leading cause of nosocomial-acquired diarrhoea. Prolonged hospital stay and diagnostic and therapeutic procedures due to CDAD cause additional costs. Risk factors for the acquisition of *C. difficile* in an outbreak situation were evaluated.
Methods: From June to April of 2003, 89 cases of diarrhoea were reported from a geriatric ward of a clinic for Internal Medicine. *C. difficile* – toxin ELISA and culture were done on all stool samples. Anamnestic data for 88 patients were evaluated. Statistical analysis were done using chi-square test.
Results: Fourty-eight stools were positive for *C. difficile* using culture and/or toxin ELISA. Vascular, heart, renal and pulmonal diseases were associated with *C. difficile* infection. Additionally, treatment with more than five drugs was related to *C. difficile* infection. Comparing patients with diarrhoea but without diagnosis of *C. difficile* and patients with CDAD, these factors were statistically significant correlated with the latter group. Interestingly, *C. difficile* infection was not associated with other gastrointestinal diseases.
Conclusion: Risk factors for the acquisition of *C. difficile* in outbreak situations seem to differ from risk factors in the normal hospital setting.

Infection in the immunocompromised host (except HIV)

R2085 The affigene(R) OPI Panel for Opportunistic Infections – viral load monitoring of CMV, EBV and HHV-6

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Background: Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Human Herpes virus 6 (HHV-6) infections are significant causes of disease and mortality among immunocompromised patients e.g. AIDS patients, cancer patients and transplant recipients. Anti-viral regimens are available and needs to be started at an early timepoint in order to achieve the best effect. Continuous monitoring of these viruses in patients at risk of developing disease, using sensitive diagnostic tests, is therefore important. Today, quantitative PCR is used in several laboratories for the monitoring of viral loads. However, truly standardised procedures are not available for all three viruses. The affigene(R) OPI (Opportunistic Infections) panel for Patient Disease Management (PDM) is comprised of three assays for efficient monitoring of CMV, EBV and HHV-6 viral load in serum/plasma specimens.
Methods: The assays were performed according to method manuals and evaluated utilising QCMD reference panels, clinical specimens and artificial specimens comprised of serum/plasma spiked with each specific virus. The three assays share a common sample preparation, which enables the user to analyse all three viruses from one single specimen preparation.
Results: All three assays can reliably measure decrease and increase in viral loads over time in clinical samples and good correlation was determined compared with expected viral loads e.g. reference panels, electron microscopy and in-house validated assays. Limit of detection (LOD), defined as more than or equal to 95% positivity rate, is 500, 500 and 1000 copies/mL for affigene(R) EBV VL, affigene(R) HHV-6 VL and affigene(R) CMV VL, respectively.
Conclusions: The affigene(R) OPI panel facilitates the monitoring of active viral infection in patients at risk of opportunistic infections. The performance of the assays make them suitable for preemptive monitoring.

R2086 Evaluation of predictive values of serum amyloid-A (SAA) and C-reactive protein (CRP) for infection and mortality in febrile neutropenic patients

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Introduction: Highly sensitive and specific indicators are being searched as early predictors of infection to identify the cause of fever and to determine the presence of infection in febrile neutropenic patients with malignancy.
Objective: In one of our previous studies, we observed that CRP values in the first 48 h of the FEN (febrile neutropenia) episode were statistically significant to indicate infection ($P = 0.001$), but insignificant to predict mortality ($P = 0.595$). Based upon this work, this study is performed to identify the value of SAA and CRP in early diagnosis of infection and prediction of mortality in FEN patients.
Methods: We evaluated 65 FEN episodes of 52 patients covered by FEN protocol in our Infectious Diseases Clinic. SAA and CRP levels were measured daily by Latex Agglutination Nephelometric Immunoassay during the course of antibiotherapy.
Results: Of total 26 infections, 23 were microbiologically and three were clinically defined, mortality was seen in only five cases. When we examined the predictive values of initial SAA and CRP levels regarding infection and mortality, it is not assumed that a significant difference exists (in case of infection, $P = 0.244$ for CRP, $P = 0.399$ for SAA; in case of mortality, $P = 0.949$ for CRP and $P = 0.929$ for SAA). SAA and CRP levels on the last day of the follow-up were found to be significant regarding infection and mortality (in case of infection, $P = 0.003$ for CRP, $P = 0.026$ for SAA; in case of mortality, $P < 0.001$ for CRP and $P = 0.021$ for SAA). Both initial and daily-measured values of SAA and CRP were positively and significantly correlated with each other ($P < 0.001$). When the changes of the two parameters in consecutive days were examined, increase in the levels of both parameters was greater on the second and third days in comparison to the first day but statistically significant gradual decline was observed after the

third day ($P < 0.001$). The difference between daily serum levels was more definite for SAA (for SAA $\times 2 = 186$, for CRP $\times 2 = 153$) therefore it is argued that SAA is a better parameter to predict infection and degree of inflammation and is more sensitive than CRP.
Conclusion: Despite low predictive values of these parameters in decision of initial therapy, they would be helpful in decision of modification and evaluation of response to therapy.

R2087 Microbiologic monitoring (MM) of infectious complications in patients undergoing radical mastectomy (RME)

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Objectives: To study pathogens and possible ways of developing wound infections (WI) in breast cancer pts, who underwent RME.
Methods: MM included culturing of tumour (from cutting place), taken intraoperatively, lymph – on the 2, 5 and 10 days after operation, wound discharge – if WI was seen. Nasal swabs were cultured twice before operation. If *Staphylococcus aureus* was isolated, phagotyping of strains was made.
Results: Growth of aerobic pathogens from cutting place of tumour was seen in 14.6%. All were coagulase-negative staphylococci (CNS). All these pts had no WI postoperatively, so it was decided these strains to be contaminants. Lymph was cultured in 61 pts. Growth of microorganisms was seen in 12 (19.7%): *S. aureus* – in five cases, CNS – in five cases, *Morganella morganii* – in one and *Corynebacterium* spp. – in one case. WI was seen in seven pts and in five of them (71.4%) *S. aureus* was isolated. The two other pathogens were: *Morganella morganii* and CNS, isolated twice from wound discharge. Single isolation of CNS in five pts and *Corynebacterium* spp. in one pt from lymph without WI was considered contamination. A total of 49 pts were taken nasal swabs and in 16 of 49 pts asympthomatic nasal carriage of *S. aureus* was seen. In two of 16 (12.5%) nasal carriers of *S. aureus* WI, caused *S. aureus* were seen, but it means that two of five pts (40%) with WI caused *S. aureus* were nasal carriers of *S. aureus*. Phygotyping confirmed identity of microorganisms isolated from lymph and from basal swabs.
Conclusion: *Staphylococcus aureus* is the most common cause of WI in pts undergoing RME. In pts who are nasal carriers of *S. aureus* autoinfection may be the mechanism of development of WI. Clinicians must consider eradication of nasal carriage of *S. aureus* with mupirocin before operation.

R2088 Investigation of bacterial peritonitis in haemodialysed patients (30-month study)

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Introduction: The microbial cause of peritoneal dialysis related peritonitis is an important determinant of clinical outcome and the basis of widely used treatment guidelines.
Objective: The aim of our study was to investigate the cause of bacterial peritonitis in undergoing chronic haemodialysis patients.
Materials and methods: A total of 27 patients on chronic HD were studied for the period January 2000–June 2003. All the clinical samples were examined in our laboratory. We did aerobic and anaerobic culture with the BD BACTEC SYSTEM. All isolates were identified by API or VITEK-II automated system, (Biomerieux, France). Susceptibility testing was performed by disc diffusion method, according to the NCCLS guidelines.
Results: During the study period we found 51 cases of peritonitis in 16 pts from 27 pts (59.2%). In three cases we had negative cultures of peritoneal fluid. We found Gram-positive peritonitis in 27 from 49 cases (55%). *S. epidermidis* was the most common cause of peritonitis (30.6%) and the second common cause was *E. coli* (20.4%). *P. aeruginosa* (12.7%), *Streptococcus* spp. (10.2%), *Enterococcus* spp. (10.2%), *S. aureus* (4%), and *Klebsiella pneumoniae* (4%) were the other common causes. Methicillin-resistant staphylococci were the 66.7% of *Staphylococcus* spp. We found in the Gram-negative strains multiresistant to antibiotics. About 20% of the strains were resistant to ampicillin and cephalothin and 20% resistant to ciprofloxacin.
Conclusions: Our study showed significant trends of causative pathogens of peritoneal dialysis-related peritonitis and increase in antibiotic resistance.

R2089 A case of *Salmonella enteritidis* soft tissue and joint infection in a patient with rheumatoid arthritis under anti TNF-α treatment

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Background: Focal extraintestinal infections from nontyphoid salmonellae have increased in incidence during the past decade. Isolated salmonella soft tissue infections are relatively rare, occurring mostly in adults with chronic underlying conditions, such as diabetes mellitus and cell-mediated immunity defects. Patients treated with anti TNF-α antibodies exhibit increased susceptibility to infections caused by intracellular microbes, such as *Salmonella*.
Purpose: To present a female patient with rheumatoid arthritis on antiTNF-α treatment with a postoperative soft tissue and joint infection due to *Salmonella enteritidis*.
Case presentation: A 52-year-old female patient with rheumatoid arthritis, initially treated with corticosteroids and naproxen, was operated for a right knee ruptured Baker's cyst. Two weeks postoperatively, arthritis symptoms rapidly deteriorated, and leflunomide plus infliximab were added. Despite prompt clinical response, she developed a painful, inflammatory swelling of the right popliteal area, which was automatically ruptured, draining pus and serosanguinous material. Cultures revealed *Salmonella enteritidis*, and markedly elevated ESR and CRP were noted. Steroid-induced diabetes mellitus was also discovered. The patient had no gastrointestinal symptoms and stool cultures were negative for *Salmonella* spp. At that point, the patient was referred to our clinic. MRI of the affected area showed a marked inflammatory process of the surrounding soft tissue with increased intra-articular fluid collection and thickening of the synovium. No radiological sign of osteomyelitis was evident. Immunomodulatory agents were stopped and ciprofloxacin 750 mg q12 h and glibenclamide were administered. Inflammatory markers soon dropped and optimal glucose control was achieved. Surgical debridement and reconstruction of the knee joint was performed under antibiotic coverage uneventfully. The patient completed a 12-week course of ciprofloxacin without adverse events, and infliximab is scheduled to be restarted.
Conclusion: *Salmonella* spp. although rare, should always be considered as a potential pathogen in soft tissue and joint infections in patients with certain comorbidities (e.g. diabetes) or under immunosuppressive medications (such as antiTNF-α antibodies). Opportunistic infections during antiTNF-α regimens do not necessarily preclude its future use in the same patient, once infection is treated, although secondary prophylaxis could be an issue.

R2090 Severe *Clostridium difficile* infection in solid organ transplant recipients

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Objectives: *Clostridium difficile* infection can lead to severe intestinal disease in immunosuppressed patients (pts). However, the importance of this infection is not well assessed in transplant

recipients. Our objective was to review the clinical presentation and treatment outcome of *C. difficile* infection in our SOT population.
Methods: We retrospectively reviewed the medical files of SOT recipients with the *C. difficile* infection (diagnosed by positive stool culture and/or toxin detection) hospitalised in our academic hospital over a 2-year period.
Results: *Clostridium difficile* was detected by culture (21) and toxin (10) in 21 pts (liver (seven), kidney (four), lung (four), heart-lung (four), heart (one) and liver–kidney (one) transplantation). Six of them (28%) had cystic fibrosis. Fourteen pts developed *C. difficile* infection during hospitalisation (eight during the first month after TX) and seven were admitted for severe diarrhoea. Clinical presentation consisted of uncomplicated diarrhea in 16 patients (five of whom had diarrhoea severe enough to require admission). Toxin was detected in stools in six of them. Eight pts were treated with metronidazole alone (six) or combined later with oral vancomycine (two) because of failure. A kidney transplant recipient presented four relapses in a few months and received multiple courses of treatment. Severe clinical manifestations were observed in four cystic fibrosis patients, 3, 4, 8 and 9 years after transplantation. They had major abdominal pain associated with severe diarrhoea in one and constipation in three. They were admitted in ICU for peritonitis and severe sepsis. Toxin was detected in all four. Pancolitis was found on abdominal CT and pseudomembranes or ulcerations were found on colonoscopy. A laparotomy was performed in two pts for suspicion of perforation, which was found in one (colic perforation). Treatment consisted in a combination of IV metronidazole with IV and intracolic vancomycin (and IV Ig in one pt). Two pts died during treatment.
Conclusion: In this study of *C. difficile* infection in SOT, we identified cystic fibrosis patients with lung transplantation as a group at risk for complicated disease (sepsis and perforation) and atypical clinical presentation (constipation instead of diarrhoea).

R2091 Correlation of *Helicobacter pylori* infection with gastric histopathologic and ultrastructural changes in chronic end-stage renal failure patients

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Objectives: The aim of this study was to determine the histopathologic and ultrastructural changes in *Helicobacter pylori* (Hp) positive and negative patients with end stage renal disease (ESRD).
Methods: We studied 36 ESRD patients with dyspeptic symptoms. Hp was detected 12 of them with microscopic, cultural and rapid urease techniques under endoscopic examination. For control group 36 patients with normal renal function was selected. All patients had dyspeptic symptoms. In control group out of 36, 12 patients were Hp positive and 24 patients were Hp negative. Biopsies were obtained from antrum and corpus to determine histopathologic and ultrastructural changes.
Results: There were no significant histopathologic differences between Hp positive and Hp-negative patients with ESRD. In addition no significant differences were found between patients with ESRD and patients without ESRD in regards of the status of Hp positivity. Chronic active gastritis was the main lesion in both Hp-positive patients with ESRD and Hp positive control group. In both Hp-negative groups beside chronic active gastritis, superficial gastritis, and chronic erosive gastritis were also the main lesions. Ultrastructural changes in Hp-positive tissues were similar in both groups but tight junction depletion was seemed more distinct in patients with ESRD than control group.
Conclusions: Our study showed us histopathological and ultrastructural changes not exactly differentiated ESRD group than the control group. However, more studies should be preformed in larger groups.

Immunology, host defenses, immunotherapy

R2092 Cytokine induction in peripheral blood mononuclear cells by various heat-stable serotypes of *Campylobacter jejuni*

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It is well known that some serotypes of *Campylobacter jejuni*, a common enteropathogen, are associated with postinfectious autoimmune-mediated disorders, like the neurologic demyelinating Guillain-Barré syndrome (GBS), a result of the production of cross-reactive antibodies to human peripheral nerve gangliosides. However it has been reported that besides antibodies, other soluble substances, like cytokines may contribute to this phenomenon of molecular mimicry. This prompted the hypothesis, that GBS-triggering *C. jejuni* heat-stable (HS, Penner) serotypes could induce in peripheral blood mononuclear cells (PBMC), cytokine patterns promoting inflammatory processes. To test this, three proinflammatory [IL-2 soluble receptor a (IL-2sRα), IL-6 and IFN-γ] and one anti-inflammatory (IL-10) cytokines were measured, after induction in PBMC by seven GBS-associated *C. jejuni* serotypes (HS:2, HS:5, HS:19, HS:37, the complexes HS:1,44, HS:4, 13, 16, 43, 50 and HS:23, 36, 53), five not yet reported as associated with GBS (HS:8, HS:11, HS:15, HS:31, HS:52) and one serotype known as not capable to cause GBS (HS:3). Interestingly no differences were found for IL-6, IFN-γ and IL-10 among all serotypes tested, whereas a decreased production of IL-2sRα characterised serotype HS:3, the only nonsialylated serotype. Provided that IL-2sRα is found in high concentrations in the serum of GBS patients, our results could lead to the question suggesting, as to whether the lack of sialylation as such, might be related to mechanisms involving inflammatory factors like cytokines.

R2093 Expression of phagocyte Fc gamma receptors in bacteraemia

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Background: Early markers of bacteriaemia are useful for prognosis and, in decision making for i.v. antibiotic therapy.
Objectives: To assess the diagnostic power of the surface expression of Fc receptors for IgG (FcgRs) for the prediction of bacteriaemia in febrile patients.
Methods: We performed a prospective case-control study on 75 consecutive patients (pts) with an episode of bacteriaemia when compared with 83 randomly selected concurrent febrile pts with negative blood cultures (control). Demographic and clinical data were collected by chart review and/or questioning their attending physicians. Plasma levels of C-reactive protein (CRP), TNFg, IL-1a, IL-6, IL-8 and IL-10 were determined. The surface expression of Fc receptors for IgG (FcgRs): FcgRI, FcgRII and FcgRIII on peripheral blood monocytes (M) and granulocytes (G) was assessed by flow cytometry. These studies were done concomitantly with blood cultures.
Results: Both groups were not different for age, sex, previous administration of immunosupressants or antibiotics, clinical sever-

ity index or comorbid conditions. In univariate analysis, cases had significantly higher levels of CRP ($P < 0.001$), TNFg ($P < 0.001$), IL-1a ($P < 0.001$) and IL-6 ($P < 0.01$) than controls. The expression of FcgRIIA and FcgRIII by M and, that of FcgRI on G was significantly enhanced ($P < 0.001$) in bacteriaemic patients when compared with culture-negative febrile pts; while the expression of FcgRIIB by either M or G was significantly decreased ($P < 0.03$). Setting a cut-off value (25%) of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence of bacteriemia of 5-10% among hospitalized patients undergoing blood cultures, results in a sensitivity, specificity, positive and negative predictive values of: 77%, 95%, 74%, and 98%, respectively for M-FcgRIIA, 73%, 96%, 74% and 97%, respectively for M-FcgRIII, 58%, 91%, 49% and 96%, respectively for G-FcgRI and 71%, 81%, 57% and 73%, respectively for G-FcgRIIB.
Conclusions: Our results suggest that the surface expression of Fc receptors for IgG on peripheral blood monocytes and granulocytes may help clinicians to rule out bacteriaemia in febrile patients.

R2094 Expression of phagocyte Fc gamma receptors in active tuberculosis

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Background: Surface receptors for IgG (FcgRs) on phagocytic cells are important in host defence against infection.
Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), monocytes cultured for 72 h (M/Mø), and granulocytes (G) in patients with active Tuberculosis (TB), during anti-tuberculous therapy (anti-TB-Rx) and, after completition of anti-TB-Rx.
Methods: The surface expression of the three type of FcgRs, FcgRI, FcgRII and FcgRIII, on M, M/Mø and G were analysed by flow cytometry in 52 HIV-negative patients with TB (43 men and nine women), at diagnosis of TB, and monthly thereafter until completion of anti-TB-Rx. FcgRs expression was assessed on resting M, M/M⊘ and G, and on these cells after stimulation by culture in the presence of IFNg.
Results: The expression of FcgRI and FcgRIII by M, M/M⊘ and G was significantly enhanced in patients with active TB by: $41 \pm 4\%$ and $23 \pm 2\%$ for M, respectively ($P < 0.001$), $57 \pm 6\%$ and $40 \pm 4\%$ for M/M⊘, respectively ($P < 0.001$) and, $128 \pm 9\%$ and $35 \pm 3\%$ for G, respectively ($P < 0.001$). The expression of FcgRIIA by M, M/Mø and G was significantly decreased by $-32 \pm 1\%$ ($P = 0.02$), $-43 \pm 3\%$ ($P < 0.001$), and $-24 \pm 1\%$ ($P = 0.002$), respectively. These alterations of phagocyte FcgRs expression returned to normality after 8 weeks of effective anti-TB-Rx and, remained normal until the end of TB treatment. The expression of FcgRI, FcgRIIA and FcgRIII by M, M/M⊘ or G from patients with TB was significantly increased by culture in the presence of IFNg ($P < 0.001$). Nevertheless, the expression of FcgRIIB by M, M/Mø or G from patients after effective anti-TB-Rx was not significantly increased by culture in the presence of IFNg.
Conclusions: Macrophages and granulocytes from HIV-negative patients with active tuberculosis exhibit an increased expression of FcgRI, FcgRIIA and FcgRIII and, an impaired expression of FcgRIIB, that disappear after effective anti-TB therapy.

R2095 A durable response to relapsing *Clostridium difficile* colitis may require combined therapy with oral vancomycin and intravenous gamma globulin

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Objectives: Since being first described in the late 1970s relapsing pseudomebranous colitis occurs in 10–20% of patients after being treated with oral metronidazole, vancomycin or bacitracin. Previous studies in children with relapsing *C. difficile* colitis have shown low or absent levels of toxin A antibodies. We studied eight adult patients with relapsing *C. difficile* colitis who were hospitalised with profound diarrhoea and dehydration.
Methods: Eight adult patients were studied ranging in age from 55 to 78 years. All had at least 1 positive stool for *C. difficile* Toxin A or B by ELISA assay. Toxin A specific-immunoglobulin was also assayed by ELISA using flat-bottom microtitre plates and were incubated overnight with toxin A in carbonate buffer. Horseradish peroxidase conjugated affinity-purifed goat anti-human IgG was added. The OD was measured at 405 nm on a micro-ELISA plate reader. Patients received oral vancomycin at a dose of 500 mg thrice daily together with IVIG, 30 g twice within 48 h. Five patients also received *Saccharomyces boulardii* orally twice daily.
Results: All eight patients failed to produce detectable *C. difficile* antibody. Treatment with oral vancomycin and IVIG resulted in disappearance of diarrhoea and normalisation of leucocytosis within 4 days of initiating treatment. *C. difficile* toxin was no longer demonstrable in the stool by day 5 of treatment, although rectal bleeding persisted in two patients with severe pseudomembranous colitis. None of these eight patients had recurrent disease after treatment.
Conclusion: A combination of oral vancomycin together with IVIG appears to be successful treatment in patients with relapsing *C. difficile* colitis. All eight patients in this small study were defective in their ability to produce specific antibody to toxin A. Further studies evaluating the nature of the defect in specific antibody formation that likely is responsible for this syndrome are currently underway.

R2096 Alteration of phagocyte Fc gamma receptors expression in HIV-infected patients with tuberculosis

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Background: Macrophage and granulocyte receptors for IgG (FcgRs) are important in host defence against infection.
Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), monocytes cultured for 72 h (M/Mø), and granulocytes (G) in HIV-infected patients with active tuberculosis (TB), during anti-tuberculous therapy (anti-TB-Rx) and, after completion of anti-TB-Rx.
Methods: The surface expression of FcgRI, FcgRII and FcgRIII, on M, M/Mø and G were analyzed by flow cytometry in 36 HIV-infected patients with active TB (33 men and three women), at diagnosis of TB, and monthly thereafter until completition of anti-TB-Rx. FcgRs expression was assessed on resting M, M/Mø and G, and on these cells after stimulation by culture in the presence of IFNg.
Results: The expression of FcgRI and FcgRIII by M, M/M⊘ and G was significantly enhanced in HIV-infected patients with active TB by: $32 \pm 3\%$ and $16 \pm 2\%$ for M, respectively ($P < 0.001$), $41 \pm 4\%$ and $27 \pm 2\%$ for M/Mø, respectively ($P < 0.001$) and, $52 \pm 5\%$ and $18 \pm 1\%$ for G, respectively ($P < 0.001$). The expression of FcgRIIB by M, M/M⊘ and G was significantly decreased by $-22 \pm 1\%$ ($P = 0.02$), $-31 \pm 2\%$ and, $-17 \pm 1\%$ ($P < 0.005$), respectively. These alterations of phagocyte FcgRs expression returned to normality after 12 weeks of effective anti-TB-Rx and, remained normal until the end of TB treatment. The expression FcgRI, FcgRIIA and FcgRIII by M, M/Mø and G from HIV-infected patients with active TB was significantly increased by culture in the presence of IFNg ($P < 0.005$).
Conclusions: Macrophages and granulocytes from HIV-infected patients with active tuberculosis exhibit an increased expression of FcgRI and FcgRIII and an impaired expression of FcgRIIB. These alterations of phagocyte FcgRs expression during active tuberculosis in HIV-infected patients disappear after effective anti-tuberculous therapy.

R2097 High prevalence of phagocytic-resistant capsular serotypes of *Klebsiella pneumoniae* in liver abscess

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Objectives: To assess the role of *K. pneumoniae* CPS K1 or K2 in liver abscess and development of endophthalmitis, we used an experimental model of phagocytosis for *K. pneumoniae* with different CPS isolated from patients with and without liver abscess and compared the resistance of the isolates to neutrophil-mediated phagocytosis.
Methods: Neutrophil phagocytosis of 70 CPS isolates included K1 ($n = 23$)/K2 ($n = 10$), non-K1/K2 ($n = 37$) were evaluated by flow cytometry, fluorescence imaging, and electron microscopy.
Results: K1/K2 isolates were significantly more resistant to phagocytosis ($P < 0.0001$) than non-K1/K2 isolates, and displayed increased resistance to intracellular killing. Although mucoid phenotype (M-type) K1/K2 isolates were significantly resistant to phagocytosis ($P = 0.0029$) than M-type non-K1/K2, no significant difference in the phagocytosis rate was observed between K1/K2 isolates with M-type and non-M-type ($P = 0.0924$). Mucoidy is an associated factor that was predominant in K1/K2 isolates, but which itself is not an independent influence to phagocytic resistance. The K1/K2 CPS proved significantly more resistant to phagocytosis than non-K1/K2 CPS in liver abscess isolates ($P < 0.0001$) and nonabscess isolates ($P = 0.0001$), implicating K1/K2 isolates were generally more virulent in both liver abscess and in nonliver abscess conditions.
Conclusions: Resistance of CPS K1 or K2 *K. pneumoniae* to phagocytosis and intracellular killing may thus contribute to their high prevalence in liver abscess and uniquely in endophthalmitis.

R2098 Hierarchy of baby-linked immunogenetic risk factors in vertical transmission of hepatitis C virus

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Objective: Mother-to-infant transmission of hepatitis C virus (HCV) represents the major cause of paediatric HCV infection today but the rate of vertical transmission is low (5–6%). Data about specific predictors of HCV transmission are conflicting. Although the hypothesis regarding the role of host defences is highly intriguing, immunogenetic influence has been poorly investigated. All existing studies on associations between HCV and genetic markers have been done in adults and are mainly confined to HLA-class II serological polymorphisms.
Methods: Among 290 parities of HCV-RNA infected women, 21 babies (7%) resulted infected (HCV-RNA positive steadily positive over 20 months of age). All the 21 infected babies, 44 randomly selected uninfected ones (steadily negative for HCV-RNA during a follow-up of 2 years) and their mothers were investigated for HLA-G, -C, -DRB1, -DQA1 and -DQB1 molecular polymorphisms. Several nonimmunogenetic parameters were also considered and their contribution was weighted by multivariate analysis.
Results: Among the different covariates, a hierarchy of susceptibility has been settled using multiple logistic regression analysis: HLA-Cw*07,-G*010401,-DRB1*0701,-DRB1*1401, maternal viral genotype 1b, male sex, first birth and breast feeding can be considered as risk factors for HCV vertical transmission. On the contrary, protection was conferred by the HLA-DQB1*06,-G*0105N,-Cw*0602,-DRB1*1104,-DRB1*1302 alleles and by formula feeding.
Conclusions: Our study demonstrates that the immunogenetic factors related to maternal and neonatal HLA profile may affect HCV vertical transmission and is independent from the other nonimmunogenetic parameters. The finding of babies genetically able to fight the virus so precociously could be a tangible demonstration of the feasibility of a successful vaccine.

R2099 The role of interleukin-16 in juvenile idiopathic arthritis: proinflammatory or antiinflammatory

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Objectives: Juvenile idiopathic arthritis (JIA) is a systemic autoimmune disease of childhood characterised by chronic inflammatory reaction in joints. Cytokine response in JIA patient is well defined. The proinflammatory and anti-inflammatory roles of different cytokines are also determined. But the role of newly defined interleukin (IL)-16 (proinflammatory or anti-inflammatory) cannot be clearly defined in rheumatoid arthritis patients. The aim of this study is to investigate the IL-16 levels in JIA patient as a novel laboratory data and to determine the exact role (proinflammatory or anti-inflammatory) of this cytokine.
Methods: Seventy-one JIA patients (47 female, 24 male) were studied in this study. Twenty-nine of JIA patients had active disease during the evaluation. Control group consisted of 17 healthy children. Serum samples of all subjects were studied. IL-1β, IL-6 and TNF-α levels were determined to demonstrate the proinflammatory response. Interleukin 1β, 6, 16 and TNF-α were measured by ELISA methods. C-reacting protein (CRP) was studied turbidimetric methods.
Results: IL-6, TNF-α, IL-16 and CRP levels of JIA patients were significantly higher than those of healthy children ($P = 0.014$, 0.0001, 0.008 and 0.001, respectively). IL-6, TNF-α, IL-16 and CRP levels of JIA patients with active disease were significantly higher than those of patients who were in inactive state of disease ($P < 0.05$). Interleukin-16 levels of JIA patient showed significant correlation with IL-1β, IL-6, TNF-α, CRP levels, erythrocyte sedimentation rate and platelet count. ($P = 0.16$, 0.047, 0.001, 0.013, 0.026 and 0.034, respectively).
Conclusions: The results of this study that investigated the IL-16 levels of JIA patients for the first time, demonstrated the correlation between the levels of IL-16 and other well known proinflammatory cytokine. Thus, IL-16 can be defined as a proinflammatory cytokine.

R2100 Effect of thalidomide on serum pro-inflammatory mediators in experimental sepsis by multiresistant *Pseudomonas aeruginosa*

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Objectives: Thalidomide has been proved to be a potent immunomodulator in experimental sepsis by susceptible *Escherichia coli* (Giamarellos-Bourboulis *et al*. AAC 2003). Its effect was tested in sepsis by multidrug-resistant (MDR) *P. aeruginosa*.
Methods: Sepsis was induced in 64 rats after intraperitoneal injection of an 8 log 10 inoculum of one isolate resistant to ceftazidime, imipenem, ciprofloxacin and amikacin. Animals were divided in three groups: A ($n = 15$) controls; b ($n = 24$) administered seed oil; and C ($n = 25$) administered 50 mg/kg of thalidomide. Thalidomide was diluted in seed oil and it was given by an orogastric catheter 30 min before bacterial challenge. Five hours after bacterial challenge animals were sacrificed and blood was sampled by their inferior vena cava for determination of endotoxins (LPS), tumour necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), nitric oxide (NO) and malondialdehyde (MDA). LPS were estimated by the QCL-1000 LAL assay, TNF-α and INF-γ by EIA, NO by a colorimetric assay and MDA by the thiobarbiturate assay.
Results: Median LPS of groups A, B and C were 13.1, 13.3 and 13.3 EU/mL, respectively. Respective values of serum TNF-α were 72.5, 62.5 (pNS compared with A) and <5 pg/mL ($P = 0.012$ compared with A); of serum INF-γ 835.6, 125.0 and 385.0 pg/mL; of serum NO 1150.0, 132.5 and 192.5 μM; and of serum MDA 5.5, 2.0 and 4.5 mM.

Conclusions: In an experimental model of sepsis by MDR *P. aeruginosa* with similar levels of enodoxaemia in all groups of treatment, thalidomide decreases serum levels of TNF-α without affecting the other pro-inflammatory mediators.

R2101 Assessment of the influence of IL-8 concentration in the cerebrospinal fluid on the course of bacterial meningitis

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Objectives: To determine the role of IL-8 in the pathogenesis of bacterial meningitis (BM).
Methods: A total of 42 patients (13 female, 29 male, age range 20–80 years, mean age 45.5 years) with BM were investigated. Control group consist of 25 patients (two female, 23 male, mean age 31 years) with viral meningitis (VM) and 23 patients (nine female, 14 male, mean age 30years) without meningitis. In all investigated group concentration of IL-8 (cIL-8) in CSF was determine by ELISA at the day of admission (CSF-I). In the group with BM cIL-8 was checked in CSF taken 72 h later (CSF-II).
Results: cIL-8 in CSF-I in patients with BM was significantly higher ($P < 0.001$), mean 1696 pg/mL (min. 852 pg/mL, max. 2631 pg/mL) than in VM (mean 405 pg/mL, min. 0 pg/mL, max. 2089 pg/mL) and in the group without neuroinfection (mean 32.59 pg/mL, min. 0 pg/mL, max. 324.9 pg/mL). In BM, cIL-8 in CSF-II was lower ($P < 0.001$) than in CSF-I, mean 693.7 pg/mL (min. 58.4 pg/mL, max. 2181 pg/mL). However during the analysis of selected cases increase of cIL-8 in CSF-II was observed among patients whose conditions had deteriorated. Correlation between cIL-8 and pleocytosis ($r = 0.46647$, $P = 0.002$), protein ($r = 0.42710$, $P = 0.005$) and glucose ($r = -0.4323$, $P = 0.004$) concentration in CSF was observed. Inversely proportional dependence between cIL-8 in CSF-I and number of points aggregated by patients in GCS was found. There was no correlation between the course of BM and cIL-8 in the CSF-I. Whereas, mean cIL-8 1369.8 pg/mL in CSF-II was associated with increase risk of patient death, and was higher ($P < 0.001$) than in group of patients who recovered without sequels (mean cIL-8 29.84 pg/mL). Also in the group of patients who developed neurological complications cIL-8 in CSF-II was increase (mean cIL-8–940 pg/mL).
Conclusion: (i) Assessment of cIL-8 in CSF can be useful in differential diagnosis of meningitis. (ii) Correlation between cIL-8 and severity of inflammation in subarachnoid space has been observed. (iii) Persistence of high levels of IL-8 in CSF in spite of therapy is associated with increase mortality rate and risk of neurological sequels.

R2102 Moxifloxacin modulates the immune responses of human neutrophils

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Background: The new quinolones such as Moxifloxacin are reported to accumulate in active form in phagocytic cells. Thus their modulatory role on phagocyte functions is of great interest. Neutrophils control infection by a sequential multistep process leading to oriented motility, the chemotaxis. The parallel changes in the expression pattern of surface molecules can be regarded as a reflection of neutrophil reaction (adhesion, oxidative burst etc.). In addition human neutrophils recognise conserved pathogen-associated microbial products (PAMPs) through the mammalian toll-like receptors (TLRs). Toll-like receptor 4 acts as the primary effector of LPS recognition, TLR-2 mediates responses to Gram-positive bacterial proteins and TLR9 is involved in recognition of bacterial DNA. The objective of our study was to investigate the immunomodulatory properties of Moxifloxacin, a 8-methoxyquinolone on human neutrophils (PMN).
Methods: We used a two-compartment chamber system (Transwell) to analyse the influence of Moxifloxacin on the migration of neutrophils towards the chemotactic factors interleukin-8 (IL-8), leucotriene B4 (LTB4), and C5a. The surface expression of function-associated surface molecules, such as CD11b, CD44, CD69, CD66b, the fMLP receptor and the Toll-like receptors 2- and 4 were studied on chemoattracted and on nonmigrated PMN by FACS analysis and by quantitative PCR (TLR-2, TLR4). Moxifloxacin was used at total amounts between 0.008 and 80 μg.
Results: The presence of Moxifloxacin resulted in an increased migration of PMN towards IL-8 by up to 166 + 8%, but suppressed the migration towards LTB4 down to 1.15 + 0.87% and showed no effects with regard to C5a. With regard to the activation status of chemoattracted PMN Moxifloxacin only modulated C5a-induced CD66b expression; a downregulation by up to 26% was observed. Moxifloxacin modulated TLR2, TLR4 and TLR9 mRNA expression in chemoattracted neutrophils. All modulatory effects of Moxifloxacin were observed in a dose-dependent manner (0.008–80 μg).
Conclusion: Our data emphasises that the antimicrobial agent Moxifloxacin has the potential to modulate the innate immune response during host defence via defined cellular signalling pathways. This immunomodulatory capacity of Moxifloxacin may thus contribute to the early host response against microbial infections.

R2103 Lipopolysaccharides of *Proteus mirabilis* O3 and O18 induce cell-specific IL-8 response within the human urinary tract

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Objectives: *Proteus mirabilis* is commonly associated with complicated urinary tract infections and may cause cystitis, eventually acute pyelonephritis or even septicaemia. Patients with functional urinary tract anomalies or with chronic instrumentation run the greatest risk. The objectives of present study was to evaluate the role of different cell types within the human urinary tract, uroepitelial cells, renal cells and monocytes, and different *Proteus mirabilis* strains O3 and O18 in relation to the local inflammatory response. The immunodeterminant oligosaccharide characteristic for *P. mirabilis* O3 (1959) is D-galacturonyl 1,4-D-galactosamine disaccharide substituted by lysine, while Proteus O18 is characterised by a phosphocholine substitute.
Methods: Cells were stimulated with LPS from *Proteus mirabilis* O3 and O18. The production of interleukin-8 was measured on the protein and mRNA levels using ELISA and real-time PCR, respectively. Expression of CD-14 on the cell surface was studied using flow cytometry.
Results: Monocytes and uroepithelial cells reacted to LPS by higher production of IL-8 when compared with renal epithelial cells. The response to LPS of *P. mirabilis* O18 was higher when compared with *P. mirabilis* O3 both in monocytes and renal epithelial cells but without statistical significance. Since *P. mirabilis* O18 is characterised by its phosphocholine substitute, we stimulated cells also with phosphocholine alone and in combination with *P. mirabilis* O3 LPS. However, an IL-8 response was not induced after stimulation with phosphocholine, nor was it augmented after simultaneous stimulation together with LPS. Despite previous controversies, uroepithelial cells expressed CD-14 and accordingly reacted to LPS by higher production of IL-8, which was in contrast to renal epithelial cells.
Conclusion: Our findings suggest that phosphocholine-rich O18 lipopolysaccharide is not biologically more active in monocytes and different cells of human urinary system. Likewise, IL-8 response was not induced or augmented by adding of free phosphocholine to the cells. Our data suggest that different cells within the human urinary tract play distinctive roles during urinary-tract infection and that the IL-8 response to lipopolysaccharides does not differ between strains with different pathogenicity.

R2104 Innate immunity as a site of production of procalcitonin

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Objectives: Although there are conflicting data in the literature, it appears that procalcitonin (PCT), which is a reliable marker of sepsis, is produced, at least in part, by cells of the monocyte/macrophage lineage. Possible differences in production of PCT by human monocytes after stimulation by susceptible and multidrug-resistant (MDR) isolates of Gram-negative bacteria were studied.
Methods: Human monocytes were isolated from healthy volunteers after centrifugation of heparinised blood over Ficoll Hypaque, incubation of the mononuclear fraction in RPMI 1640 with 10% FCS and 2 mM glutamine, and removal of nonadherent cells. Monocytes were subsequently harvested and resuspended at a final concentration of 50 000 cells/well. Inocula of 4.6 log10 and 6.6 log10 of eight susceptible and 12 MDR isolates of *P. aeruginosa* and 10 susceptible and 10 MDR isolates *E. coli* were then added, one in each well and supernatants were collected after 2 and 4 h of incubation. PCT was measured by immunochemoluminometric assay.
Results: PCT (mean ± SE ng/10 000 cells) after stimulation by a 4.6 log10 inoculum of *Pseudomonas aeruginosa* was 0.27 ± 0.16 after 2 h. Respective values after stimulation with a 6.6 log10 inoculum were 2.98 ± 0.54 after 2 h and 2.08 ± 1.04 after 4 h (P: NS). Values of PCT after stimulation by a 4.6 log10 inoculum *E. coli* were 0.10 ± 0.02 at 2 h and after stimulation by 6.6 log10 inoculum were 0.05 ± 0.05 and 0.13 ± 0.05 at 2 and 4 h, respectively. Compared with stimulation by *P. aeruginosa*, production of PCT after stimulation by *E. coli* was significantly decreased at both 2 ($P < 0.001$) and 4 h ($P = 0.04$).
Conclusions: It appears that PCT production by human monocytes depends on the challenge microorganism. It is possible that different signal transduction pathways might be involved after stimulation by various microorganisms. Correlation to clinical data remains to be studied.

R2105 Clarithromycin: immunomodulatory therapy of experimental sepsis and acute pyelonephritis by *Escherichia coli*

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Objectives: Clarithromycin (CL) has been proved an effective immunomodulator when administered intravenously in parallel to bacterial challenge in experimental sepsis by *Pseudomonas aeruginosa* (Giamarellos-Bourboulis *et al.* AAC 2004). Its effect was assessed in experimental sepsis by *Escherichia coli* when applied on sepsis-associated pulmonary edema (SAPO).
Methods: Acute pyelonephritis was induced after ligation of the right ureter and injection of one isolate susceptible to amikacin (AM) into the renal pelvis of 70 rabbits assigned into seven groups of treatment. A controls; B–D therapy initiation in parallel to bacterial challenge as follows: B: CL; C: AM; D: both agents; E–G: therapy initiation on SAPO as follows: E: CL; F; AM, G: both agents. SAPO was presented six hours after bacterial challenge. CL was infused at two iv doses with a 2-hour interval between them, 80mg/kg the first dose and 50mg/kg the second dose. AM was given iv bolus at 15 mg/kg following the first dose of CL. Tumor necrosis factor-alpha (TNF) was estimated by a bioassay on L929 fibrosarcoma cell line and malondialdehyde (MDA) by the thiobarbiturate assay. Blood monocytes were isolated after centrifugation of whole blood over Ficoll Hypaque, incubation of the mononuclears in RPMI with 10% FCS and removal of nonadherent cells. Monocytes were lysed and caspase-3 activity was estimated in the cytosolic extract by a chromogenic assay.
Results: Mean ± SE survival of animals of groups A, B, C, D, E, F and G were 2.51 ± 0.61, 16.00 ± 2.43, 13.45 ± 2.55, 15.75 ± 2.97, 7.60 ± 2.79, 10.25 ± 2.90 and 11.40 ± 3.05 days, respectively. Median TNF at 48 h of groups A, B, C and D were 37.5, 5.75, 5.75 and 5.75 pg/mL respectively and of MDA 6.6, 2.7, 1.8 and 3.3 mM, respectively. Median caspase-3 activity at 24 h of groups A, B, C and D were 272.8, 18.1, 6.1 and 0 pmol/min/10 000 cells, respectively. On SAPO median TNF of groups E, F and G were 8.62, 8.00 and 11.5 pg/mL and at 48 h 11.5,11.5 and 5.75 pg/mL respectively. On SAPO median MDA of groups E, F and G were 4.1, 5.1 and 5.2 mM and at 48 h 1.6, 2.3 and 4 mM, respectively. On SAPO median monocyte caspase-3 activity of groups E, F and G were 4.9, 100 and 17 pmol/min/10 000 cells and at 24 h 0,10 and 0 pg/mL, respectively.
Conclusions: Intravenous CL attenuates systemic inflammation and apoptosis being a promising immunomodulator for sepsis since its efficacy was proven after administration on SAPO.

R2106 Absence of booster effect 6 months after vaccination in individuals with initial long-term protection against diphtheria

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Objectives: In the 1990s, the Newly independent States of the former Soviet Union experienced the largest diphtheria outbreak since the 1960s. This resulted in a change in the Russian vaccine programme, including extra booster doses. Increased herd immunity was a result of this initiative but additional boosters also caused vaccination of highly protected individuals especially among children and young adults. The main objective of the investigation was to study the booster response in individuals with initially high antitoxin levels.
Methods: Sixty participants of the study got a single dose of diphtheria-tetanus vaccine (5 Lf diphtheria toxoid). The mean age was 18.7 years and the mean time since the last vaccination was 4.3 ± 3.6 (SD) years. Blood samples were taken before the booster doses were given and 1 week and 1, 2, 3 and 6 months after. A double antigen enzyme linked immunosorbent assay was used for the assessment of the antibody levels. The study population was divided into two groups based on antibody levels to investigate if high initial antibody levels influenced on the antibody response. The 'low' group included participants with initial antibody level <1 IU/mL, the 'high' group with initial antibody level higher than or equal to 1 IU/mL (long-term protection).
Results: The initial antibody level was high in all individuals; geometrical mean titre (GMT) was 0.73 IU/mL. The highest antibody levels in both groups were reached 1 month after the vaccination (GMT 5.07 IU/mL); the difference between the groups was not significant. The ratio between initial and 1 month antibody concentrations was >10 times higher and after 6 months still four times higher in those with initial antibody levels <1 IU/mL. In individuals with initial antibody levels >1 IU/mL a twofolddecrease was observed after 6 months compared with the initial levels.
Conclusions: Both groups responded to the booster by significant enhance of the GMT, but 6-month after the booster effect was maintained only in the 'low' group. Thus, vaccination of individuals with initial long-term protection against diphtheria is unnecessary and should be avoided.

R2107 The influence of LPS on AMPs expression in HaCaT keratinocytes

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Objectives: Antimicrobial peptides (AMPs) – very important component of innate immunity acts either directly, destroying bacteria, fungi and some viruses or indirectly, stimulating some elements of inflammatory and immune reactions. They are present usually in regions, where contact with environmental pathogens is more frequent (on mucosal and epithelial surfaces). Constitutive (hBD-1) or inducible (hBD – 2–4; hCAP18/LL-37) expression of AMPs has been observed in keratinocytes. We expected that LPS – important external stimulus, originated from G(-)bacteria cell wall and acting via TLRs should increase expression of genes coding for β-defensins and cathelicidines. The aim of this study was to evaluate

correlation between LPS concentrations, time of acting and AMPs expression in immortalised human keratinocyte line HaCaT.
Methods: Human immortalised keratinocytes HaCaT were cultured in NUNCs 6-well culture plates in 37°C and 5% CO_2, using K-SFM medium (Gibco BRL). Upon reaching 100% confluency, keratinocytes were stimulated with LPS from *E. coli* K-235 (Sigma Chemical Co.) in concentrations of 1 and 10 μg/mL, acting 1 and 24 h. Every variant was repeated three times. mRNA was isolated from each well with TRIZOL (Gibco BRL) according with Chomczynski. Primers for LL-37, hBD-1 and hBD-2 were manufactured in Proligo LLC and used to determine mRNA levels in RT-QPCR reaction performed in ABI Prism 7000 device. AMPs expression was measured in numbers of copy/1 μg of total RNA.
Results: A low LPS concentration (1 μg/ml) in culturing medium resulted in decreasing of expression of all examined AMPs after 1 h and 24 h of incubation. 10 μg/ml concentration was most effective for hBD-2 stimulation, reaching over 2 times higher expression after 1 h incubation and almost 5 times higher concentrations after 24 h incubation with LPS. Less effective was this LPS concentration for hBD-1 expression, almost 2 times higher expression has been observed only after 24 h incubation with this stimulant. LPS concentrations used in the experiment were ineffective for kathelicidine stimulation.
Conclusion: There are different requirements for LPS concentrations and time of action for different AMPs. LPS acts on expresion of AMPs by time-and-dose-dependent manner.

Pathogenesis, animal models including experimental treatment

R2108 Moxifloxacin pleural concentrations in a human like treated experimental pneumococcal pneumonia rabbit model

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Pleural effusion, most often purulent, occur in approximately 25% of pneumococcal pneumonia cases and are associated with poor prognosis and therapeutic failure. Little is known concerning the pleural diffusion of drugs.
Objective: To investigate the pharmacokinetics (PK) of MXF 400 mg od in the pleural space.
Methods: A total of 140 rabbits were intrabronchially inoculated with 10 log CFU/mL of penicillin-resistant pneumococci. Pleural punctions were done once to thrice during the second day of the experiment. MXF was infused through central venous catheter by computer controlled pump in order to simulate the human PK of MXF 400 mg o.d. for 48 h; then, MXF doses were modified in order to simulate low (6%) to high doses (600%). Blood samples (5–7 per rabbits) were obtained through the second central venous catheter. A microbiologic assay was used. PK data were analysed by using specialized software.
Results: The human MXF 400 mg regimen was correctly simulated in rabbits ; the variations in doses were associated with proportionnal variations of both seric concentrations and AUCs. Pleural MXF concentrations varied with the dose and proportionnally with the blood concentrations. For MXF 400 mg o.d., a final model was constructed with the following pleural concentrations [mg/L (SD)] vs. time (hour : H):H1: 5.2 (2); H3: 3.8 (0.7); H4: 4.4 (0.3); H5: 3.2 (0.5); H6: 2.4 (1.2); H7: 2.9 (0.6); H21: 1.8 (1.2); H24: 1.6 (1.2). Positive pleural cultures were associated with lower MXF concentrations both in blood and pleural effusion.
Conclusion: MXF concentrations were similar in blood and pleural space during parapneumonic pleuresia; however the interindividual variations were high.

R2109 The procalcitonin expression in stimulated human peripheral blood mononuclear cells

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Objectives: Procalcitonin (PCT) is well known as a marker of severe systemic bacterial infection. PCT consists of fragments katacalcin and calcitonin, which are precursors of calcitonin in thyroid. The potential role in the pathophysiology of PCT in inflammatory process is still unclear. This study was aimed to determine whether PBMC can induce PCT when they are stimulated with endotoxin or other stimuli including pro-inflammatory cytokine. PCT was investigated in terms of its biologic role and its role as a surrogate marker in sepsis.
Methods: Mononuclear cells were prepared from heparinized venous blood from donor by density gradient. PBMC were cultured in RPMI with 10% FCS and penicillin/streptomycin. Stimulated cells were incubated overnight in media containing each LPS (1 mg/mL), PMA (5 ng/mL), TNF-α (10 ng/mL), and IL-18 (5 nM). Intracellular PCT content was estimated using anti-katacalcin Ab-FITC, and anti-calcitonin Ab-FITC each. Monocytes were identified by phycoerythrin-conjugated CD14 Ab. Flow cytometric analysis was performed in treated PBMC with brefeldin A and permeabilising solution. Ten micrograms of soluble TNF receptor (sTNFR) were pretreated in PBMC 1 h prior to adding stimuli. Quantitation of PCT was performed in U937 cells and whole blood by immunoluminometer.
Results: (i) LPS slightly increased the expression of intracellular PCT from 9.0 to 13.2% in CD14-positive monocytes from healthy donors (one representative, $n = 5$). When PMA was used, the expression of intracellular PCT in CD14-positive cells increased from 2.9 to 7.6%. (ii) For PBMC from patients with sepsis, PMA induced much higher expression of PCT than in cells from healthy donors (from 5.8 to 36.9%, $n = 3$). LPS slightly increased PCT in the same cells (from 1.0 to 4.0%). In the same cells, sTNFR mildly inhibited the PCT expression in PMA stimulated cells. However, LPS-induced PCT expression rather decreased in the presence of sTNFR. (iii) PCT was slightly increased by various stimulants including LPS, PMA, TNF-α, IL-18 (control 0.51 ng/mL, vs. 0.76, 0.87, 0.61, 0.62 ng/mL, respectively), but PCT in whole blood was not significantly increased by stimuli.
Conclusion: The PCT expression increased in monocytes of PBMC. The induction of PCT in stimulated cells was higher when using PMA compared with LPS. Also, the monocyte response to inflammatory stimuli was enhanced in septic patients. The PCT expression is partly mediated through TNF-α production.

R2110 Influence of 82MDa plasmids *Yersinia pseudotuberculois* on morphological changes in mice

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Objectives: Two strains of first serotype *Y. pseudotuberculosis* were used in the study. One of them had a plasmid 47 MDa and the second had plasmis 47 and 82 MDa.
Methods: The mice CBAj were inoculated per os by the agent in isotonic solution. The doze contained 1000 millions microbial cells on one animal. Ileums, livers and liens of the animals were investigated by light and electronic microscopy. The distribution rate of agent in mice was investigated by indirect immunofluorescence during 1–10 days after inoculation.
Results: The morphological changes in ileum included of the inflammatory of gut, desquamation of epithelium, enlargement of Peyer's platelets. Inflammatory process in case of *Y. pseudotuberculosis* (82:47 MDa) initiated in earlier periods and was more intensively, then in case of *Y. pseudotubtrculosis* (47 MDa) infection. In liver we observed the infiltration by granulocytes, macrophages and granulomas later. Same as process in ileum we saw more quickly and early changes if infectious agent had plasmids 47 and 82 Mda from second day vs. fourth if it had only 47 MDa plasmid.

By lien investigation we observed interested immunomorphological changes – reactive centres in white pulp developed from second day in mice with only 47 MDa *Y. pseudotuberculosis* induced infection vs. fourth day in case with 47 and 82 MDa. Mice which were inoculated two-plasmid strain, had white pulp necrosis from seventh day. Immunofluorescence analysis of different tissues developed more quick spread of *Y. pseudotuberculosis* (82:47 MDa) vs. *Y. pseudotuberculosis* (47 MDa) in organisms of animals.
Conclusions: There are some morphological differences in investigated organs at the animal infection by agent strains possessing various plasmid spectrum. Mice infected by two-plasmid agent strain (82:47 MDa) had the morphological changes in investigated organs occurred in earlier periods that were more expressed in comparision with a contamination by a one-plasmid strain (47 MDa). In controversy of this immunomorphological process in lien was more expressed in case with 47 MDa only strain. If infectious agent has both 47 and 82 MDa plasmids we observed suppression of immunological response.

R2111 Rabbit and murine models of *Borrelia burgdorferi* infection for evaluating immune protection and the treatment of Lyme disease

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Objectives: To further evaluate the usefulness of two experimental animal models of Lyme borreliosis for providing key insights on borrelial transmission and the development of immune mechanisms associated with, and on therapeutic options for, *Borrelia burgdorferi* (Bb) infection.
Methods: New Zealand white rabbits were exposed to *Ixodes scapularis* ticks that had been collected from an area of Westchester County, NY, that is highly endemic for Lyme disease. Ten to 15 adult deer ticks were placed onto the ears of each of 24 rabbits, and the ability of ticks to transmit infection was based on the recovery of live organisms from the draining nodes and by serologic testing. At 2 and 6 weeks post-tick exposure, the rabbits were humanly killed, bled out and cultures in BSK medium were established in extracts derived from the cervical nodes. In related experiments involving mice, high titre antiborrelial rabbit sera were passively transferred to normal C3H mice who were subsequently challenged intradermally with low-passage, culture-grown strain B31. The mice were then evaluated for protection against borrelial infection, based on whether or not extract cultures of their urinary bladders (a key target site) contained motile spirochetes. Control mice were treated with either normal rabbit serum or the antibiotic ceftriaxone.
Results: Spirochetes were culturable from the nodes of 75% of the tick-exposed rabbits. Reactivity with a specific panel of monoclonal antibodies identified all of the isolates as Bb. Borrelial antibodies were detctable in 67% of the rabbits, based on an indirect haemagglutination antibody test. Most of the seropositive rabbits had high titres ($\geq$4096) and immunoblot analysis revealed a serologic response reactive with multiple low to high molecular weight protein bands. It was also found that passively transferred high titre rabbit sera fully protected the mice against borrelial challenge infection in a manner analogous to those receiving ceftriaxone alone.
Conclusions: These experiments show that these animal models are extremely useful for understanding tick–Borrelia–host interactions related to immune protection, that may have a bearing on vaccine development, and to possible treatment for Lyme disease.

R2112 Influence of probiotic antibacterial and antioxidative lactobacilli on gut mucosa in generalised experimental *Salmonella typhimurium* infection

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Objectives: The purpose of the present study was to test in a mouse model the ability of the probiotic *Lactobacillus* sp. with high antimicrobial and antioxidative potential to compete the invasive *Salmonella enterica* serovar Typhimurium infection and protect the gut mucosa against excessive oxidative stress during inflammatory tissue damage.
Methods: Altogether 47 mice were divided into four groups. The mice of control groups were treated either with PBS (group 1) or with 0.5×10^8 CFU/mL of human intestinal lactobacilli *L. fermentum* ME-3 (DSM 14241) and *L. acidophilus* 0.5×10^8 CFU/mL (Gr 4) daily during 15 days. Group 2 and 3 mice were challenged with a clinical isolate of *S. typhimurium* (0.5×10^5 CFU/mL). The Gr 3 mice were additionally orally inoculated with lactobacilli for 5 days before and 10 days after the challenge with *S. typhimurium*. The counts of salmonellas and lactobacilli in blood, intestine, liver and spleen were found, the morphological indices of inflammation in the same organs and oxidative stress-indicative biochemical status (lipid peroxidation, total antioxidative activity, redox ratio of glutathione and iron content) of gut mucosa were assessed on the tenth day after oral inoculation.
Results: The administration of probiotic lactobacilli of human origin did not increase the total count of lactobacilli in terminal ileum of mice, however mild hyperplasia of lymph nodes was registered in Gr 4 mice compared with the Gr 1. In *S. typhimurium* challenged mice the administration of *in vitro* antagonistic lactobacilli did not decrease the count of salmonellas in the gut and prevent their invasion into tested organs. The reduced level of iron, lipid peroxidation and increased total antioxidative activity, glutathione redox value in gut mucosa was found. In conclusion, the administration of probiotic lactobacilli of human origin did not increase the colonisation resistance against *S. typhimurium* in mice gut and prevent the generalisation of the infection. The possibility to influence the pro- and antioxidant balance of gut mucosa by specific probiotic lactobacilli with antioxidative properties could have significant therapeutic implications in management of inflammatory tissue damage.

R2113 Synergistic cytotoxic effects of verotoxin1 and monophosphoryl lipidA on vero cells in culture media

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Objectives: Verotoxigenic *E. coli* strains have a high rate of morbidity and mortality such as chronic renal failure in effected patients. We studied the interaction of monophosphoryl lipidA (MPL) and verotoxin1 on cell death and proliferation in Vero cells in culture system. Cytotoxicity of purified verotoxin1 was compared with the MPL alone or in combination with verotoxin1 and controls.
Methods: Cells were cultured on glass slides to confluency (18 h). After 12 h incubation with verotoxin1 and MPL or verotoxin1 plus MPL toxicity and cell deaths was determined by direct examination with invert microscope and trypan blue exclusion dye. Cell proliferation determined by MTT assay.
Results: Cytotoxicity and cell death was time-dependent and increased for 3 days after treatment. MPL-treated cells have mainly undergone necrosis as studied by Hoechst 33258/propidium iodide staining. Cell proliferation was significantly decreased in verotoxin1 and MPL plus verotoxin1 groups in comparison with MPL alone. The mechanism of cell death was studied by DNA fragmentation and nuclear condensation by Hoechst 33258 staining.
Conclusion: Results were analysed by univariate analysis of variance and concluded that MPL could profoundly increase the cytotoxicity of verotoxin1 in *in vitro*, and suggest that MPL beside adjuvant properties could have synergistic effects with other bacterial metabolits such as verotoxin1.

R2114 Concentration of IFN-g, Il-6, Il-12 and Il-15 in cerebrospinal fluid of patients with neuroborreliosis

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Objectives: To evaluate concentration of IFN-g, Il-6, Il-12 and Il-15 in cerebrospinal fluid (csf) of patients with neuroborreliosis presenting as meningitis.
Material and methods: The study group consisted of 25 patients, aged from 21 to 64 years. Neuroborreliosis was confirmed by detection of IgM and/or IgG antibodies in serum and/or csf by ELISA (Biomedica, Austria). Before and after 4 weeks of treatment with cefotaxime, csf concentration of IFN-g, Il-6, Il-12 and Il-15 was measured with ELISA kits (Quantikine, R&D Systems, USA and Human Interleukin-12, Endogen, USA). The control group consisted of csf fluids of 10 patients with discopathy.
Results: In control csf, moderate concentrations of IFN-g ($x = 0.93$ pg/mL), Il-6 ($x = 0.79$ pg/mL), Il-12 ($x = 1.11$ pg/mL) and Il-15 ($x = 0.27$ pg/mL) were observed. Inflammatory changes (cytosis of 38–80 cells/mm^3, protein concentration 32.0–91.0 mg/dL) were initially observed in csf of all patients, but resolved after treatment. Concentrations of all measured cytokines were significantly increased in csf of all neuroborreliosis patients before treatment (IFN-g $x = 13.10$ pg/mL, Il-6 $x = 9.8$ pg/mL, IL-12 $x = 167.19$ pg/mL, Il-15 $x = 7.71$ pg/mL), compared with controls. After 4 weeks of antibiotic treatment csf cytokine concentrations decreased significantly (IFN-g to $x = 4.32$ pg/mL, Il-6 $x = 4.56$ pg/mL, IL-12 $x = 43.67$ pg/mL, IL-15 $x = 4.8$ pg/mL), but still remained significantly higher than in control group.
Conclusions: Significant and substantial increase of csf concentrations of IFN-g, Il-6, Il-12 and Il-15 was observed in neuroborreliosis patients when compared with control group. It persisted after the end of 4-week antibiotic therapy, in spite of clinical recovery and normal csf parameters.

R2115 Serum concentration of IFN-g, Il-6, Il-12 and Il-15 in patients with neuroborreliosis

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Objectives: To evaluate concentration of IFN-g, Il-6, Il-12 and Il-15 in serum of patients with neuroborreliosis presenting as meningitis.
Material and methods: The study group consisted of 25 patients, aged from 21 to 64 years. Neuroborreliosis was confirmed by detection of IgM and/or IgG antibodies in serum and/or cerebrospinal fluid by ELISA (Biomedica, Austria). Before and after 4 weeks of treatment with cefotaxime, serum concentration of IFN-g, Il-6, Il-12 and Il-15 was measured with ELISA kits (Quantikine, R&D Systems, USA and Human Interleukin-12, Endogen, USA). The control group consisted of 10 healthy volunteers.
Results: In control sera, moderate concentrations of IFN-g ($x = 0.9$ pg/mL), Il-6 ($x = 1.04$ pg/mL), Il-12 ($x = 5$, 19, 15 pg/mL) and Il-15 ($x = 0.29$ pg/mL) were observed. Inflammatory changes (cytosis of 38–80 cells/mm^3, protein concentration 32.0–91.0 mg/dL) were initially observed in csf of all patients, but resolved after treatment. All measured serum cytokine concentrations in neuroborreliosis patients were significantly, many times higher than in control sera (IFN-g $x = 6.77$ pg/mL, IL-6 $x = 5.67$ pg/mL, IL-12 $x = 319.39$ pg/mL, IL-15 $x = 0.96$ pg/mL). After 4 weeks of cefotaxime treatment cytokine concentrations decreased significantly to: IFN- $x = 1.82$ pg/mL, IL-6 $x = 2.93$ pg/mL, IL-12 $x = 217.4$ pg/mL and IL-15 $x = 0.11$ pg/mL. However, they remained significantly higher than in control group, in spite of normal csf parameters and lack of clinical symptoms of neuroborreliosis.
Conclusions: Significant and substantial increase of serum concentrations of IFN-g, Il-6, Il-12 and Il-15 was observed in neuroborreliosis patients when compared with control group and persisted even after the end of 4-week antibiotic therapy.

R2116 Concentration of MCP-1 in serum and cerebrospinal fluid of patients with Lyme borreliosis

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Objectives: Chemokines are family of chemotactic cytokines, playing important role in pathogenesis of inflammatory response. Chemokines of CC subfamily act mainly on different populations of mononuclear cells. They are synthesised after stimulation with *Borrelia burgdorferi*, but there are few data as for their role in pathogenesis of Lyme borreliosis *in vivo*. In our previous study concentration of CC chemokines: macrophage inflammatory protein 1a and 1b (MIP-1a, MIP-1b) was only moderately increased in borreliosis and was lower in csf than in serum in neuroborreliosis, thus not creating chemotactic gradient for cell migration into the csf. It suggests other chemokines, probably also of the CC family, may be responsible for mononuclear cells recruitment in Lyme disease. MCP-1 (monocyte chemoatractant protein 1) is CC chemokine acting on T cells and monocytes, which is synthesised by human cells after incubation with small quantity of *B. burgdorferi* spirochetes. We investigated its synthesis in patients with different clinical forms of Lyme borreliosis.
Methods: A total of 29 patients were included: 13 with erythema migrans (EM), 10 with Lyme arthritis (LA) and six with neuroborreliosis (NB). Controls (K) were eight healthy volunteers (control blood samples) and eight patients in whom lumbar puncture excluded the possibility of meningitis or neuroborreliosis (control csf). Samples were obtained before (examination 1) and 2–4 weeks after start of antibiotic treatment (examination 2). MCP-1 concentration was measured by ELISA. Statistical significance was considered at $P < 0.05$.
Results: MCP-1 concentration (mean $\pm$ SD, ng/mL) in serum was: in EM: 277.17 ± 86.35 in ex.1 and 323.50 ± 140.55 in ex.2; in LA 388.97 ± 208.00 in ex.1 and 429.06 ± 216.64 in ex.2; in NB 245.78 ± 78.24 in ex.1 and 497.47 ± 215.87 in ex.2; while in K – 253.71 ± 55.25. MCP-1 concentration in csf in NB was 589.11 ± 235.11 in ex.1 and 510.95 ± 253.17 in ex.2, compared with 356.01 ± 36.94 in K. MCP-1 tended to increase in borreliosis patients, but the increase was only significant in serum in NB in ex.2 and of borderline significance in LA in ex.1 and in csf in NB in ex.1. MCP-1 concentration in csf was significantly higher than in serum in ex.1. No correlation was observed between MCP-1 concentration and inflammatory changes in the csf.
Conclusions: MCP-1 synthesis seems to be increased in Lyme borreliosis. MCP-1 may participate in stimulating mononuclear cells flux into the csf in neuroborreliosis.

R2117 Concentration of I-TAC in serum and cerebrospinal fluid of patients with Lyme borreliosis

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Objectives: Chemokines are family of chemotactic cytokines, including CXC-chemokines which act either on neutrophils (interleukin 8, Il-8) or on active T cells (interferon-g-inducible T-cell chemoatractant - I-TAC). Chemokines are synthesised after stimulation with *Borrelia burgdorferi*, but data on their role in Lyme borreliosis *in vivo* are scarce. In previous study we observed raised levels of Il-8 in serum and cerebrospinal fluid (csf) of patients with borreliosis, as well as chemotactic gradient created by Il-8 between csf and serum in neuroborreliosis. However, concentration of chem-

okines possibly acting on T-cells: macrophage inflammatory proteins 1a and 1b was only moderately increased and was lower in csf than in serum. It suggests other chemokines, possibly I-TAC, being responsible for T-cells recruitment in Lyme disease. We investigated I-TAC synthesis in patients with different clinical forms of Lyme borreliosis.
Methods: A total of 29 patients were included: 13 with erythema migrans (EM), 10 with Lyme arthritis (LA) and six with neuroborreliosis (NB). Controls (K) were eight healthy volunteers (control blood samples) and eight patients in whom lumbar puncture excluded the possibility of meningitis or neuroborreliosis (control csf). Samples were obtained before (examination 1) and 2–4 weeks after start of antibiotic treatment (examination 2). I-TAC concentration was measured by ELISA. Statistical significance was considered at $P < 0.05$.
Results: I-TAC concentration (mean ± SD, ng/mL) in serum was: in EM 73.00 ± 54.18 in ex.1 and 88.66 ± 12.16 in ex.2; in LA 78.87 ± 132.51 in ex.1 and 90.26 ± 191.43 in ex.2; in NB 87.25 ± 38.58 in ex.1 and 96.76 ± 28.82 in ex.2 and in K 29.86 ± 15.49. I-TAC concentration in csf in NB was 36.69 ± 13.12 in ex.1 and 44.84 ± 11.70 in ex.2, compared with 23.23 ± 3.38 in K. The difference between I-TAC concentration in study atients and in controls was significant only in serum and csf in NB and of borderline significance in EM in ex.1. I-TAC concentration was significantly higher in NB than in LA in ex.2. I-TAC concentration in csf was lower than in serum and did not correlate with csf cell count.
Conclusion: Synthesis of I-TAC in Lyme borreliosis is increased and depends on clinical form of the disease. I-TAC does not seem responsible for lymphocyte flux into csf in neuroborreliosis. The role of other chemotactic factors in Lyme disease should be investigated.

R2118 The susceptibility of different mouse strains to *Legionella longbeachae* infection

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Objectives: Since the first isolation of *Legionella longbeachae* in 1981 it was considered as an uncommon pathogen of Legionnaires' disease. Today we are aware that, besides some individual cases observed in Japan, USA, Spain, Germany and France, *L. longbeachae* is responsible for up to half of the legionellosis in Australia. In contrast to *L. pneumophila*, this bacteria has been detected only occasionally in water. More commonly, it has been isolated from soil and decomposing materials. In this study we examined the pathogenesis of *Legionella longbeachae* (strain D4968) lung infection in different mice strains, since previous studies pointed to a genetically driven permisivity of mice to *L. pneumophila* (BALB/c and C57Bl/6 mice are highly resistant and A/J mice susceptible).
Methods: A/J, BALB/c, and C57Bl/6 mice were infected by intratracheal inoculation of *L. longbeachae* using different doses from 102 to 105 CFU. In the infected animals we followed the bacterial clearance from lung tissue, mortality assay and histopathological changes in the lungs.
Results: Irrespective of mouse strains the intratracheal inoculation of 105 *L. longbeachae* caused death in 90% of the animals within six days.The LD50 dose of *L. longbeachae* for all mice strains was determined at 104 CFU. The animals that received 102 or 103 bacteria survived 14 days post inoculation. During that period the multiplication of bacteria in the lungs reached a peak at 72 h after inoculation (106–108 CFU/lung) and thereafter decreased gradually to the detection level on day seven. Histological appearance of the lungs taken from infected animals was constraint with bronchoalveolar pneumonia.
Conclusion: Our results indicate that all three mice strains are susceptible to infection with *L. longbeachae*. The LD90 and particularly LD50 dose was unusually low indicating a high infectivity potential of *L. longbeachae*. Histopathological changes in the lung tissue, indicate on a bronchopneumonia rather than on interstitial pneumonia as seen in *L. pneumophila* infection.

R2119 Study of cell surface hydrophobicity and its correlation with bacterial adherence capacity to catheter sections

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Introduction: Generally, bacteria with hydrofobic surface are less adherent to the cellular substratum, but they are intensively adherent to hydrofobic materials causing medical devices associated infections, very difficult to treat because their phenotypic resistance to antibiotics.
Purpose: Determining cell hydrophobicity of 10 selected enterobacteria opportunistic strains with different isolation sources (previously tested for adhesion and invasion on cellular substratum) and to investigate the adherence capacity to an hydrophobic substratum (poliuretan silicon coated catheter sections).
Material and methods: Bonner's method (1997) is based on the spectrophotometric assay of bacterial suspensions capacity to mix with organic solvents and to pass from the aquatic phase to the organic one, having as consequence the decrease of optic density in the aquatic phase. The adherence capacity to an inert substratum was tested *in vitro* on a model in 24 – multi-well plate, incubating the catheter sections with bacterial suspensions. After incubation, the catheter pieces were washed in sterile PBS, fresh medium was added, further incubated for 24 h and the optic density of the bacterial cultures was assayed. An *Enterobacter cloacae* strain, selected for its intense adherence capacity to a cellular substratum, also proved to be moderately hydrophobic and was studied for its interaction with catheter surface by SEM. With this aim, bacterial culture on nutrient broth was introduced in a circuit including a fragment of catheter and recirculated with a peristaltic pump. After 24 h, the catheter was removed and examined by SEM.
Results: Our study demonstrated that cell surface hydrophobicity promotes strong bacterial adherence capacity to the hydrophobic, inert substratum represented by plastic catheter. SEM results demonstrated that an opportunistic enterobacterial strain isolated from food could become an aetiologic agent of prosthetic devices associated infections due to its adherence capacity to the inert substratum and to its capacity to form biofilms.
Conclusion: The hydrophobic bacterial surface, even moderate, could constitute a virulence feature of opportunistic enterobacteria. In this sense, an *Enterobacter cloacae* (43) strain isolated from food and previously proved to be able to generate A/E lesions on a cellular substratum was also able to adhere and to form biofilms to the internal surface of a catheter fragment, as SEM results clearly demonstrated.

R2120 Biofilm formation of pathogenic *Escherichia coli* isolates

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Objective: Infectious diseases caused by *E. coli* are initiated by adhesion to the surfaces of the human genitourinary, gastrointestinal or respiratory tracts. Although the utilised adhesion mechanisms have been extensively studied they are only part of the story since additional postadhesion events are required for bacterial cells to establish themselves and to initiate infection. *In situ* studies indicate that as in most natural bacterial surface-associated communities (biofilms), many adhered bacterial cells at a given infected site do not have a direct cell-surface contact but are rather attached to other *E. coli* cells. We therefore aim to understand the genetic repertoire that allows pathogenic *E. coli* to adhere to each other withstanding the strong shear forces present at many host sites. In a first step towards that goal, we started to characterise the potential of pathogenic *E. coli* isolates to form biofilms *in vitro*.
Methods: *E. coli* strains (each collection $n > 80$) isolated from faeces of healthy children, from faeces of children with diarrhea, from

blood of patients with bacteraemia, and from urine of male patients suffering from complicated UTI were tested for biofilm formation on the surface of polystyrene microdishes. Bacteria were cultured in standard media such as LB medium and minimal medium supplemented with glucose or casaminoacids but also in physiologically more relevant environments such as urine and mucus. In addition, the prevalence of factors known to promote biofilm formation of *E. coli* K-12 strains *in vitro* (curli expression, AAF fimbriae and conjugative IncF plasmids) was assessed in the different strain collections. Finally, molecular approaches were applied to dissect the genetic repertoire that allows pathogenic *E. coli* strains to form biofilms *in vitro*.
Results: In LB medium only about 5% of the strains were able to form biofilms to a similar extent as *E. coli* K-12 strains expressing biofilm-promoting factors. In minimal medium however, 10–20% of the strains formed strong biofilms. Our initial analysis shows that strains able to form biofilms in standard media are equally distributed among the different strain collections.
Conclusion: Our data suggests that the ability to form strong biofilms *in vitro* is not clearly associated with pathogenicity. However, additional analysis also indicates the presence of previously unrecognised genetic pathways that enable biofilm formation of *E. coli*.

R2121 Studies on biofilm formation by opportunistic species of *Corynebacterium* and coryneform

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Ability of biofilm production by some microorganisms after adhesion to different surfaces is important feature influencing course of the infection. Frequency, kinetics and amount of produced biofilm may be correlated with pathogenesis course, clinical symptoms and treatment. In the case of biofilm producing species chemiotherapeutics penetration to bacterial targets is hindered. Moreover, solitary cells from primary colonisation site may be released and create new colonisation sites associated with persistent infections. In immunocompromised subjects it may be clinically important. The important feature pointing that opportunistic corynebacteria may produce biofilm is the course of their pathogenesis. Some of them were observed to cause difficult to treat persistent infections.
Objectives: The aim of the study was to determine the biofilm formation in vitro by opportunistic species of *Corynebacterium* and coryneform isolated from either different clinical materials or model strains from Institute of Serum and Vaccines (Copenhagen).
Methods: Studied material constituted model strains of *C. pseudodiphteriticum* 3633, *C. afermentans*.subsp. *afermetans* AB2746, *C. jeikeium* 6717, *C. striatum* AB2545Ci, *C. ulcerans*, *C. amycolatum*, *C. macgineleyi*, *C.urealyticum*, *C.propinquum*, *Rhodococcus equi*, *Brevibacterium* spp.). Culture (bacterial suspension 0.5 McFarland diluted 1:100) was assessed in BHI medium growth enriched with rabbit serum and performed in 96 flat-bottomed plastic tissue culture plates (37C). Produced biofilm was fixed with Bouin's Solution and stained with 1% crystal violet. Optical density (OD) was measured after 24, 48, 72, and 96 h by an enzyme immunosorbent assay reader at 540 nm. The procedure was performed in triplicate.
Results: The most abundant biofilm formation was observed after 24 h for *C. striatum* (OD = 0.128–0.134) and *C.macgineleyi* (OD = 0.202). After 72 h biofilm formation of *C. propinquum* and *C. jeikeium* reached OD = 0.148 and OD = 0.123, respectively (blank OD = 0.068).The most dynamic biofilm production was observed in *C. macgineley* (OD = 0.202–0.438) and *C. striatum* (OD = 0.128–0.400). After 96 h of incubation the formation was seen in *R. equiculture*, however, it was not persistent.
Conclusions: As we expected, corynebacteria showed various intensity of biofilm formation. *C. macgineleyi* (sputum, throat), *C. striatum* (throat, pus), and *C. striatum* AB2545Ci were characterised with the most efficient level of the production.

R2122 Investigation of translocated *E. coli* isolates from general surgical patients

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Objectives: Translocation, the passage of viable enteric bacteria across the intact mucosa of the gastrointestinal tract into sterile tissues, is a key cause of postoperative septic morbidity. Host factors that predispose to it have been studied, but little is known about whether or not bacterial pathogenicity factors are involved in the phenomenon. *E. coli* is one of the most common translocating species. This study aimed to determine if translocated isolates have any unusual adherence or invasive properties that might facilitate or explain their passage across the lamina propria.
Methods: Thirty-four *E. coli* strains isolated between 1991 and 2003 from 30 general surgical patients at a District Hospital were studied. Patients were aged 23-85 (mean 71) yrs, none had intra-abdominal sepsis at the time of isolation. A single mesenteric lymph node was harvested during laparotomy, suspended in sterile saline and sent for culture. *E. coli* isolates were identified by standard techniques then stored at −70°C. For adherence assays, HEp-2 and T84 epithelial cells were grown on glass coverslips, then co-incubated with aliquots of overnight cultures of *E. coli* for 3 h, in 5% CO_2 at 37°C. After washing, fixing and staining, cells were examined for adherent bacteria. The gentamicin protection assay was used to assess invasion into HEp-2 cells. PCR was used to screen invasive isolates for the ipaH gene, characteristic of enteroinvasive *E. coli* (EIEC).
Results: Only two of the 34 (5.9%) isolates were adherent. Both of these adhered to HEp-2 and T84 cells with a diffuse pattern, similar to that seen with diffusely adherent *E. coli* (DAEC). Both of these isolates were highly invasive in the gentamicin protection assay compared with an EIEC control strain, but tested negative for the ipaH gene.
Conclusions: The majority of the *E. coli* isolates were nonadherent and may represent members of the normal commensal flora that have been translocated as a result of changes in the patient's GI tract. However, some cases of translocation were associated with unusual adherent/invasive *E. coli*. Similar strains have previously been reported from cases of Crohn's disease. The origin and contribution of such strains to postoperative septic complications remains to be elucidated.

R2123 Effects of different antioxidants and amrinone on vancomycin-induced nephrotoxicity

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Objectives: In the present study, we investigated the effects of three antioxidant agents and amrinone, an inotropic agent, on vancomycin-induced nephrotoxicity.
Methods: Thirty adult female Sprague Dawley rats (168–234 g) were divided into six groups. Saline-treated group (group 1) served as a control. The other five groups were treated with vancomycin (VCM, 100 mg/kg, intraperitoneally) alone (group 2) or with combination. Group 3 received VCM plus amrinon (Amr, 100 mg/kg i.p.). Group 4 received VCM plus melatonin (Mel, 10 mg/kg i.p.). Group 5 received VCM plus α-lipoic acid (ALA, 10 mg/kg i.p.). Group 6 received VCM and Ginkgo Biloba extract (EGb 761) 100 mg/kg p.o. All the drugs and vancomycin were administered for 7 days. All rats were sacrificed by decapitation, kidney tissues were excised immediately at day 8, and blood and kidney samples were collected. Blood urea, and creatinine levels, and kidney tissue malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPHx) levels were measured. Kidney samples were examined histologically and were assigned scores 0–4 as reported by Houghton *et al*.

Results: The biochemical and pathological findings were shown at table.

Table. Effects of vancomycin and different antioxidants on different parameters (mean±SD)

Parameter	Control	VMC	VMC+AMr	VMC+Mel	VMC+ALA	VMC+EGb
BUN	25.6±03.28	54.00±4.95*	37.60±3.97	39.60±6.65	34.20±5.26	31.40±4.56
Creat	0.42±0.13	0.76±0.11*	1.52±0.08	0.48±0.08	0.38±0.08	0.44±0.11
MDA	2.26±0.25	3.84±0.21*	2.21±0.76	1.44±0.29	1.63±0.58	1.60±0.68
SOD	22.71±1.92	12.82±1.66*	19.77±2.93	21.49±2.66	21.97±3.42	22.20±3.31
GPHX	2.12±0.52	1.21±0.12*	2.04±0.38	2.07±0.45	2.27±0.44	2.17±0.40
Pathological Score	0.00±0.00	3.80±0.45*	1.40±0.55	1.60±0.55	1.60±0.55	2.80±0.45

*p<0.05, the levels of all parameters at VMC group were statistically significant different from other groups.

Conclusion: The present study indicates that the three antioxidants and amrinone protect vancomycin-induced nephrotoxicity. Amrinone was the most effective drug based on pathological score.

R2124 Effect of EPs7630 (Umckaloabo®) on the adherence of *Streptococcus pyogenes* to human epithelial cells

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Introduction: EPs7630 (Umckaloabo®,ISO-Pharmaceuticals, Germany) is an extract of the root of the South African geranium *Pelargonium sidoides*. The phytotherapeutic remedy has been successfully used for the treatment of upper-respiratory tract infections (*Phytomedicine* 2003; **10**, 7–17).
Study objective: To investigate the biological effects of EPs7630 in the pathogenesis of upper-respiratory tract infection.
Material and methods: Using a flow cytometric technique, we assessed the influence of EPs7630 on streptococcal adherence (i) to the human HEp-2 cell line (90% viable) and (ii) to human buccal epithelial cells (BEC; 90% dead), sloughed off from the oral mucosa. *Streptococcus pyogenes* (DSM 2071) was stained with Calcein-AM which is retained by viable bacteria. Adherence was determined after incubating bacteria and epithelial cells for 120 min. EPs7630 was applied in five concentrations, 30, 10, 3.0, 1.0 and 0.3 μg/mL. Alternatively, either streptococci or epithelial cells were preincubated with EPs7630 and washed before further incubation. The readout was the percentage of epithelial cells with adhering fluorescent bacteria.
Results: Streptococcal adherence to HEp-2 cells decreased significantly with rising concentrations of EPs7630: for 30, 10 and 3.0 μg/mL adherence was reduced by factors 0.54, 0. 6 and 0.7, respectively. In contrast, streptococcal adherence to BEC increased significantly under treatment with EPs7630: for 30 μg/mL by factor 7, for 3.0 μg/mL by factor 2.9, and for 1.0 μg/mL by factor 1.7. Preincubation of bacteria with EPs7630 showed similar results, whereas preincubation of epithelial cells had no effect.
Conclusions: The decrease in bacterial adherence to viable HEp-2 cells and the increase in bacterial adherence to dead BEC under the influence of EPs7630 could be an important mechanism in preventing and mitigating upper respiratory tract infections.

R2125 *Chlamydophila pneumoniae* and atherosclerosis: the eventual role of iron

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Several studies (serological, pathological and in animal models) suggested that the arterial chronic infection by *C. pneumoniae* (more frequent in males, smokers an in the elderly) plays an important role in the atherosclerosis pathogenesis. Some authors postulated that stored iron interacts with *C. pneumoniae* in the pathogenesis of atherosclerosis, stimulating the organism's growth. Reinforcing iron's key role, infected cells present an increased number of transferrin receptors.
Objectives: To evaluate the importance of iron's intracellular concentration in the association between chronic infection by *C. pneumoniae* and atherosclerotic lesion.
Methods: Serum from 26 patients with coronary disease confirmed by angiography, was stored at 80°C. Simultaneously, we also studied a control goup (26 persons), sex and age matched. IgG and IgA antibodies to *C. pneumoniae* were determined by microimmunofluorescence (MIF), using purified elementary bodies of the organism (MRL Diagnostics). Serum ferritin (sFt), soluble transferrin receptors (sTfR) and C-reactive protein levels were assessed by kinetic nephelometry (Behring Nephelometer II). We have also calculated the ratio of sTfR/sFt, apparently independent of inflammation.
Results: IgG and IgA antibodies to *C. pneumoniae* were present in 55.5 and 18.5% of the coronary disease group, and in 62.9 and 7% of the control group, respectively. Mean levels of sFT (μg/L) and sTfR (mg/L) were higher in the coronary disease group (167.6 and 1.55) compared with the control group (153.2 and 1.23). C-reactive protein mean levels were low in both groups (<8 mg/dL).
Conclusions: The positive association between chronic infection by *C. pneumoniae* and iron concentration in tissues seems to be supported by this study. Higher sTfR blood levels in the coronary disease group may signify an increase of soluble transferrin receptors within infected cells.

Molecular bacteriology

R2126 The comparison of two diagnostic tolls in Lyme neuroborreliosis: the proof of intrathecally produced antiborrelial antibodies and PCR

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Objective: Aim of the study was the comparison of the diagnostic effectivity of antibody index CSF/serum (AIBb) and PCR in neuroborreliosis.
Methods: In 55 patients with neurological symptoms of Lyme borreliosis was evaluated secretion of the CSF specific antibodies (AIBb) comparing with PCR in CSF, plasma and urine. AIBb was established according to Reiber *et al*. DNA of Borrelia burgdorferi sensu lato was detected by three sets of primers encoding: plasmid gene for OspC protein and two chromosomal genes for 16SrDNA and flagellin. The nested PCR was examined in the CSF, plasma and urine in parallel at the beginning, testing of the urine was repeated still after 3 and 6 months.
Results: Spirochetal DNA was found in 20 patients (46%) in CSF and in 10 (18%) in plasma. In urine DNA was detected one times in 36 (65%) patients at least. Five times was spirochetal DNA found in CSF, plasma and urine when examinated in parallel. The AIBb was positive in 50 patients, in the other five patients antibodies remained negative and only DNA was found.
Conclusion: The results show that the AIBb is much more sensitive in neuroborrelios (96%) comparing with the detection of DNA in CSF (46%), plasma (18%), and urine (50%), when we have examined before antibiotic treatment. PCR can not replace antibody diagnostic in Lyme neuroborreliosis, but it can supplement it very well.

R2127 LAL activity of lipopolysaccharides extracted from reference strains of the *Bacteroides fragilis* group

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Objectives: To determine a biological activity of lipopolysaccharides from reference strains of the *Bacteroides fragilis* group (BFG) using quantitative Bacterial Endotoxins Test (BET) with Limulus amoebocyte lysate (LAL reagent).
Methods: Lipopolysaccharides were extracted from eight strains of the *Bacteroides fragilis* group (BFG): *B. thetaiotaomicron* NCTC 10582, *B. ovatus* ATCC 8483, *B. vulgatus* ATCC 8482, *B. distasonis* ATCC 8503 and *B. fragilis* NCTC 9343, IPL E 323, IPL E 2360, ATCC 43858 (enterotoxigenic strain, ETBF 1). LPS preparations were extracted by the hot phenol–water method and purified by nuclease treatment and ultracentrifugation. Quantitative, photometric BET method (formerly LAL assay) with LAL reagent and chromogenic substrate S-2423 (ENDOCHROME kit, Charles River Endosafe Ltd., USA) was applied to determine biological activities of *Bacteroides* lipopolysaccharides.
Results: The highest LAL activity showed the LPS from *B. fragilis* NCTC 9343 strain followed by the LPS of *B. thetaiotaomicron* NCTC 10582 strain. Lipopolysaccharides from *B. vulgatus* and *B. distasonis* strains demonstrated considerably lower activities. LPS of ETBF one strain was much less active than LPSs from nonenterotoxigenic (NTBF) strains. Lipopolysaccharides of the *Bacteroides* genus were less active than the *E. coli* O55:B5 LPS (Sigma chemical Co., USA) used as positive control.
Conclusions: Lipopolysaccharides from reference strains of the *B. fragilis* group are active in reaction with LAL reagent. Lipopolysaccharides of BFG rods demonstrate some differences in endotoxic potential and lower LAL activity than the LPS of *E. coli* O55:B5 strain.

R2128 LAL activity of lipopolysaccharides extracted from clinical *Bacteroides fragilis* and Bacteroides thetaiotaomicron strains isolated in Central Europe

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Objectives: To examine a biological activity of lipopolysaccharides derived from eleven clinical *B. fragilis* and *B. thetaiotaomicron* strains isolated in Poland and Roumania using quantitative Bacterial Endotoxins Test (BET) with Limulus amoebocyte lysate (LAL reagent).
Methods: Lipopolysaccharides were extracted out of one *B. thetaiotaomicron* and nine *B. fragilis* strains isolated from different clinical specimens from patients of a university hospital in Warsaw, and one *B. fragilis* strain cultured from blood sample in Bucharest. Purified LPS preparations were examined by the quantitative, photometric BET mehod (previously LAL test) with Limulus amoebocyte lysate and chromogenic substrate S-2423 (ENDOCHROME kit, Charles River Endosafe Ltd., USA).
Results: Lipopolysaccharides of strains cultured from pancreatic ulcers and blood were much more active in BET than LPS preparations of strains isolated from other clinical specimens. Moderate activities showed LPSs of strains cultured from wounds after appendectomy. Considerably less active in reaction with LAL reagent were LPSs extracted out of strains originating from following samples: postoperative abdominal wound, traumatic wound of perineum, wound after removal of colon tumour and perirectal abscess. LPS of *E. coli* O55:B5 (Sigma Chemical Co., USA), used as positive control, was the most active compound in reaction with LAL reagent.
Conclusions: LAL activities of investigated *Bacteroides* lipopolysaccharides were differentiated, but lower than the activity of *E. coli* O55:B5 LPS. Some correlations between the origin of clinical strains and activities of their lipopolysaccharides in reaction with LAL reagent were observed.

R2129 Identification of the novel α-like protein gene in group *B. streptococci*

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Objectives: *Streptococcus agalactiae* (group *B. streptococcus*, GBS) is important cause of severe infections of human and animals. Certain surface antigens, e.g. Rib protein and Bca (alpha) protein, are potentially involved in GBS virulence. Both Rib and alpha proteins belong to the family of surface proteins containing repetitive elements, which produce variations in protein size and antigenicity. Recently some α-like protein genes (alp2, alp3 and alp4) were identified in GBS. In this study the novel α-like surface protein gene alp5 discovered in bovine GBS strains is described.
Methods: A collection of 35 human and 44 bovine GBS strains was analysed. The GBS chromosomal DNA was isolated by phenol/chloroform extraction. The strains were tested for the presence of bca, alp2, alp3, alp4 and rib genes. PCR was performed using the primers for conserved regions of bca, alp2, alp3, alp4 and rib genes including start codon and stop codon of the genes under study. PCR products were sequenced using ABI Prism 377 Perkin-Elmer Sequencer.
Results: PCR analysis of bovine GBS for the presence of bca, alp2, alp3, alp4 and rib genes was performed. In two strains, the PCR products of unusual size (850 bp) were received. After sequencing of one of these two PCR products, the complete open reading frame (ORF) consisted of 852 bp was identified. The 5′-end and 3′-end of this ORF contained the sequences of 145 bp and 258 bp highly homologous to the conserved regions of bca, alp2, alp3, alp4 and rib genes. However, the internal region of this ORF was not homologous to bca, alp2, alp3, alp4, rib genes or any other bacterial sequences. The complete ORF identified in this study was named as novel α-like surface protein gene alp5. The alp5 gene did not contain repetitive regions found in bca, alp2, alp3 and rib genes. The alp5 gene was revealed in two of 44 GBS strains isolated from the dairy cows (4.5%) and was not found in human GBS. The possible evolution relationship of bca, alp2, alp3, alp4, alp5 and rib genes and the possible role of the novel Alp5 protein in GBS virulence are discussed.
Conclusions: In this study the gene encoding for the novel GBS surface protein is described. Features of this gene organisation allow considering it as a novel α-like protein gene alp5. This gene was revealed only in 4.5% of bovine GBS and was not found in human GBS. This work was supported by VEGA 1-8021-01, Russian Grants MK-2782.2003.04, NSh-2206.2003.4, RFBR 03-04-49760.

R2130 Evaluation of the BD ProbeTec (TM) ET *Legionella pneumophila* amplified DNA assay

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Objectives: New assays for diagnosing atypical pneumonia caused by *Mycoplasma pneumoniae*, Chlamydiaceae family and *Legionella pneumophila* are currently under evaluation on the BD ProbeTec™ ET System (BD Diagnostics) from throat swabs and lower respiratory specimens (LRS). These assays are based on strand displacement amplification (SDA) technology with real time detection. We took part in the evaluation of the BD ProbeTec ET *Legionella pneumophila* (LP) Assay with retrospective (frozen) lower respiratory specimens.
Methods: Seventy-eight samples were included in this study. Sensitivity and specificity of the assay were calculated against the gold standard (culture positive) and against the expanded reference method [culture positive or Binax urinary antigen (UA) positive and PCR positive]. The in-house PCR assay for detection of *Legionella pneumophila* and *Legionella* spp. was performed on the BD ProbeTec ET processed samples (Qiagen DNA extraction) for some samples, including those that yielded discrepant results and from the original LRS on another subset of the samples.

Results: Gold standard: of 19 samples with positive cultures for *L. pneumophila*, 8 were LP Assay positive (sensitivity 94.7%). The negative LP Assay sample was culture and PCR positive but from a patient with a negative UA result. Of the 59 samples with negative cultures, 51 were LP Assay negative (specificity 86.4%) including one specimen from which *L. micdadei* was isolated. Expanded reference method: 30 samples were culture positive or from UA-positive patients and positive by PCR from the processed samples. Of the 30 samples, 24 were positive by the LP Assay (sensitivity 80%). There were 27 specimens that were culture and PCR negative and that were from patients with negative UA. Of the 27 specimens, eight samples were PCR negative from the BD ProbeTec ET processed samples and 19 were PCR negative from the original LRS. The specificity of the LP Assay was 100% (27/27).
Conclusion: The BDProbeTecTM ET LP Assay is a sensitive and specific assay for the rapid identification of *Legionella pneumophila* from lower respiratory specimens.

R2131 Development of a real-time PCR assay for the detection and biovar discrimination of *Ureaplasma urealyticum*

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Objectives: Intrauterine infection with *Ureaplasma urealyticum* has been implicated in premature births. *U. urealyticum* is clustered into two distinct biovars, parvo and T960, and it is suggested that biovar difference might play a role in the association with prematurity. The aim of this study was to develop a diagnostic assay which can simultaneously detect *U. urealyticum* and discriminate between its two biovars.
Methods: Vaginal swab specimens were obtained and inoculated into liquid culture media for screening *U. urealyticum*. DNA was extracted from culture-positive liquid media as well as from each standard *U. urealyticum* biovar strain, and used as a template for conventional PCR of *U. urealyticum* urease gene with specific primers. DNA sequences were determined for the amplicons of this conventional PCR. Based on the obtained sequence data, primers and probes for real-time PCR (*Taq*Man assay) were developed. In this real-time PCR assay, two common primers and two biovar-specific probes each labelled with different reporter dye were designed to detect *U. urealyticum* and to discriminate between the biovars simultaneously in one reaction tube. The previously extracted DNA samples positive for the conventional PCR were subjected to the real-time PCR.
Results: Forty-four specimens including standard biovar strains were positive for the conventional PCR. On analysing these amplicon sequences, the parvo biovars were present in 38, the T960 in five, and the both in one. With the real-time PCR assay, we obtained the same result with the conventional PCR and subsequent analysis of amplicon sequence except that one specimen which was expected to be the parvo biovar did not yield any significant fluorescent signal. Overall turnaround time of the real-time PCR was within 6 h.
Conclusion: The real-time PCR assay we developed can simultaneously detect *U. urealyticum* and discriminate between its biovars. This assay is also efficient and rapid.

R2132 The genome macrorestriction analysis, serological typing and antimicrobial susceptibility patterns of sequential mucoid *Pseudomonas aeruginosa* strains from patients with bronchiectasis without cystic fibrosis

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Objective: The respiratory tracts of bronchiectasis patients without cystic fibrosis may be persistently colonised with *Pseudomonas aeruginosa*, especially mucoid type, in spite of intensive chemotherapy. It is questionable and beneficial whether it is recurrence with the same strain or reinfection with a new strain. This information is useful and vital for epidemiologic study and patients management.
Methods: We studied 25 bronchiectasis patients over a period of 1 year(1–3 year) with more than 3-months intervals. Of these patients, 65 sequential mucoid *P. aeruginosa* were isolated. They were characterised by a genotyping method, pulsed-field gel electrophoresis (PFGE), and serotyping method using 14 antisera against O-group antigens of *P. aeruginosa* (Denka Seiken, Japan). Antimicrobial susceptibility tests were determined by disc diffusion method according to the NCCLS guidelines with six antibiotics (ciprofloxacin, imipenem, gentamicin, cefotaxime, ceftazidime, amikacin).
Results: The intrapatient macrorestriction similarity pattern of all of the 25 patients were 96–100%. However, diversity was observed in the *P. aeruginosa* isolates from all 25 patients, with relatedness of only 50–70% in PFGE. Serotyping yielded seven different patterns (type A, E and M were 12%, respectively, and type B and G were 8% respectively and type K and I were 4%, respectively) but 32% of isolates were nontypable. No remarkable relationships were observed between serological types and PFGE patterns. Antimicrobial susceptibility patterns were all susceptible for all 65 isolated *P. aeruginosa* to all test antibiotics.
Conclusion: The study showed the mucoid *P. aeruginosa* infection in bronchiectasis without cystic fibrosis are recurrence with the same strain rather than reinfection and PFGE is the most useful tool rather than serotyping. Although the antimicrobial susceptibility patterns could not discriminate the strains, the recurrence of infection might be uneradicated colonisation of mucoid *P. aeruginosa* after chemotherapy, not by the antibiotic resistance.

R2133 Gene expression profiling of patients with chronic fatigue syndrome

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Objectives: To identify genes in peripheral blood mononuclear cells (PBMCs), which may play an important role in pathogenesis and diagnostics of chronic fatigue syndrome (CFS), using microarray technology.
Population and methods: The study cohort consists of 23 patients (six men and 17 women), from a clinic for infectious diseases, fulfilling criteria for CFS and 19 healthy age- and sex-matched controls. The average age of the patient group is 38 years (26–60 years) and the median illness duration time is 3.5 years (1–27 years). Twelve patients had an insidious onset of illness and 11 a sudden onset. Several onset triggers were identified among the group, e.g. Ehrlichia, Herpes Encephalitis, Borrelia, sarcoidosis, flu, upper respiratory infection, tonsillitis and abortion. For gene expression analysis PBMCs were isolated from freshly drawn blood, from both patients and controls, using a lymphocyte separation media (LymphoprepTM, Medinor AB). Three micrograms of extracted PBMC total RNA [TRIzol(R) Reagent, Invitrogen] was used to synthesise biotin or fluorescein-labelled cDNA. Matched patient and control samples (biotin/fluorescein) were hybridised to human cDNA microarrays representing approximately 30000 genes. The HiLight dual-color kit (Qiagen) with anti-biotin and anti-fluorescein antibodies, conjugated with gold and silver particles, respectively, was used for resonance light scattering (RLS) detection. Experiments were performed in duplicates or triplicates using dye swap. Signal intensities were evaluated using Gene Pix(R) Pro 5.0 and data preprocessing is performed using the R-package.
Results: Good quality total RNA was achieved from 21 patients and 18 controls (average of 8.5 μg from 10 million PBMCs). Preliminary biological results from the initial data analysis will be demonstrated together with data showing excellent technical quality and reproducibility for the hybridisations.
Conclusion: This study will contribute to increased understanding of the so far unknown pathogenesis of CFS and could possibly be used for the diagnostics.

R2134 Advances in diagnostic testing of *Chlamydia trachomatis*

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Objectives: The diagnostic testing for genital chlamydia infection has changed in the past few years. New highly sensitive tests based on nucleic acid amplification technology (NAATs) have been developed to allow noninvasive specimen collection and to create new opportunities for innovative screening programs. We evaluated the performance of the RealArt C. trachomatis RG PCR Kit (artus, Germany) on the Rotor-Gene 3000 instrument (Corbett Research, Australia) with swab, urine and sperm specimens to improve our ability to diagnose chlamydia infections in invasive and noninvasive specimens.
Methods: The RealArt assay combines sequence-specific primers and a dual labeled fluorogenic probe. In addition, it contains a second amplification system (internal control) to identify possible PCR inhibition and to control the isolation procedure. Swab specimens were purified using the QIAamp DNA Mini Kit, urine and sperm specimens by the QIAamp Viral RNA Mini Kit (QIAGEN, Germany). The evaluation of the assay was performed with 298 retrospective swab specimens (233 eye, four Douglas, 31 urethral, 15 cervical, one penis and 10 vaginal swabs) as well as for 322 prospective specimens (187 urine, 100 cervical swabs and 35 sperm samples).
Results: On retrospective specimens the RealArt *C. trachomatis* RG PCR Kit showed an inhibition rate of 0.3% appearing on one eye swab. One of 11 previously positive tested swab samples could not be detected due to this inhibition. The specificity for retrospective specimens was 100%. For prospective specimens the inhibition rate was 0.3%, too. The inhibition occurred on one urine sample. The results for the prospective samples showed a positive rate of 1.6% for urine, 4% for cervical swabs and 2.9% for sperm specimens. The positive result for the sperm sample was confirmed by retesting and parallel testing of a urine sample of the same person.
Conclusions: Our results showed that the new real-time PCR assay provides a sensitive and specific way to detect *C. trachomatis* not only in invasive but also in noninvasive specimens. These collection methods are more acceptable to patients and allow for testing outside traditional clinic settings by eliminating the need for painful urethral swabs in men and uncomfortable pelvic exams in women. This increases the access to care for high-risk groups.

R2135 Evaluation of a real-time PCR assay for the detection of *Chlamydia trachomatis* in different specimen types

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Hamburg, D

Objectives: Bacteria of the species *Chlamydia* (C.) are of great epidemiological importance worldwide. Until recently, the gold standard for identifying *C. trachomatis* has been the culture method. Although specific, this procedure is time consuming, laborious and is unfit for the routine screening of *C. trachomatis* in clinical specimens. We evaluated the performance of the RealArt *C. trachomatis* TM PCR Kit (artus, Germany) on the ABI PRISM 7000 SDS instrument (Applied Biosystems, USA) to improve access to screening methods in routine clinical practices.
Methods: This new real-time PCR assay combines sequence-specific primers and a dual labelled fluorogenic probe. In addition, it contains a second amplification system (internal control) to identify possible PCR inhibition and to control the isolation procedure. The results of the RealArt *C. trachomatis* TM PCR Kit for 150 retrospective swab specimens (50 urethral, 50 cervical and 50 vaginal swabs) and for 100 retrospective urine specimens (50 male and 50 female) were compared with those of Abbott's ligase chain reaction (LCx CT/GC assay) and GEN-PROBE's transcription mediated amplification (APTIMA Combo 2 assay).
Results: On retrospective specimens the RealArt *C. trachomatis* TM PCR Kit showed a correlation to LCx CT/GC and APTIMA Combo 2 of 98.8%, respectively. Two RealArt *C. trachomatis* positive, LCx and APTIMA-negative samples were further analysed by sequencing which revealed a *C. trachomatis* specific sequence.
Conclusion: Our results showed that the new real-time PCR assay is a sensitive and specific way to detect *C. trachomatis* in swab and urine specimens, enabling fast screening of this important target group.

R2136 Rapid detection of *Salmonella* sp. in clinical veterinary samples

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Salmonella sp. is the main cause of human gastroenteritis worldwide. Salmonellosis, which symptoms may vary from mild diarrhoea to severe sepsis, affects 2–4 million individuals per year in the USA, with a mortality rate of 1–10%. Pork and derivate products is an important vehicle of *Salmonella* transmission. Since their consumption has increased in Portugal in the last decade, it is urgent to develop rapid methods for the detection of *Salmonella* in slaughterhouse samples. Using a specific probe described by Nordentoft *et al*. (1997), a FISH protocol for Salmonella sp. detection was developed. This protocol was applied to 129 samples from a slaughterhouse, which included ileum, intestinal ganglia, amygdales, mandible ganglia, carcass and liver. These samples were also analysed through VIDAS. It was observed that 78 samples (60%) revealed to be positive through FISH analysis, while VIDAS originated 22 positive results (17%). These results show that the FISH protocol developed allows the detection of *Salmonella* sp. in clinical samples in 48 h, with high sensitivity and low cost. This technique is currently applied to the microbiological analysis of faeces, urine and blood samples.

R2137 Inactivation of pbp4b gene in *Salmonella enterica* serovar Typhimurium SL1344, *Escherichia coli* BW 25113, CS-801-4 and RP1 by using PCR products

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Objectives: To understand what are the molecular mechanisms (and the proteins implied in them) by which *Salmonella* carries out the cellular division and peptidoglycan biosynthesis within the cell eucariota, and contribute for the design of therapeutic strategies that can limit the infection and prevent the antimicrobial resistance. An important virulence factor in STM is its capacity to replicate itself intracellularly in epithelial cells. Studies made in mutants of STM, that normally invade nonphagocitics cells in culture, but that are not able to proliferate intracellularly, suggest the existence of a genetic loci-specific and necessary to be able to multiply intracellularly and confer to STM the pathogenic character. By this reason we have designed a mutant of STM for the pbp4b gene implied in the process of cellular division and the peptidoglycan biosynthesis. This pbp4b-mutant, was obtained by a new technique of PCR, using primers of 60 nucleotide of length, which amplify the cassettes of Cloranfenicol and Kanamicina resistance that are flanked by regions FRT (FLP Recognition Target) and regions homologous to adjacent genes to the pbp4b gene and then transform bacterial strain containing the pKD46 vector of low number of copy and termosensible that codifies for the recombinas red of the Lambda phage that is under the control of an inducible promoter by arabinosa, and the mutants generated were selected by their resistance to antibiotic. These resistance genes were eliminated using a vector thermosensible that codifies for FLP recombinase. This mutant pbp4b- have been put under studies of kinetic of intracellular proliferation using mice model and *in vitro* tests using cellular lines Hela, Swiss 3T3 and NRK for the obtaining of the respective rate of intracellular proliferation in

nonphagocytics cells. The results obtained until the moment indicate that STM displays a different infection behavior and intracellular proliferation depending on the cellular type that it invades. Despite it is important to make a mention and indicate that this research is actually in developing process, and we are sure that the results that will obtain allow an experimental but deep boarding in the identification of new useful targets in STM in the design of new drugs with therapeutic application.

R2138 Correlation between pigment production and β-haemolysin of *Streptococcus agalactiae*

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Ulm, Jena, D

Objectives: *Streptococcus agalactiae* (group B streptococci) is the leading cause of bacterial sepsis, pneumonia and meningitis in neonates. The production of an orange-red pigment is frequently used for diagnostic purposes. Pigment formation and production of haemolysin is associated with the cyl genes, a locus that comprises a cluster of 12 different genes.
Methods and results: To investigate the linkage between pigment and haemolysin formation, nonpigmented mutant strains harbouring a deletion in the cyl A, cyl G, cyl E or cyl K gene were compared with pigment-forming wild-type strains. It was possible to extract haemolytic activity from pigmented intact cells using an extractor solution containing starch as stabilising agent. However, at room temperature this activity was rapidly lost. No haemolysis was found associated with the analysed nonpigmented mutant strains or in wild-type strains grown under conditions not leading to pigment production. Interestingly, the formation of haemolysin and pigment is inhibited by an excess of glucose in the growth medium. *Streptococcus agalactiae* pigment is produced in the late exponential phase and is associated with the bacterial cells. However, in presence of a suitable carrier molecule it could also be found in the medium. Pigment from lysed wild-type-cells was isolated under acidic and basic conditions, resulting in orange-red solutions with different spectral properties. Further analysis was performed on the extracts, a lyophilised extract, and precipitated extract preparations. As a result the association of the pigment with a high-molecular weight carrier molecule was observed.
Conclusion: Our findings confirm the proposed linkage between the expression of hemolytic activity and pigment production of *Streptococcus agalactiae*. The described isolation methods enable further analysis of the pigment and will give new insights into the nature of this molecule. The pigment isolation along with the knowledge on stability and production of the haemolysin will allow the elucidation of the correlation between haemolysis and pigment production.

R2139 Detection of common agents of bacterial meningitis with a low density DNA array

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Bacterial meningitis is a serious infection of the central nervous system, whose aetiology may be missed in 10–15% of cases. Rapid diagnosis is a prerequisite for the initiation of prompt and effective treatment. Traditional laboratory diagnostic methods with culture for the identification of bacterial meningitis pathogens may take 36 h or more. Furthermore, following an increase in the practice of starting antimicrobial therapy prior to clinical sample collection, the ability to confirm the pathogenic microorganisms of bacterial meningitis may not always be possible. Compared with conventional methods, assays based on nucleic acid detection have the potential of greater speed and sensitivity. In recent years, PCR techniques have increasingly been used to amplify and detect microbial DNA in clinical samples. By using a combination of PCR methods and array technology we describe a method for identifying common bacterial agents of pyogenic meningitis (*S. pneumonia*e, N. meningitidis, *H. influenzae* by amplifying and detecting both universal and variable regions of bacterial 16S ribosomal DNA and pathogen-specific virulence or pathogenicity-associated genes on an oligonucleotide DNA array platform.

R2140 Improving method of chromosomal DNA extraction of *Brucella melitensis* for multiplying gene pieces by PCR

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Brucella melitensis is a Gram-negative bacterium, which has a cell wall with a complex structure. Because of cell wall characteristics, the common methods for chromosomal DNA extraction of Gram-negative bacteria have some problems with this bacterium. In this research, chromosomal DNA extraction of this bacterium was carried out for multiplying gene pieces by PCR by CTAB and common method. Because of low efficiency of these two methods, chromosomal extraction of this bacterium was performed by combining these two methods. First, we treated bacteria by SDS and TE, and then we destroyed peptidoglycan by lysozyme. After that, we precipitated protein with phenol–chloroform and polysaccharide by CTAB. In this research, appropriate time for chromosomal extraction from liquid culture was 48 and 72 h, Then we obtained appropriate amount of 10% SDS and lysozyme, 90 ml and 85 μl, respectively (5 g/mL). Our results showed that the amount of DNA extracted with this method is higher than the other two methods and it reaches 500 g/mL. In addition, purity of extracted DNA in this method is better than the other two methods. The extracted chromosomal DNA was used for multiplying l7/l12 (a bacterial ribosomal protein). Because of improved efficiency of this method, it may be used as a method for chromosomal DNA extraction in clinical samples of blood and milk for diagnosing brucellosis.

R2141 Detection of *Listeria monocytogenes* in fresh FISH using real-time PCR, PCR and ISO methods

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The objectives of the present study were the assessment of *Listeria monocytogenes* contamination of fisheries and the evaluation of standard ISO methods and modern molecular techniques. Seventy samples of fresh and saltwater edible fish were examined for *L. monocytogenes* contamination, using the standard ISO cultural methods and two different polymerase chain reactions (PCR). The samples were collected from local fish markets and local aquacultures. Half-Fraser broth and Fraser broth were used for pre-enrichment and enrichment reciprocally, while Oxford and Palcam agar (ISO 10560) were used as selective agars. Two PCR automated systems, the LightCycler (Roche) with detection limit 103 CFU/mL, and the Bax (Qualicon), with detection limit 105 CFU/mL were used for the performance of PCR. Two strains of *L. monocytogenes* were isolated with the ISO cultures and also detected by both PCR systems. One isolate (*L. monocytogenes* serotype 4) was detected from a fish of local capture (cephalus) and the other strain (*L. monocytogenes* serotype 1) was isolated from imported fresh salmon filet. Both isolates were susceptible to routinely used antibiotics. The results indicate the possible risk of *L. monocytogenes* infection transmitted from fisheries to humans through inadequately cooked seafood and/or through contamination of culinary tools. The results also suggest that molecular techniques minimise considerably the recovery time of *L. monocytogenes* from food, enabling a more efficient safeguarding of public health with regard to food-borne infections.

Diagnostic methods (other than molecular)

R2142 Development and application of a Dot blot assay for detection of human immunodeficiency virus types 1 and 2 using recombinant p24, gp41 and gp36 proteins from Iranian strains

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Recombinant p24, the capsid protein region and recombinant gp41, the transmembrane protein of human immunodeficiency virus type 1 (HIV-1) and also recombinant gp36, the specific human immunodeficiency virus type 2 (HIV-2) antigen had been expressed in *Escherichia coli* BL21 (DE3). The antigenic reactivity of all recombinant proteins was confirmed by Dot blot with human antisera to HIV-1 and HIV-2 separately. HIV-negative human sera failed to recognise the recombinant proteins, thereby showing the specificity of the HIV Dot blot. Sixty-five human-positive serum samples as well as human-negative serum samples were tested for the presence of anti-HIV antibodies by the recombinant protein-based Dot blot and a reference assay. There was a 100% concordance when all recombinant proteins were used in the Dot blot. However, the Dot blot using the recombinant proteins as its test antigen identified two HIV-positive human sera, which had tested negative in the reference assays. The results of this study showed that the recombinant p24, gp41 and gp36 proteins could be used as test antigens for detection and confirmation of HIV-infection in human.

R2143 Standardisation of an ELISA test for detection of hydatid cyst antigen/s in human serum

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Introduction and objectives: Serological methods to confirm the diagnosis of hydatidosis are mainly based on detection of antibodies against cyst antigen/s in patients serum, but as antibody production is time consuming (several days) as well as low or no antibody responses to cyst antigens are recognised in some human patients as well as animals such as sheep, so serology based on antibody detection may not be fully dependable. There for, serology based on antigen/s detection may be more valuable. In this study a capture ELISA for detection of hydatid cyst antigen/s in human serum was designed and standardised.
Methods: Antibodies used for coating ELISA plate wells and for conjugation with HRP were prepared in rabbits and guinea pigs. ELISA test was standardised by coating one microgram antibody /well of microtitre plate in carbonate-bicarbonate buffer at 4°C over night, blocking with 3% BSA, conjugated antibody 1/75 dilution, and use of H2O2 as substrate and OPD as chromogen.
Results: Results show that the standardised ELISA test is able to detect as less as 312 ng of cyst antigens/mL of patients serum or in PBS with cut off 0/13 i.e. mean of negatives plus two standard deviations. The sensitivity of the standardised test for hydatid cyst fluid, protoscolex soluble antigens, and mixture of both was same.
Discussion and conclusion: The standardised test seems to be useful for diagnosis as well as follow-up of treatment.

R2144 Selected markers in the monitoring of infections in an abdominal surgery setting

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The aim of the study was to construct our own model of the monitoring of severe inflammation in patients after surgical operations within the abdominal cavity.

Methods: For evaluation of the inflammation we have taken the scale, which included clinical state, also, the results of microbiological cultures and biochemical parameters. The level of PCT, TNF-alpha, IL-1beta, IL-6 in the blood serum was designated. The blood samples were taken in the 0-3-5 day after the operation and, in case of need, later depending on the clinical state of the patient. 103 determinations of the PCT level in 34 patients were performed, 71 determinations in 29 patients to measure TNF- alfa and IL-1 beta levels and in nine patients 21 determinations for IL-6.
Results: The mean PCT level in the group of patients with local infections was 0.39 ± 0.52 ng/mL, in acute pancreatitis was 2.46 ± 3.05 ng/mL, in diffuse peritonitis 4.98 ± 6.46 ng/mL, and in septic shock was 12.8 ± 7.9 ng/mL. Significant statistical differences between particular groups were noticed with $P < 0.001$. In the group of patients who survived this value was: $l.63 \pm l.22$ ng/mL and in those who died 7.36 ± 4.4 ng/mL/ $P < 0.001$. The median plasma concentration of TNF-alpha was 13.55 ± 10.15 pg/mL in the examined group and 12.44 ± 3.62 pg/mL in the control group; II-1beta respectively 5.46 ± 3.43 pg/mL and 2.14 ± 2.15 pg/mL; IL-6: 828 ± 573 and 254 ± 78 pg/mL. Significant statistical differences were noticed only for IL-6/ $P < 0.05$/. In the group of patients who survived, TNF-alpha level was 12.11 ± 7.90 pg/mL and in those who died 14.53 ± 16.37 pg/mL; IL-1beta, respectively, 6.19 ± 4.47 pg/mL and 4.24 ± 1.05 pg/mL; IL-6: 763.5 ± 120.4 and 1243 ± 906.6 pg/mL/ n.s.
Conclusions: The dynamics of changes in the values of each parameter in time were compared. PCT is the most sensitive and precise prognostic factor in the group of patients with severe infections of the abdominal cavity. This marker can facilitate the diagnosis of sepsis and permits objectivisation of the dynamics of the clinical picture. It's difficult to evaluate cytokines' usefulness in monitoring of infections.

R2145 Performance of TechLab C. DIFFICILE ANTIGEN CHEK for the detection of *Clostridium difficile* in stool specimens

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Objectives: TechLab Inc. (Blacksburg, VA) has recently developed an ELISA test for the detection of glutamate dehydrogenase, a *C. difficile* antigen. We evaluated the performance of this kit, TechLab C. DIFF CHECK (TL-AG) and compared its results with those of cultures and the component of Triage *C. difficile* panels detecting GDH (Tr-AG).
Methods: Stools submitted for the diagnosis of *C. difficile*-associated diarrhoea (CDAD) were inoculated onto *C. difficile* selective media (Oxoid, Canada) and tested with TL-Ag and Tr-AG according to the manufacturers' instructions. After 48 h incubation under anaerobic conditions, suspected colonies were identified as *C. difficile* by the MicroScreen *C. difficile* slide agglutination test (Microgen Bioproducts Ltd, Surrey, UK). *C. difficle* isolates were also tested for the presence of toxin A and B genes by PCR.
Results: The study included 497 specimens. Because of blackening, Triage panels could not be evaluated in 15 (3%) of cases. *C. difficile* was isolated from 93 specimens. Twenty (21.5%) were nontoxogenic. TL-Ag correctly identified the presence of *C. difficile* in 87 specimens, and Tr-Ag detected *C. difficile* in 79/93 cases. Compared with culture results sensitivity, specificity, positive- and negative-predictive values were 93.5, 98, 91.6 and 98, respectively, for TL-Ag, and 84.9, 98.8, 94 and 96.6%, respectively for Tr-Ag.
Conclusions: ELISA methods for the detection of *C. difficile* were more rapid than cultures. TL-Ag was more sensitive than Tr-AG. Like cultures, the tests for GDH detect both toxogenic and nontoxogenic strains of *C. difficile* therefore, these tests should only be

used as screening tests. Should TL-Ag be used in the diagnosis of CDAD, it should be used in combination with a toxin detection test.

R2146 *Mycoplasma hominis* in the genital tract of women

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Mycoplasmas are the smallest free-living micro-organisms able to replicate on cell-free media. They possess the smallest genome of all prokaryotes. *M. hominis* belongs to the sensitive micro-organisms and its determination is very difficult. The role of *M. hominis* in pathogenesis of genital infections has not been proved yet. *Mycoplasma hominis* can be isolated with different frequency from the women urogenital tract and it is thought to cause various syndromes such as pelvic inflammatory disease, infertility, amniotic fluid infection, aborts, lowborn weight, postpartum endometritis and fever. The aim of this study was to determine the incidence of *M. hominis* in the genital tract of women and its susceptibility to antimicrobial agents. Specimens were taken from the cervix of randomly selected women and cultivated on PPLO agar. Isolates were tested in PPLO-arginine broth and confirmed by PCR method. 252 cervical swabs were investigated. *M. hominis* was isolated in 44 cases (17.5%) from this cervical specimens. Susceptibility of clinical isolates of *M. hominis* was studied to doxycyclin, ciprofloxacin and erythromycin. This research was supported by MSM 253100002.

R2147 Prevalence of *Staphylococcus aureus* in the university dairy herd

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The prevalence of *Staphylococcus aureus* in the university dairy herd during two seasons (winter and summer) was investigated. A total of 12 Friesland cows (42 quarters because the other six quarters were blocked) were used during the research. California mastitis test (CMT) and bacteriological culturing were employed. All tests were negative in all cows during winter. During summer, 10 quarters were negative in CMT and all bacteriological cultures. The remaining 19 quarters were positive on both CMT and bacteriological culturing of *S. aureus*. *S. epidermidis* was isolated in the remaining 13. These results suggest that the incidence of *S. aureus* was high in summer as compared with winter (0% in winter vs. 45% in summer). Interestingly, *S. aureus* was also isolated from other milking equipments in the dairy, probably because of poor sanitary measures.

R2148 Distribution of systemic inflammatory response syndrome (SIRS) according to the cause in emergency ward admissions Esfahan, Iran, 2002–03

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Objectives: The systemic inflammatory response syndrome may have an infectious or noninfectious aetiology. SIRS is usually presented by signs and symptoms such as fever or hypothermia, tachycardia, leucocytosis or leucopenia, and appearance of band cells in peripheral blood. Any of the above can happen in severe infections. This study is conducted to evaluate share of any clinical entity inducing SIRS.
Material and methods: The study included every patient over 12 years with a minimum of two measures for SIRS during 1 year (21 October 2002 to 21 October 2003). A criteria selection form was filled by resident physician and rechecked by specialist. Those who proved to be SIRS were evaluated for the cause and final diagnosis was registered in the form.
Results: Totally 904 cases were referred to the emergency room because of different conditions. A total of 429 cases had criteria to be SIRS. The most frequent causes of SIRS presentation were trauma (34.7%), infection (26.3%), CVA (5.6%) GI bleeding (4.9%) and other conditions including convulsion, vaginal delivery, CHF, unstable angina, malignancy, acute myocardial infarction, pamphigus, thrombocytopenic purpura, thromboembolism, hypoglycaemia, cardiomyopathy, atrialfibrilation, pancreatitis, Steven–Johnson syndrome, DKA and pancytopenia.
Conclusions: Although SIRS may be the preliminary stage of sepsis and septic shock but a wide range of diseases initially may present with this syndrome. Physicians have to evaluate and observe these critically ill patients course of the illness. Many noninfectious causes may trigger SIRS which are not responsible to antibiotics and do not need this kind of drugs.

R2149 Evaluation of *Helicobacter pylori* stool antigen test for diagnosis of infection and posteradication follow-up

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A total of 54 patients with epigastric discomfort referred to lmmam Khomeini teaching hospital for upper gasterointetinal endoscopy were enrolled in this study. *Helicobacter* infection was confirmed with rapid unease test; culture and histological methods. Cold standard tests were positive culture or positive RUT plus histology with which HpsAT was compared; and gold standard for negative infection; was negative Rut; negative culture and histology.

A total of 26 patients with positive infection with omeprazol, tetracycline; metronidazol; bismuth subsalisylate; for 2 weeks. Then 1 and 3 months later revaluated with gold standard tests and HpsAT.
Results: Sensitivity and specificity and accuracy of *Helicobacter pylori* stool antigen test before eradication was 78.6, 92.3 and 85.2% respectively. One month after eradication sensitivity and specificity, and accuracy of test was 100 and 59.5%, and 61.5%, respectively, and 3 months after treatment was 85.7, 89.5 and 88.5%, respectively. These results implicate that HpsAT for primary diagnosis of *Helicobacter pylori* infection is an excellent method; but its specificity and accuracy in early period fallow-up of eradication are low. Also 3 months fallowing eradication and later than that, HpsAT has very good and acceptable results.

R2150 CPS ID 3 – a new chromogenic media used in detection of urinary infection agents

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Objectives: In our university hospital, the chromogenic media recently started to be integrated and used in parallel with Columbia 5% sheep blood, Cled and Mac Conkey agar. We have evaluated the fertility, identification performance for the microorganisms isolated in our laboratory and the enumeration speciality of the new media called CPS ID 3 (bioMérieux S.A. France).
Methods: In total, 105 urine specimens were included in this study, collected between September and November 2003 in the university hospital of Kahramanmaras Sutcu Imam. The specimens were inoculated in parallel on the CPS ID 3 medium, Columbia 5% sheep blood, Cled and Mac Conkey agar. They were incubated at 37 °C during 18–24 h.
Results: Seventeen specimens were sterile (<1000 CFU/mL), they were not included in the study. 65 of 79 specimens had a single type colony, and 14 of 79 had mixed type colony (12 of 14 two types, two of 14 three types). The distribution of the bacteria were respectively: 44 *Escherichia coli*, 10 *Enterobacter* spp., 8 *Proteus* spp., 8 *Klebsiella* spp., 3 *Pseudomonas aeruginosa*, 4 B group streptococci, 7 *Enterococcus* spp., 6 *Staphylococcus* spp, 2 *Candida* spp. and 2 *Morganella* spp. The sensitivity of CPS ID 3 for identification of

E. coli, *Klebsiella*, *Enterobacter*, enterococci were 93.2, 100, 100 and 100%, respectively. CPS ID 3 efficiently prevented the swarming of *Proteus*.
Conclusion: CPS ID 3 is a useful chromogenic media to perform urine specimen testing because of rate of identification of the most frequent bacteria, efficient enumeration and fertility.

R2151 An evaluation of MRSA ID: a new chromogenic medium for the isolation and identification of methicillin-resistant *Staphylococcus aureus*

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Objectives: MRSA ID is a new chromogenic medium, which relies upon alpha-glucosidase detection for the visualisation of methicillin-resistant *Staphylococcus aureus* (MRSA) as green colonies. The medium is selective against Gram-negative bacteria, enterococci and fungi and also inhibits methicillin-sensitive staphylococci due to the inclusion of a beta-lactam antibiotic. The aim of this project was to evaluate the effectiveness of MRSA ID for the isolation of MRSA from nasal swabs. Its performance was compared with that of two commercially available agars.
Method: A total of 192 nasal swabs from distinct hospital patients were each emulsified in 750 μL of saline (0.85%). A 50 μl sample of the resulting suspension was inoculated onto MRSA ID, CHROMagar MRSA and ORSAB medium. The inoculum was spread for single colonies and all media were incubated at 37°C. The culture plates were examined for the presence of MRSA after 20–22 h incubation and were examined again after a total of 48 h incubation. All suspect colonies of MRSA on all media were confirmed using standard methods including the tube coagulase test and detection of the mecA resistance determinant.
Results: A total of 28 strains (14.6%) were isolated on at least one of the three media. After 20–22 h incubation 25 of these strains (89%) were isolated as coloured colonies on MRSA ID compared with 20 (71%) on CHROMagar MRSA and 21 (75%) on ORSAB medium. After 48 h incubation, 26 strains (93%) were isolated as coloured colonies on MRSA ID and ORSAB medium compared with 25 (89%) on CHROMagar MRSA. The chromogenic reactions of all three media were highly specific for MRSA after 20–22 h incubation. The specificities of the media were 100, 100 and 95% after 20–22 h for MRSA ID, CHROMagar MRSA and ORSAB, respectively. After 48 h, some strains of coagulase-negative staphylococci generated pale green colonies on MRSA ID, they were clearly distinguishable from *S. aureus* colonies.
Conclusions: MRSA ID is a highly effective medium for the detection of MRSA from nasal swabs and has an excellent sensitivity and specificity after 20–24 h incubation.

R2152 Disk diffusion (DD) synergy test with cefotetan/cloxacillin (CTT/CLX) for discrimination between *Klebsiella pneumoniae* (Kp) producing plasmid-mediated AmpC-type beta-lactamase (pACBL+) from other cefoxitin-resistant (FOX-R) strains

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Background: It is important to differentiate between Kp pACBL+ from other FOX-R strains because of diagnostic, therapeutic, and epidemiological reasons.
Methods: Sixty Kp clinical strains were studied, including 26 pACBL+, 12 strains producing extended-spectrum beta-lactamases and deficient in porins (ESBL+/POR−) and 22 strains with other mechanisms of resistance (10 ESBL−/ACBL−/POR+; 10 ESBL+/ACBL−/POR+; 2 ESBL−/ACBL−/POR−). Strains were identified as pACBL+ when they transferred FOX-R by conjugation and contained a beta-lactamase inactivated CLX but not by clavulanic acid, as determined by *in situ* analysis on isoelectric focusing gels. ESBL were detected by microdilution (NCCLS method). Porin loss was determined by SDS-PAGE of outer membrane proteins. The activity of both FOX and CTT was determined by DD (30 μg disks) and by microdilution (MD) (NCCLS guidelines). Synergy between CLX and CTT was determined by MD and DD in medium containing CLX (0.25 mg/mL) or by using CTT/CLX disks (CLX content: 0.25, 0.5 or 1 mg).
Results: All 26 pACBL+, all 12 ESBL+/POR− strains, and 12/22 of other strains were FOX-R. CTT-R was only noted among pACBL+ (21/26 strains). By MD, the MICs of CTT decreased $\geq$3 twofold dilutions in the presence of CLX 0.25 mg/mL for 23/26 Kp pACBL+, and in none of the remaining groups. By DD, zones around CTT disks increased $\geq$4 mm for 19/26 Kp pACBL+ in medium containing CLX (0.25 mg/mL). DD with CTT/CLX disks gave zones of inhibition that were $\geq$4 mm in comparison with those of CTT disks alone for 18/26, 19/26 and 24/26 Kp pACBL+ when CLX content in disk was 0.25, 0.5 and 1 mg, respectively. In all other organisms this increase was $\leq$3 mm.
Conclusion: A $\geq$4 mm increase in the diameter of inhibition zone of a CTT/CLX disk (30 μg/1 mg) in comparison with the diameter of a CTT disk (30 μg) alone allows an easy-to-perform discrimination between clinical isolates of Kp pACBL+ from other FOX-R Kp.

R2153 Diagnostic accuracy of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis

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Objective: The aim of the study is to evaluate the positivity of anti-cyclic citrullinated peptide antibodies (anti-CCP) in patients with juvenile idiopathic arthritis (JIA) and its relationship with disease subtype and erosive form of the disease.
Methods: The study population consisted of 122 patients (72 girls, 50 boys) with JIA. 16 of them were evaluated both during active disease and remission period. 19 children with systemic lupus erythemathosus (SLE), 27 patients with rheumatoid arthritis (RA) and 15 healthy children were also included in the study. RF positivity was observed only in 12 children with JIA and 34 patients had persistent erosive lesions in their joints. Anti-CCP antibody levels were determined by via ELISA and values above 5 relative units (RU) were regarded as positive.
Results: Only three girls with seropositive polyarticular JIA and erosive joint disease had positive anti-CCP values. Children evaluated both during active and remission period of the disease as well as those with SLE and healthy children all had negative anti-CCP antibody levels. However, 19 (70%) of adult patients with RA showed positive anti-CCP antibody values.
Conclusions: In contrary to RA, in patients with JIA, which has a heterogeneous nature, anti-CCP antibody positivity is only rarely found. In patients with RF positivity and/or in patients with erosive joint disease, anti-CCP antibodies can be detected. For that reason, in patients with JIA, anti-CCP antibodies should not be used as a screening method, but as early indices of joint damage, especially in children with polyarticular JIA.

R2154 Procalcitonin level measurement in juvenile idiopathic arthritis

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Objectives: The aim of this study is to determine the role of procalcitonin (PCT) in diagnosis and follow ups of JIA as acute phase reactants.

Methods: Eighty-one JIA patients (50 females, 31 males) were studied. Sixty of the patients had active disease during the evaluation. Control group consisted of 15 healthy children and 21 children diagnosed with bacterial meningitis. PCT was measured with immunoluminometric method. ROC curve was obtained with results and cut-off points were accepted as 0.856 ng/mL.
Results: Procalcitonin levels of JIA patients [1.04 (range 0.52–7.42) ng/mL] were significantly higher than those of healthy children [0.71 (range 0.6–1.17) ng/mL] and was lower than those of children diagnosed as bacterial meningitis [13.5 (range 0.8–209) ng/mL]. The PCT levels of JIA patients with active disease were significantly higher than the JIA patients whose disease is in remission. The PCT level showed significant correlation with leucocytes and CRP in JIA patients. There was no correlation between PCT levels and erythrocyte sedimentation rate. Twenty-one (25.9%) of JIA patients studied had positive PCT levels according to range limit of 2 ng/mL. All of these children had active disease and 13 (62%) of them also had bacterial upper respiratory infection findings.
Conclusions: PCT level shows only a slight elevation in inflammatory disease although very significant elevations are seen during bacterial infection. The presence of active disease and infectious findings in most of the patients may be useful to predict the antibacterial treatment.

R2155 Protein C6 in serodiagnosis of Lyme borreliosis in patients from Poland – a premilinary study

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Objectives: The purpose of the study was to analyse the percentage of seropositive results in patients with Lyme borreliosis in comparison with blood donors, using ELISA (C6-Immunetics and Biomedica Recombinant kits).
Methods: The study included 136 patients treated in the outpatient clinic or hospitalised in the Department of Infectious Diseases and Neuroinfection of the Medical University of Bialystok. They were divided into two groups: Group I $n = 36$ patients with erythema migrans, group II $n = 68$ patients with Lyme arthritis. Group III n consisted of 42 healthy blood donors. Serum samples were obtained from patients before treatment. The diagnosis was based on the criteria of CDC and EUCALAB. The tests of Immunetics Quick ELISA C6 Borrelia Assay Kit (USA) and Biomedica Recombinant IgM, IgG (Austria) were used according to the producers' recommendations.
Results: Only one individual of 42 blood donors had a positive result in the test of Immunetics Quick ELISA C6 Borrelia Assay Kit. It showed antibodies in IgG class (+++)59 BBU and in IgM class (+)13 BBU. One individual with a doubtful result in the test of Biomedica Recombinant had a negative result in the test of Immunetics Quick ELISA C6 Borrelia Assay Kit. Twenty-two patients of 36 patients with erythema migrans had positive or doubtful results and four patients – negative results in the test of Immunetics Quick ELISA C6 Borrelia Assay Kit. In the test of Biomedica, 10 patients of 34 examined had positive results in IgM class and four patients of 34 in IgG class. Antibodies in IgG class were found in 68 patients with chronic borreliosis using the test of Biomedica Recombinant and 20 of them had antibodies in IgM class. In the test of Immunetics, positive or doubtful results were proved in 57 patients and negative results were found in 11 patients.
Conclusions: ELISA test based on C6 protein is highly sensitive not only at the early stage of borreliosis and erythema migrans but at the later stage, as well.
– This test enables to detect antibodies in patients without clinical symptoms.
– The results proved to be useful and necessary especially in patients who had doubtful serum results in other tests.
– This test can be used as a screening and verifying test.

R2156 The isolation and characterisation of *Actinomyces* strains in patients suffering canaliculitis in a teaching hospital, Tehran

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The main purpose of the study was to isolate and detect *Actinomyces* species and *Propionibacterium propionica* from lachrymal duct infection. A total of 112 patients with advanced symptomatic canaliculitis and dacryocystitis, who had been referred to Farabi Teaching Hospital eye clinic in Tehran, were studied over a period of 18 months. From each sample three thin smears were prepared for microscopic observations. The samples were cultured anaerobically and micro-aerophilically in appropriate rich media. Medical histories of patients were also recorded in the certain questionnaire for further data analysis. The results demonstrated that eight strains of bacteria (five strains of *Actinomyces* and three strains of *Propionibacterium propionica*) from the canaliculitis patients and 12 other micro-aerophilic bacteria from dacryocystitis patients were isolated. The incidence of canaliculitis in women (20%) was significantly higher than the men (2.9%) ($P < 0.05$). The rate of actinomycotic canaliculitis was 48.6% in household women and 11.4% in students and 40% in other professions, which indicates the role of occupation in the incidence of this infection.

R2157 Isolation and characterisation of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* with aggressive peridontitis

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Statement of problem: One of the best ways for treatment of Aggressive Periodontitis (AP) is identification and eliminations of aetiological factors specially micro-organisms including Aa and Pg in patients referring to Tehran University, Dental clinic. The main purpose of the present study was to determine the prevalence of Aa and Pg in active sites of AP Patients. The correlation between the incidence of Aa and Pg and such variables as age and sex was also investigated. This investigation is a cross-sectional and descriptive study. At first marginal gingival dried and Sampling Performed by number 30 paper point. The samples obtained from 54 sites (Pocket depth Pd > 5 nm) in 15 patients. From each sample thin smears were prepared for microscopic observations. The samples were cultured in selective medium for isolation of Aa and in TSBV and *Brucella* agar for isolation of Pg. Stalistical tests were fisher and X2. A total of 13 patients or 38 sites (%70.4) identified Aa positive and three patients or 10 sites (% 18.4) Pg positive. There was not significant difference between incidence of Aa and sex ($P = 0.177$) or age ($P = 0.086$). Pg in men found more than women ($P < 0.0001$) but about Pg it was not the same. Aa with Pd had a significant positive correlation. ($P = 0.002$). It means with increasing pd, the probability of Aa existence would be high but about Pg it was not the same. Also Aa had significant negative correlation with family number that means with increasing them, Aa+ sites decrease. Our results revealed that the most of A.P Patients could consider Aa positive but not Pg positive.

R2158 ELISA vs. routine tests in the diagnosis of patients with brucellosis

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Objective: Brucellosis is one of the endemic disease in Zanjan province and a zoonosis disease among animal and human beings. Because of the importance of the brucellosis, there are

many serological techniques for diagnosing the disease. Each of them has advantages or disadvantages. Amongst serologic methods ELISA test is a choice method. The major aim of this study is comparison of routine tests vs. ELISA in the diagnosis of brucellosis in Zanjan.
Methods: In this survey, 176 patients, showing clinical symptoms of malta fever. The routine serological test for wright (rapid), standard tube agglutination (STA), Coombs wright and IgM, IgG ELISA were performed in serum samples. Sensitivity, specificity, ppv and npv of them vs. total ELISA is compared.
Results: A total of 176 cases (92 males and 84 females) were affected by *Brucella* ranging from 15 to 65 years. Sensitivity, specificity, ppv, npv wright(rapid) vs. ELISA sequencially is: 62.5, 100, 100 and 79.3%. STA vs. total ELISA sequencially is: 68.0, 100, 100 and 81.8% and coombs wright vs. total ELISA sequencially is: 80.0, 100, 100 and 88.1%.
Conclusion: ELISA is the most sensitive and more specific of the *Brucella* serologic routine tests and is useful to monitor antibodies in patients undergoing treatment, isotype determining and phase of disease. Over all extrapolation of data from our study indicate that the ranking of tests according to their reliability of diagnosing human brucellosis is as follows: ELISA > coombs > STA > wright (rapid).

R2159 Survey of Rose Bengal test vs. ELISA in the diagnosis of acute and chronic brucellosis

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Objective: Brucellosis is one of the endemic diseases in Iran and because of the importance of the brucellosis, there are many serologic techniques for diagnosing the disease. Each of them has advantages or disadvantages. Amongst serologic methods ELISA test is a preferable (choice) method. The major aim of this study is comparison of Rapid Rose Bengal test vs. ELISA in the diagnosis of acute and chronic brucellosis (2002) in Zanjan.
Methods: In this survey, 176 patients, showing clinical symptoms of malta fever. The serological test for Rose Bengal and IgG and IgM ELISA were performed in all serum samples. Sensitivity, specificity, ppv and npv of Rose Bengal vs. ELISA are compared.
Results: Of 176 cases (92 male and 84 female) affected by *Brucella* ranging from 15 to 65 years old. Sensitivity, specificity, ppv and npv Rose Bengal vs. IgM ELISA is 100, 95.6, 70.8 and 100% sequentially and for IgG ELISA is: 36.9, 100, 100 and 73.0% sequentially.
Conclusion: ELISA is the most sensitive and more specific of the *Brucella* serologic tests and is useful to monitor antibodies in patients undergoing treatment. Isotype and phase of disease determining Rose Bengal tests highly correlated with IgM ELISA in acute phase of brucellosis but not correlated with IgG ELISA in chronic diseases.

R2160 Laboratory diagnostic of suspect neuroborreliosis – results from 1997 to 2003 in patients from West Slovakia

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Background: Neuroborreliosis affects peripherial and central nervous system.
Objectives: Point out on possibilities of laboratory diagnostics of neuroborreliosis.
Subjects and methods: During the years 1997–2003 we tested 902 pair samples of CSF and serum from 894 patients with different neurological diagnosis by ELISA, Western blot, PCR, completed by biochemical and cytological investigations.
Results: Forty-five of 894 patients were anti-borrelia seropositive (5%), 19 of them had specific IgG only (2.1%), 12 had only specific IgM (1.3%) and 14 (1.6%) had both classes of specific antibodies in serum. We confirmed intrathecal specific IgG antibodies production by AI in 22 cases (2.44%) of total 902 samples tested. From those in 12 cases there were present also IgM antibodies in CSF. We found borderline AI values in three cases (0.33%) and isolated intrathecal production, antibodies present only in CSF, in two cases (0.22%). There were normal AI values found in 29 cases (3.2%). Specific antibody positivity in CSF without seroposivity was detected by WB method only in two cases. DNA positivity by PCR was detected in one CSF from 112 samples during the last 4 years.
Conclusions: There should be used a complex of methods in algorithm of microbiological testing for suspect neuroborreliosis. The results have to be interpreted only in correlation with the biochemical and cytological tests and even with clinical symptoms and epidemiological data to lead to a correct overall clinical diagnosis.

R2161 Impact of CPS ID 3, a new chromogenic medium, on identification and susceptibility results

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Objectives: CPS ID 3 is a new chromogenic medium for the diagnosis of urinary tract infections, enabling the enumeration of micro-organisms, the identification of *Escherichia coli* and *Proteus* as well as the preliminary identification of enterococci and the *Klebsiella–Enterobacter–Serratia–Citrobacter* group (KESC). We have verified that the use of this medium is compatible with the bioMerieux identification and susceptibility testing products API, ID 32, ATB strips and the VITEK system. CPS ID 2, the previous formula, was used for comparison as well as another medium when it was recommended by the products information.
Methods: A set of 60 strains of micro-organisms representative of those commonly encountered in urine and various mechanisms of resistance were streaked on CPS ID 3, CP ID 2 and a reference medium. A 24-h-old plate was used to inoculate API 20E, ID 32GN, Rapid ID 32E, ID 32 Staph, Rapidec Staph, Rapid ID 32 Strep, ID 32 C, VITEK GNI+, GPI, YBC and Rapid ATB UR, ATB UR, ATB G(−), ATB Staph, ATB Strep, VITEK cards GNS 513, GNS 506, GNS 528. Identifications and MICs generated from the different media were compared.
Results: When compared with CPS ID 2 or the recommended medium, identification testing on strips or on VITEK cards is not affected by isolation on CPS ID 3. Agreement rates for each disposable were for API 20E: 80%, ID 32GN: 90%, Rapid ID 32E: 95%, ID 32 Staph: 90%, Rapidec Staph: 100%, Rapid ID 32 Strep: 85%, ID 32 C: 80%, VITEK GNI+: 90%, GPI: 70% and YBC: 100%. Concerning ATB strips and the VITEK cards GNS 513, GNS 506, GNS 528, the MIC agreements were at least 97%. No major discrepancy was encountered. There was no trend to induce a higher susceptibility or resistance for specific species or drugs.
Conclusion: In conclusion, no interference due to the detection of enzymatic activities is observed on identification and susceptibility testing. Moreover the colours obtained and the improved growth considerably reduces the risk of a mixed inoculum. Therefore, the complete solution including CPS ID 3 medium and bioMerieux identification and susceptibility products is perfectly adapted to the isolation of most common uropathogens and even most complex specimens, as it reduces misidentification and consequently time and cost.

R2162 Evaluation of MRSA Select, a new chromogenic medium, for the detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*

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Objectives: MRSA Select is a new chromogenic medium for presumptive identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens as pink colonies after 24 h of incubation. The performances of this new medium and of the oxa-

cillin resistance screening agar base (ORSAB) for detection of nasal carriage of MRSA were evaluated.
Methods: First, a well-defined collection consisting 70 *S. aureus* (29 susceptible to methicillin and 41 MRSA mecA + with various levels of methicillin resistance) and 13 methicillin-susceptible coagulase-negative staphylococci (CNS) was used. A standard inoculum (1 μL of a 0.5 McFarland suspension) was spread onto MRSA Select, ORSAB and oxacillin-salt agar screening (Oxa-Screen) plates. A low inoculum (a 10^{-3} dilution of a 0.5 McFarland suspension) was also tested. Then, 101 nasal swabs were taken from 95 patients at high risk for MRSA carriage and discharged in 1 mL of glycerol–BHI broth. A 50-μL of the suspension was immediately inoculated onto MRSA Select, ORSAB and Chapman salt agar plates. All media were incubated for 24 and 48 h at 37°C. Pink colonies on MRSA Select, blue colonies on ORSAB and mannitol-fermenting colonies on Chapman salt agar were identified by a coagulase test. Suspected MRSA colonies on MRSA Select and ORSAB and five mannitol-fermenting colonies on Chapman salt agar were checked for methicillin resistance by using an Oxa-Screen medium.
Results: With the collection strains at the standard inoculum, sensitivity for MRSA detection on MRSA Select, ORSAB and Oxa-Screen after 24 h of incubation was 95.1, 87.8 and 95.1%, and specificity was 100, 93.3 and 100%, respectively. Using the low inoculum, sensitivity for MRSA Select and ORSAB was 92.7 and 63.4%, and specificity 100 and 97.7%, respectively. Of 101 nasal specimens examined, 19 grew MRSA on at least one medium. As compared with the Chapman-salt agar, after 24 h of incubation, sensitivity for MRSA Select vs. ORSAB was 100% vs. 95.2%, and specificity was 97.6% vs. 83.6%, respectively. Predictive positive values for MRSA Select and ORSAB were 0.90 and 0.54, and predictive negative values were 1 and 0.97, respectively.
Conclusion: In this preliminary study, on the basis of ease of identification of positive colonies, high sensitivity and specificity, and unnecessary complementary coagulase testing, MRSA Select appears to be a promising routine technique for presumptive identification of MRSA in nasal specimens.

R2163 Comparison of four laboratory tests for diagnosis of *Clostridium difficile*-associated diarrhoea

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Objective: To evaluate four assays for the detection of *C. difficile*-associated diarrhoea (CDAD) in clinical practice.
Methods: Over a 20-month period (1/3/2002 to 31/10/2003) 642 liquid or semiliquid stool samples were tested for *C. difficile* by culture and for *C. difficile* toxin A by three immunoassays. Control samples were examined from 50 patients without diarrhoea. *C. difficile* strains were isolated from cycloserine–cefoxitin–fructose agar, supplemented with 5% egg yolk and identified by conventional methods. Toxin A was detected in stool specimens by an ELISA assay (Vidas, bioMerieux, France) and two chromatographic assays [Color Pac (CP); Becton Dickinson, USA and Novitec, Hiss Diagnostics, Germany]. *C. difficile* isolated strains were tested for toxigenicity (Toxigenic culture, TC). The overall efficiency of the tests was compared with results of TC assay.
Results: *C. difficile* was isolated in 45/642 (7%) of stool samples. Toxin A was detected in 43 strains using two assays, so TC was positive in 43/642 (6,7%) of stool specimens. Toxin A was detected in 41 (6.3%), 33 (5.1%), and 25 (3.9%) stool samples using CP, Vidas and Novitec assays, respectively. The sensitivities and specificities of CP, Vidas and Novitec were 77 and 99%, 70 and 99%, and 44 and 99%, respectively. Positive and negative predictive values were 80 and 98%, 91 and 98%, and 76 and 96%, respectively. None of the 50 control specimens was positive by any of the diagnostic tests.
Conclusions: All immunoassays appear useful rapid methods for ruling out CDAD due to high negative predictive value. Of the immunoassays, CP was the most sensitive, followed by Vidas and Novitec but all had high specificity.

R2164 Prevalence of myocardial infarction caused by *C. pneumonia* among residents of Yazd, Iran

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Objectives: Miocardial infarction (MI) is one of the most important death factors in the whole world. Today, it is well known that some bacterial and virus species play a vital role in MI manifestation. *C. pneumoniae* is considered to be such a bacterium. The general purpose of the cross-sectional study was to determine the correlation between the MI and *C. pneumoniae* infection.
Methods: A total of 103 infracted patients along with 75 healthy people as control with the similar demographic status were selected for this study. Following tittering IgG and IgM using ELISA technique for detecting of antibodies against *C. pneumoniae*, all patients' serum was furthering examined for determination of sugar, CRP, cholesterol and triglyceride. Simultaneously, demographic status such as BMI, blood pressure, family history and addiction to cigarettes were recorded.
Results: Results showed that 92 (89.3%) of serum samples from patients and 58 (77.2%) of controls were positive for IgG; whereas only two patient's serum samples were positive for IgM. Prevalence of IgG positive in the patients regarding sex was in significant, although it was found to be significant for age ($P = 0.03$). When the history of heart problem was searched, it was found that the correlation of IgG+ patients with their mother was significant ($P = 0.04$). In general, the relationship of patients with IgG positive with BMI, smoking, chest pain and blood pressure was not insignificant.
Conclusion: Although, the case numbers were limited, it may be concluded that the chlamydial infection is prevalent among the residents in Yazd and the silent infection could be the causative factor in MI.

R2165 Electromigration techniques – rapid methods for the detection and identification of urinary tract pathogens

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Objectives: Rapid detection of micro-organisms is instrumental in the timely beginning of the therapy. Sometimes, especially in urinary infections, it is useful to quantify the micro-organisms in the sample. The capillary electromigration separation techniques, especially capillary zone electrophoresis and capillary isoelectric focusing, seem to be suitable for this purpose. These techniques, based on surface properties of microbes, were recently used for on-line rapid separation, identification and quantification of bacterial cultures. They can be used also for the detection of some of factors of pathogenicity, e.g. the biofilm formation.
Methods: The micro-organisms often causing urinary tract infections, i.e. *Escherichia coli*, *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae* and *Staphylococcus epidermidis* (including biofilm-forming strains), were detected by capillary zone electrophoresis and by capillary isoelectric focusing and their isoelectric point has been assessed. The pyrenebutanoate and nonionogenic tenside based on pyrenebutanoate, which were used as fluorescent compounds, dynamically modified microbial samples and strongly increased the sensitivity of detection.
Results and conclusions: By means of the capillary isoelectric focusing the isoelectric point of particular micro-organisms has been assessed. This technique as well as the capillary zone electrophoresis was successful in the detection of micro-organisms causing urinary tract infections. The identification of respective micro-organisms and their separation has been successful also from the mixed culture and in the presence of human epithelia and proteins as well. The sensitivity of the fluorimetric detection was

10E3–10E4 bacterial cells per ml, which is the limit of clinically significant findings. The study was supported by the grant of Grant Agency of the Academy of Sciences of the Czech Republic IAA4031302.

R2166 Use of time to positiveness to predict the presence of *Staphylococcus aureus* in blood culture with clustered Gram-positive cocci on direct smear examination

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Introduction: Rapid differentiation between *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) in positive blood culture can help clinician to decide to initiate antibiotic treatment.
Objective: To assess the value of time to positiveness of blood cultures with clustered Gram-positive cocci on direct smear examination to predict presence of *Staphylococcus aureus*.
Methods: The time to positiveness of the first positive blood culture was determined using a BACTEC 9240 system in 790 consecutive patients hospitalised in a 1200-bed university hospital over a 2-year period with clustered Gram-positive cocci on direct smear examination. Isolates were identified as *S. aureus* or CNS on the results of the rabbit plasma coagulase test.
Results: One hundred and eighty-five episodes were identified as *S. aureus* and 605 as CNS. Median time to positiveness was 14 (range: 2–115) and 26 h (2–123) for *S. aureus* and CNS, respectively. Response Operating Characteristic (ROC) curve analysis showed that the best cut-off value to differentiate between *S. aureus* and CNS was 18 h, resulting in 74% sensitivity and 86% specificity. Likelihood ratio for the presence of *S. aureus* were 16.4, 7.0, 2.7, 0.6 and 0.2 for time intervals of 2–9, 10–13, 14–17, 1–25 and >25 h, respectively. Given the prevalence of *S. aureus* bacteraemia in the sample studied, the positive predictive value for *S. aureus* was 61.7% using the 18-h cut-off, and reached 83.3 and 74.4% using the 2–9 h, and the 10–13 h intervals, respectively.
Conclusion: Analysis of time to positiveness of blood culture with clustered Gram-positive cocci on direct smear examination is an efficient mean to predict the presence of *S. aureus* and should be included in medical decision criteria.

R2167 Investigation of interleukin 8 and 10 levels and their relationship between prognosis in meningitis

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The most common form of central nervous system infections is acute meningitis and rapidly aetiological diagnosis and treatment are important for prognosis. Detection of cytokines such as TNF-alpha, IL-6, IL-8 and IL-10 in CSF may have diagnostic and prognostic value. Twenty-four patients diagnosed as meningitis according to physical findings, biochemical and microbiological evaluation of CSF were included to the study in a period 19 months. CSF of patients was examined on the first and 10 days. The IL-8 and IL-10 levels in CSF were detected by ELISA. Fourteen patients were diagnosed as bacterial meningitis and 10 patients were diagnosed as viral meningitis. IL-8 levels were found higher in bacterial meningitis than viral meningitis samples obtained on the first and 10th days. The difference between pretreatment and post-treatment levels of IL-8 was significant in 24 patients ($P < 0.05$). Pretreatment and post-treatment IL-8 levels in ABM group were found higher than viral meningitis group ($P < 0.05$). There was no difference found between pretreatment and post-treatment IL-10 levels in 24 patients. Also there was not a difference between ABM and viral meningitis groups' IL-10 levels. There was not a significant relationship between WBC, CSF cell count, CSF CRP, protein, glucose levels and IL-8 or IL-10 levels ($P > 0.05$). The results of the present study showed that detection of IL- 8 levels in CSF is useful for diagnosis of bacterial and viral meningitis. Rapidly etiological diagnosis of meningitis is critically important for treatment. Detection of cytokines in CSF is a part of diagnosis but its cost and accuracy of results are limiting. Detection of serum IL-8 and IL-10 levels may be useful.

R2168 Evaluation of the performance of *Candida* ID2, a new chromogenic agar medium for detection and differentiation of clinically important yeasts

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Objectives: *Candida* ID2 (CID2, BioMérieux, Marcy l'Etoile, France) is a new chromogenic CE-labelled medium, recently developed for the isolation of yeasts from clinical specimens. CID2 allows the direct identification of *C. albicans*, and presumptive identification of some other *Candida* species (*C. kefyr*, *C. lusitaniae*, *C. tropicalis*). The purpose of this prospective study was to evaluate the performance of CID2 in comparison with the two other chromogenic media, *Candida* ID and *Albicans* ID2 (BioMérieux).
Methods: To assess selectivity, fertility and sensitivity of CID2, 294 biological specimens and 42 collection strains were isolated on the three media. After dilution in 1 mL of sterile water, 100 μL of the suspension were plated onto each medium. Media were incubated at 37°C, and red after 24 and 48 h. The inhibition of bacterial growth, the number and the size of yeast colonies, the colouration intensity of blue colonies (*C. albicans*) or pink colonies were appreciated. A specimen was considered positive when yeast grew on at least one of the three media. After 48 h, the pink and white colonies were identified by the standard methods.
Results: (i) CID2 appeared the most selective medium at 24 and 48 h (1.4% of samples showed a bacterial growth) comparatively to *Candida* ID (5.1%) and *Albicans* ID2 (3.1%). (ii) 113 biological samples (38.4%) were positive, and a total of 156 fungi were isolated on at least one of the media: no significant difference of fertility was seen between the three media. (iii) Regarding sensitivity, CID2 was globally superior for intensity of the blue colour at 48 h, but it appears to be lightly inferior for pink coloration, especially at 24 h. Moreover, the blue colour is more homogeneous on CID2 and *Albicans* ID2. Although evaluation of filamentous fungi detection was excluded to the study, they can be reliably detected on the tested media (seven samples positive with *G. candidum*, and 10 with moulds mainly *Aspergillus*).
Conclusion: The new formulation of CID2 clearly improves the selectivity of fungal isolation compared with the previous chromogenic media. This medium gives a great ability to detect mixed cultures: indeed CID2 seems to be equivalent to other tested media for fertility and time of blue colour apparition, but homogeneity of the coloration facilitates greatly the reading of cultures. CID2 allows a direct and rapid identification of *C. albicans*, but other colonies must always be identified by the standard techniques.

R2169 Evaluation of a new chromogenic culture medium CPS ID 3 for urinary tract infections

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Over the past few years chromogenic media have become essential reagents in the bacteriology laboratory, notably for cytobacteriological examinations of urine to provide rapid and reliable organism identification to suggest the appropriate therapy to clinicians.
Objective: bioMérieux has reformulated the CPS ID 2 chromogenic medium (specific to urine specimens) and now offers a new medium, CPS ID 3, which enables increased colour intensity and nutrient capacity for *Escherichia coli*, enterococci and particularly *Proteus* species. Moreover, bacterial species previously exhibiting little or no colouration on the original medium now demonstrate

specific colouration, which can help identify Group B streptococci, and *Pseudomonas aeruginosa*. In addition, the growth of Gram-positive bacteria and yeasts is improved.
Methods: The performance of the new medium was evaluated in our laboratory during a 3-month period. In total, 104 urine samples (including 38 polymicrobial cultures) were tested in parallel on the two media CPS ID 2 and CPS ID 3. Identifications of micro-organisms recovered, were performed with both media, according to current bacteriological criteria for identifying *E. coli*, enterococci and *Proteus mirabilis* and with VITEK 2 cards for the other bacteria. The performance results of both media in terms of growth characteristics (number of different colonies isolated, enumeration, and size of colonies), as well as colony colour intensity, were compared.
Results: In total 149 bacteria were identified as follows: 54.3% Gram-negative bacilli, 38.3% Gram-positive cocci, 5.4% yeasts, 2.0% Gram-positive bacilli. The performance of CPS ID 3 was found to be better than, or equivalent to, the performance of CPS ID 2 in 97.1% of cases, for colony size, in 96.1% for enumeration and 93.3% for the number of different colonies isolated. Moreover, the colour intensity of *E. coli*, *Proteus* and enterococci was significantly increased.
Conclusion: CPS ID 3 provides a practical improvement due to increased colony size, and colour intensity for the main organisms encountered in urinary tract infection, as well as better legibility of the B-glucuronidase and deaminase metabolic activities. Furthermore, the specific colouration observed, for *Strept. agalactiae* and *P. aeruginosa* colonies enables better orientation of diagnosis. Based on the results, this medium can be recommended for use in the bacteriology laboratories for the direct examination of urine specimens.

R2170 Use of sulesomab, a radio-labelled antibody fragment, to detect spondylitis in patients with brucellosis by leukoscintigraphy

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Objective: To evaluate the role of a 99mTc-antigranulocyte Fab' fragments (LeukoScan), the diagnosis of brucellar spondylitis. This radiotracer, tank to its capacity to accumulate in the infectious foci, has been used successfully in the diagnosis of orthopaedic infections.
Patients and Methods: A total of 36 consecutive patients (mean age 52.3; SD 18) with brucellosis were enrolled; of these those with suspected spondylitis underwent Leukoscan and MRI. Leukoscan study was performed 4 and 24 h after the administration of 555 MBq of the radiotracer with dual headed gamma-camera equipped with low energy high resolution collimator. Planar and SPET images of the regional interest were acquired. Leukoscan images were interpreted as positive when uptake in the region of interest exceeds uptake in some reference point and also in presence of photopenia as demonstrated by Palestro. Sensitivity and specificity of Leukoscan were computed taking MRI as 'gold standard'.
Results: A total of 22 with suspected spondylitis underwent MRI and Leukoscan. MRI detected a skeleton involvement in 11/22 cases and showed still other alterations: nine paraspinal abscess, four a psoas abscess and 10 epidural extension of disease. Leucoscintigraphy of the 11 patients positive to MRI demonstrated normal vertebral activity in seven cases, increased in two and decreased in two. One of the two patients with antigranulocyte increased uptake was not correspondence with the skeleton involvement demonstrated by MRI. Leukoscintigraphy of the 11 patients negative MRI demonstrated increased activity in two cases and in none a decreased one. Considering both hyperactivity and hypoactivity, Leukoscan sensitivity and specificity were 27.2 and 81.1%, respectively; negative predictive value and positive predictive value were 52 and 60%, respectively.
Conclusion: Contrary to what we had been longing, Leukoscan was not useful, so we do not recommend its use in the managing of patients with suspected brucellar spondylitis.

R2171 Comparative evaluation of antimicrobial susceptibility testing methods in Indonesia and the Netherlands

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Objectives: This study was designed to comparatively evaluate the disk diffusion method with manual measurement of zones of inhibition as performed in Indonesia and the Oxoid aura image system, an automated image analyzer that measures zone diameters, as performed in the Netherlands for the antibiotic susceptibility testing of bacterial isolates.
Methods: Six hundred and eighty-three isolates, including 294 *Staphylococcus aureus*, 195 *Escherichia coli* and 194 other Enterobacteriaceae, were studied. Bacteria were collected in Indonesia in order to determine the prevalence of antimicrobial resistance in the Indonesian population. For each isolate five or six clinically relevant antibiotics were tested. The disk diffusion with manual zone measurement was performed as recommended by NCCLS in three laboratories in Surabaya, Indonesia and one laboratory in Semarang (Indonesia). The aura image system was used in Rotterdam, The Netherlands. The Vitek 2 system was used as the reference method for the assignment of clinical categories (susceptible, intermediate or resistant).
Results: In comparison with the reference method the disk diffusion with manual zone measurement gave categorical agreements of 1318/1470 (*S. aureus*), 892/975 (*E. coli*) and 859/970 (other Enterobacteriaceae) with an overall agreement of 89.9%. The aura image system gave categorical agreements of 1278/1470 (*S. aureus*), 838/975 (*E. coli*) and 851/970 (other Enterobacteriaceae) with an overall agreement of 86.9%. Very major errors (VME; resistant by reference method, susceptible by the investigated method) by the aura system occurred with 30 of 3415 readings (0.9%), and were mainly caused by oxacillin zone measurements with *S. aureus*. However, on further testing none of these strains harboured the mecA gene. The percentage of VME made with manual measurement was 3.2%. The majority of these were found with ampicillin testing for Enterobacteriaceae. An overall correlation of 0.96 was observed for zone diameters measured manually and measured using the aura image system.
Conclusions: The results of this study indicate that both the disk diffusion method as performed manually in Indonesia and by the aura image system in the Netherlands will yield acceptable susceptibility test results for medically relevant bacteria. The aura image system, however, gave less VME.

R2172 External quality assessment of antimicrobial susceptibility testing in the office of general practitioners

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Objectives: To evaluate a system for quality assessment of susceptibility testing performed on urine specimens in the office of general practitioners.
Methods: Simulated urine specimens with various common urinary pathogens were stabilised with borax and distributed by mail twice a year to general practitioners in Aarhus County. Strains were designated as susceptible or resistant by the general practitioner according to the instructions of the manufacturer of their routine susceptibility assay. Results recorded on an enclosed report form had to be mailed or faxed back to the laboratory within a week.
Results: Twelve distributions were mailed between 1999 and 2002 for quality assessment of phase contrast microscopy, urine stix, culture of urine, and susceptibility testing of urine specimens. In average 50% of the about 170 participating general practitioners reported results on susceptibility testing of the simulated urine specimens. Reports included testing of susceptibility towards sulphonamides (SU), trimethoprim (TP), ampicillin (AP), mecillinam (MC), and ciprofloxacin (CP). For quality control strains sensitive

to SU, TP, AP, MC, and CP, correct results were obtained in 77, 95, 89, 78, and 99% of the assays, respectively. For strains resistant to SU, TP, AP, MC and CP, correct results were obtained in 100, 97, 52, 13, and 48% of the assays, respectively. Repeated distribution of the same control strain showed that e.g. the percentage of correct reports on an *E. coli* strain sensitive to ampicillin and sulphonamides increased from 77 to 94% and from 76 to 96%, respectively.
Conclusion: General practitioners have difficulties interpreting the results of susceptibility testing on strains resistant to AP, MC, or CP. False conclusions about the drug susceptibility may be caused by reading zones without actual measurement of the zone-diameter. Old and dried-out agar media may also cause misleading results. Slight growth in inhibition-zones with disks containing sulphonamides or mecillinam is often interpreted as resistance. However, the reporting to the general practitioner of overall results and comments on pitfalls of the various assays seems to improve the performance on repeated distributions of a quality control strain.

R2173 Utility of 293 cells in culture of enteroviruses

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Objectives: Viral isolation in cell culture remains as a reference method for diagnosis of enteroviral infection. Enteric adenoviruses are cultivated in 293 cells. Enteroviral and enteroadenoviral tropisms for the gastrointestinal tract lead to the assumption that 293 cells would be useful in enteroviral culture. We evaluated usefulness of 293 cells in the diagnosis of enteroviral infection.
Methods: Human embryonic lung fibroblasts (HEL), HeLa, RD and 293 cells were used to evaluate viral isolation from clinical specimens and susceptibilities of the cell lines to ATCC (American Tissue Culture Collection) enteroviral strains. Type 9 echovirus positive 31 stool specimens, type 30 echovirus positive 33 stool and 59 cerebrospinal fluid (CSF) specimens were inoculated onto cell lines.
Results: Of 31 echovirus 9 isolates, 22 (71.0%), 21 (67.7%), 6 (19.4%) and 3 (9.7%) were detected in HEL, 293, RD and HeLa, respectively. Of 33 echovirus 30 isolates from stool specimens, 32 (97.0%) were detected in 293, 17 (51.5%) were detected in RD. Of 58 echovirus 30 isolates from CSF specimens, 39 (67.2%) were detected in 293, 50 (51.7%) were detected in RD. 293 cells were sensitive for coxackievirus A9 ATCC strain and echovirus 7 ATCC strain.
Conclusion: 293 cells are useful in the diagnosis of echoviral and some enteroviral infection.

R2174 Calibration of imipenem and meropenem disk diffusion susceptibility testing

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Objectives: Using single strain regression analysis (SRA) to calculate species related breakpoints and to select the appropriate disc strengths for agar disk antimicrobial susceptibility testing of imipenem and meropenem.
Methods: A total of four reference strains and 20 clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* were tested. MIC values were determined with the help of the agar dilution technique. Disk diffusion tests were performed according to the methodology of the Swedish Reference Group for Antibiotics (SRGA), see www.srga.org. The disk strengths 1, 5, 10, 50 and 100 μg imipenem and 1, 5, 10, 30, 100 and 300 μg meropenem were used for SRA.
Results: The single strain regression lines that were obtained allowed us to correlate MIC-values and zone diameters for the different disk contents. With the current SRGA breakpoints for imipenem (1 and 8 mg/L Enterobacteriaceae, 4 and 8 mg/L *Pseudomonas*) and meropenem (0.12 and 8 mg/L Enterobacteriaceae, 2 and 8 mg/L *Pseudomonas*) all tested disc strengths could distinguish between susceptible (S) and intermediately susceptible (I) strains. However, neither the 1-μg nor the 2-μg disks could distinguish between strains with MICs in the intermediate and the resistant categories.
Conclusion: Our results indicated that the imipenem and meropenem 10 μg disks were unnecessarily strong and that the 5 μg disks for both antibiotics best distinguished across the S-, I- and R-ranges of MIC-values. A disk containing 5 μg imipenem clearly distinguished between strains with and without resistance mechanisms. For meropenem, members of Enterobacteriaceae showed sufficient separation with a 5 μg disk whereas the separation for *P. aeruginosa* was less distinct.

R2175 URICHROM® III, a new chromogenic medium for urinary tract pathogens: a comparative study with UriSelect 4

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Objectives: To comparatively assess the performance of URICHROM® III agar (INTERNATIONAL MICROBIO, Signes, France) and UriSelect 4 agar (BioRad, Marnes la Coquette, France) for the detection, enumeration and direct identification of urinary tract pathogens.
Method: A total of 207 clinical urine specimens, the majority of which were identified by microscopic examination as containing leucocytes and/or micro-organisms, were streaked prospectively, in parallel and in two separate laboratories on the two agar media. Further tests, as well as reading of the enzymatic reactions were carried out according to the manufacturers' instructions.
Results: Of the 207 urine specimens tested, eight were sterile and 199 yielded positive cultures: 119 were pure cultures and 80 were mixed cultures. A total of 212 micro-organisms were considered as clinically significant including 115 *E. coli*, 29 *Enterococcus* spp., 14 Enterobacteriaceae from the group *Klebsiella–Enterobacter–Serratia*, and 14 from the group *Proteus–Morganella–Providencia*, 7 *Pseudomonas* spp., 3 *Citrobacter* spp., 2 *Streptococcus* spp., 1 *Haemophilus parainfluenzae*, 16 *Staphylococcus* spp. and 11 *Candida* spp. For all samples, enumeration of micro-organisms was comparable with the two media. URICHROM® III and UriSelect 4 gave detection rates of 99.5 and 97.6%, respectively. For the direct identification of *E. coli*, the sensitivity was 90.4% for URICHROM® III, which detects beta-glucuronidase activity and 94.8% for UriSelect 4 which detects beta-galactosidase activity. On UriSelect 4, a strain of *Citrobacter freundii*, as well as one of *Enterobacter cloacae* yielded pink colonies similar to *E. coli* colonies, thereby confirming the necessity to perform the indole test on pink colonies. The total identification rates of *E. coli*, *P. mirabilis* and *Enterococcus* spp. were superior to 67% for the two media. This resulted in a marked reduction in workload, as well as significant savings in time. For the presumptive identification of the *Proteus–Morganella–Providentia* group and the *Klebsiella–Enterobacter–Serratia* group, the two media performed equally well. On URICHROM® III, the size of staphylococci, yeast and lactobacilli colonies were slightly superior to those obtained on UriSelect 4, enabling a better detection of the contaminants. An equal inhibition of swarming of *Proteus* spp. and *Pseudomonas* spp. was observed for the two media.
Conclusion: URICHROM® III, is an efficient medium for the diagnosis of urinary tract infections.

R2176 The role of IgG avidity in the diagnosis of toxoplasmosis; a comparative study of four commercially available anti-*Toxoplasma gondii* IgG avidity assays

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Objectives: The IgG anti-*Toxoplasma* avidity test is a confirmatory method that can differentiate between acute and chronic stage of

toxoplasmosis in a single serum sample. It has proven useful in management of pregnant women with anti-*Toxoplasma* IgM and/or IgA antibodies. Our objective was to compare and analyse the results of four commercially available assays to solve the previously experienced differences in the avidity obtained by the different kits.
Methods: Thirty-three samples positive for IgG and IgM and/or IgA anti- *Toxoplasma* antibodies were used in order to examine and compare the bioMérieux 'VIDAS Toxo IgG Avidity' ELFA kit (France), the TEST-LINE 'EIA *Toxoplasma* IgG' ELISA (Czech Republic), the ABOTT '*Toxoplasma* IgG Avidity EIA RADIM' ELISA (Italy) and the FEROL 'DIESSE Enzywell *Toxoplasma* IgG Avidity' ELISA (Italy) with each other. VIDAS and TEST-LINE dilute the sera to 15 and 50 IU/mL, respectively, while RADIM and DIESSE use a 1:300 and 1:100 dilution rates, respectively.
Results: The discrepancies between the avidity results of the different kits varied between 5 and 35%, and the correlation between the results of the different tests varied between $r = 0.37$ and $r = 0.88$. The correlations between the avidity and IgG level of sera were nonsignificant in the case of VIDAS ($r = -0.07$) and TEST-LINE ($r = -0.04$), but there was good correlation in the case of RADIM ($r = 0.56$) and DIESSE ($r = 0.53$). Tests showed no correlation with the IgG level of the sera gave a good correlation with the IgM and IgA level of sera (VIDAS: -0.60, -0.69 and TEST-LINE: -0.69, -0.61, respectively) while the other tests showed a very weak correlation with the IgM and IgA level of sera (RADIM: -0.20, -0.32 and DIESSE: -0.36, -0.34).
Conclusions: The kits with different diluting methods tend to give different avidity. Two kits (RADIM and DIESSE) gave a good correlation between the avidity and the IgG level of the sera, while they showed a weak correlation between the avidity and the IgM and IgA level of sera. The dilution of the sera seemed to be very important in respect to the comparability of the results.

R2177 Comparison of Bactec plus Aerobic/F medium and BacT/Alert FA medium for detection of Gram-negative bacterial strains

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Objectives: The purpose of this study is to compare ability to cultivation of Gram-negative aerobic bacterial strains in the Bactec Plus Aerobic/F and BacT/Alert FA systems.
Methods: All strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, *Salmonella enteritidis*, *Morganella morganii* and *Serratia marcescens*) with aetiological relation were isolated from blood of hospitalised patients in the University hospital Hradec Kralove. Suspensions of bacterial strains in physiological solutions in descending CFU/mL (colonies forming units) concentrations were prepared. Each suspension was inoculated onto the plates with Columbia agar broth (2 mL) and into the bottles in both automated blood culture systems (5 mL per bottle). The colonies (CFU) on agar plates were counted after 24 h of cultivation. All results produced by automated systems were reviewed by inoculating the bottle contents onto the agar plates. The positive bottles were checked for the positivity time to detection (TTD).
Results: TTD depends in both systems on the number of inoculated CFU. All tested specimens grew faster in the Bactec Plus Aerobic/F system. All inoculated Bactec bottles had shorter TTD than BacT/Alert bottles inoculated with the same suspension of bacterial strain. The highest difference was proved in the strain of *Salmonella enteritidis*. The difference of TTD was 3.0 h. *Morganella morganii*, *Serratia marcescens* and *Klebsiella pneumoniae* had similar results, and the difference between investigated systems was not so high. The TTDs were from 2.5 to 2.9 h shorter in the Bactec system. *Escherichia coli* had the shortest TTDs with difference 1.0 h between both systems. Minimal differences of cultivation in both systems had *Enterobacter cloacae*. (difference 0.3 h). The highest TTD had Bact/alert bottle inoculated with only 10 CFU of *Enterobacter cloacae*.
Conclusion: The Bactec Plus Aerobic/F system is better defined for detection of aerobic Gram-negative bacterial strains than BacT/Alert FA Aerobic system. Cultivation in this system is shorter and difference of TTD is from 0.3 to 3 h. Both systems are able to cultivate and to detect very low numbers of inoculated bacterial cells. The TTDs of both systems are very short and both systems have a high potential for successful culture and detection of Gram-negative blood stream infection.

R2178 The value of successive Infecton scans in the management and follow-up of chronic bone and joint infection

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Objectives: The aim of the study was to evaluate the usefulness of successive Infecton scans in the diagnosis and treatment of patients with chronic bone and joint infection.
Methods: We studied 33 patients with chronic osteomyelitis, septic arthritis or both. All patients were submitted to successive Technetium-99m radio labelled ciprofloxacin scans (INFECTON) with vials supplied by St Bartholomew's Hospital, London, at the beginning and at the end of treatment. Infecton positivity was graded in four grades. Clinical evaluation, erythrocyte sedimentation rate (ESR), C reactive protein (CRP) was also recorded. Infecton and clinical outcome were classified as resolution (R), improvement (I), stabilisation (S) and progression (P). Patients ($n = 22$) had a post-treatment follow-up for 4–48 (median 32) months.
Results: Infecton was positive before treatment in 32 patients, which was in concordance (88%) with clinical evaluation (p NS). At the end of treatment resolution of grounds of the infecton was noted to eight patients compared with 15 patients with clinical resolution ($P = 0.05$). ESR and CRP were less sensitive in evaluating therapy. Clinical relapse was noted in 81% of the patients during post-treatment follow-up, when 79% of them had an R or I infecton at the end of treatment.
Conclusions: (1) Evaluation on the basis of Infecton seems to be compatible with initial clinical picture of chronic bone and joint infection. (2) Infecton positivity at the end of treatment implies a probable subclinical inflammatory process. (3) The role of Infecton scintigraphy needs further elucidation.

R2179 POLMICRO 2003 – National External Quality Control Assessment Scheme in Microbiology

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Objectives: The objective of this study was to analyse the results of 2003. Polish National External Quality Assurance Scheme in Microbiology (POLMICRO).
Methods: The following strains were distributed to participating laboratories ($n = 435$) *Enterococcus gallinarum* PM-39, and *Klebsiella oxytoca* PM-38 (ESBL-positive). Each isolate was blind-coded and laboratories were asked to identify the strains to species level and to test for antimicrobial susceptibility. The susceptibility interpretation results were assessed as correct, with minor error, major error and very major error.
Results: Almost all laboratories were able to identify properly *K. oxytoca* strain and 99% classified the strain as ESBL producer. Ninety seven per cent of laboratories reported correct susceptibility – results for gentamicin for *K. oxytoca*. The most difficult iso-

late was *E. gallinarum*, which was properly identified by 68.3% laboratories. The strain was classified as *E. faecium* by 8.7%, *E. faecalis* by 11.2%, and *E. casseliflavus* by 11.7% of laboratories. Most of the laboratories were able to detect vancomycin resistance in *E. gallinarum* PM-39. Ninety six per cent of laboratories reported test results for teicoplanin as sensitive. Over 98% of laboratories properly determined this strain as susceptible to each of aminoglycosides–streptomycin and gentamicin.
Conclusions: The majority of the participating laboratories proved to be reliable and keeping up with high standards of the microbiological diagnostics, which was confirmed by their capabilities to accurately identify species and determine susceptibility of clinically relevant strains.

R2180 Evaluation of a microdilution sensitivity test which uses 384 well plates

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Objectives: Antibiotic susceptibility tests are the most frequently used tests in clinical microbiology. Due to economic pressure new test systems are developed in order to reduce personal and reagents costs. Some of these systems are fully automatic others partly automatic. In this study we want to compare the MICRONAUT system using a 384-well microtitre plate with a conventional microdilution method according to NCCLS guidelines.
Methods: The MICRONAUT system uses vacuum dried antibiotics in a 384-well-plate. The inoculum is applied with an automatic dispenser with a final inoculum of 1×105 cells/μL in 50 μL cation-adjusted Müller–Hinton medium with the addition of 0.025% phytagel. As the comparator we used the microdilution method according to NCCLS guidelines. Evaluation was performed by the comparison of the natural sensitivity of clinical isolates of common pathogens – *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus* – of the first half of 2003. For each species–antibiotic combination meeting the conditions for comparison (at least five-dilution series with a modal value at the third dilution of the naturally sensitive population) two figures are calculated to determine the degree of similarity of the MIC distributions: the modal value and the percentage of strains within the range of ± 1 dilution step of the modal value. This way of evaluation seems more reliable than the use of already evaluated results with S, I and R categories describing errors, major errors and very major errors.
Results: The results are shown in Table 1. In 10 of 15 cases the results match very well with regard to the peak and the width of the MIC-distributions. Only for imipenem and gentamicin and *P. aeruginosa* as well as for vancomycin and *E. faecalis* the NCCLS procedure tends to show lower MIC values than the MICRONAUT system. In contrast moxifloxacin and meropenem, and *E. faecalis* show lower MIC values for the MICRONAUT system. Differences of the modal value never exceed one dilution step. The percentage of values within the range of ± 1 dilution step of the modal value for the above-mentioned exceptions, however, is nearly identical.
Conclusions: In general both methods show nearly identical results. Differences in modal MICs are rare and never exceed one dilution step.

Table 1. Comparison of naturally sensitive populations of selected species: Modal value and percentage of strains within a range of modal value $+/-1$ dilution step derived from the microdilution according to NCCLS standards and those derived from the MICRONAUT system

	NCCLS		Micronaut	
Species/Antibiotic	modal value	percentage (mv+/ − 1)	modal value	percentage (mv+/ − 1)
P. aeruginosa				
Gatifloxacin	0.5	77.7	0.5	77.6
Levofloxacin	0.5	85.2	0.5	79.9
Piperacillin	4	77.7	4	82.8
Piperacilin/Taz	4	78.8	4	80.7
Ceftazidime	2	89.8	2	88.3
Imipenem	0.5	80.9	1	83.5
Gentamicin	1	85.2	2	87.4
E. coli				
Cefuroxime	4	92.9	4	90.1
Tobramycin	1	87.2	1	92.3
E. faecalis				
Moxifloxacin	0.5	93.3	0.25	94.9
Imipenem	1	94.4	1	85.7
Merepenem	4	84.1	2	80.0
Vancomycin	1	92.7	2	97.2
Linezolid	1	99.0	1	98.1
S. aureus				
Linezolid	1	99.0	1	98.1

R2181 Verification of an automised blood culture system via correlation between micro-organism concentration and positive result

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Objective: To determine the correlation between genus, concentration and time required for the determination of micro-organisms growth by BACTEC 9240 blood culture system and to provide the result verification of the blood culture system.
Methods: The study covered 239 strains including Enterobacteriaceae family members, Gram-positive cocci (*S. aureus* and spp., *Enterococcus* spp., beta-haemolytic streptococci), *Candida* spp. and nonfermentative bacteria. For each strain six dilutions changing from 106 to 10 CFU/mL concentrations were prepared and seeded into BACTEC plus aerobic/F blood culture vials with 10 mL human blood. These vials were followed in BACTEC 9240 instrument. Acridin orange staining and subcultures were performed from both positive and negative (after an incubation period of 7 days) blood culture vials.
Results: All the vials containing strains of Enterobacteriaceae and Gram-positive cocci group were flagged positive by the BACTEC 9240 system. For each group, the correlation between the time to detection of growth (TTD) and the bacterial concentration also for each dilution, the correlation between TTD and bacterial genus was found to be statistically significant ($P < 0.05$). Twenty-six of 180 blood culture vials inoculated with *Candida* spp. at different dilutions could not be flagged positive by the system. These were confirmed to be false-negative results by Acridin orange staining and subcultures. In spite of these results, the correlations between *Candida* concentration and TTD; and correlation between bacterial genus and TTD at each dilution were found to be statistically significant ($P < 0.05$). Forty-three of 180 blood culture vials inoculated with nonfermentative bacteria could not be determined to be positive which were also proved to be false-negative results. For nonfermentative bacteria the correlation between bacterial concentration and TTD was not statistically significant ($P > 0.05$), whereas the correlation between bacterial genus and TTD at each dilution was found to be significant ($P < 0.05$).
Conclusion: The result verification of BACTEC 9240 system for -Enterobacteriaceae and Gram-positive cocci has been successfully established. However, verification of system for *Candida* species and nonfermentative bacteria could not be established. Performing Acridin orange staining and subcultures from vials flagged to be negative by BACTEC 9240 blood culture system is recommended.

R2182 An assessment of a rapid immunochromatographic strip assay for the detection of antibodies to human visceral leishmaniasis in serum samples

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The use of a simple immunochromatographic strip assay as a screening test for the rapid detection of antibodies to human visceral leishmaniasis (VL) in serum samples was assessed. A total number of 282 serum samples collected from 52 confirmed leishmaniasis cases, 200 suspect patients and 30 healthy subjects (control group) were screened using the LEISHMANIA Strip quick test, commercially available by Cypress Diagnostics (LEISMANIA Strip). This rapid assay is a qualitative membrane based immunoassay used for the qualitative determination of antibodies to VL in serum. The membrane is precoated on the test line with a novel recombinant antigen (K39) specific for visceral leishmaniasis caused by parasite members of the *L. donovani* complex and on the control line with chicken anti-protein A. During testing the serum sample reacts with the protein A colloidal gold conjugate, which has been precoated in the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with the recombinant VL antigen on the membrane and generates either a red line (positive result) or no line (negative result). During this assessment study the 52 definitely confirmed leishmaniasis cases were tested with the LEISMANIA Strip test and were found positive. The control group was also tested with the same strip test and it was found negative. When the 200 suspect patients' serum samples were tested with the LEISMANIA Strip test they were all found negative; however, 10 of 200 were found positive when the immunofluorescence test (IFA) was applied. Nevertheless, the 10 samples reacted positively with IFA in very low titres, thus definite diagnosis of leishmaniasis was excluded based upon clinical manifestations and laboratory findings. The results of the present assessment study indicate that the LEISMANIA Strip test has the potential to be used as a preliminary screening test and can be helpful especially for laboratories lacking specialised lab equipment.

R2183 The BACTEC LX, a new continuous monitoring, noninvasive and nonsensor technology for determining the growth of micro-organisms in sealed culture reagents

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Diode laser spectroscopy can be used to analyse the gas in a sample volume. This technology has been integrated into a microbial culture detection system. The instruments were constructed to incubate (35°C), agitate and continuously monitored the culture reagents for growth. The culture reagents contain 30–40 mL broth designed to accept clinical specimens (blood, sterile body fluids and processed specimens) for the detection of viable organisms. Tests were performed with aerobic and anaerobic as well media for the detection of mycobacteria. Some tests included BACTECTM Resins for antimicrobial neutralisation. The test organisms included representative strains of enterobacteria, obligately oxidative nonfermentative bacteria, staphylococci, streptococci, enterococci, obligate anaerobic bacteria (bacteroides and clostridia), mycobacteria, yeast and fungi. Control systems included the BACTEC 9000 Blood Culture system and plated media. The test conditions were designed for typical laboratory usage of the product that includes varying specimen volumes, culture delays and specimen types. The system was capable of detecting the presence of metabolising organisms at 104–105 cells/mL. Growth detection was generally equivalent in a kinetic detection mode (monitoring analytic changes during growth). Direct quantitative measurement of carbon dioxide allows threshold detection (monitoring analytic changes after growth) equivalent to kinetic detection. This technology can be applied to any microbial detection system (medical, industrial, or research) in which reagents with an unobstructed culture gas headspace path length can be monitored for changes in gas metabolites.

R2184 Contribution of urinary pneumococcal antigen detection combined with the research of *Legionella* antigen for diagnosis of pneumonia in hospitalised adult patients

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Bacteriological confirmation of pneumonia (PNM) in hospitalised patients is often erratic or belated. Because of importance of prognosis, early adaptation of treatment requires an empirical antimicrobial therapy (generally aminopenicillin and macrolide combination). The starting therapeutic strategy should profit by a fast and reliable test asserting a pneumococcal aetiology . The Binax Now *S. pneumoniae* (BNP) test allows an urinary pneumococcal antigen (UPA) detection using an immunochromatographic membrane assay within 15 min.
We first evaluated the BNP test for 28 patients [median age: 56 (30–98 years)] with pneumococcal PNM proved by culture, and 118 negative control patients [median age: 53 (15–92 years)] without PNM. The sensitivity was 71.4% (85.7% for the 21 bacteraemic PNM), and the specificity was 98.3%; that is consistent with previous published data. The BNP test was then evaluated by testing urine from 158 hospitalised patients [median age: 56 (19–98 years)] with a clinical picture of PNM (community acquired: 90, nosocomial: 68) for whom a research of urinary *Legionella* antigen (Binax Now) was prescribed and was positive for only two cases. Fifty-seven patients (36.1%) were hospitalised in ICU. UPA was detected in 17 cases of PNM (10.8%): 15 among the community acquired (16.7%) and 2 (2.9%) among the nosocomial cases. The pneumococcal aetiology was confirmed by bacteriological samples in 7/17 patients (six by blood cultures). The 10 others showed clinical and radiological features in agreement with a pneumococcal PNM. Among the 141 patients with negative AUP, *S. pneumoniae* was isolated from six of them (two in blood cultures). The Binax Now *S. pneumoniae* test allowed a fast and reliable aetiological diagnosis in 10,8% of hospitalised PNM (16.7% of the community-acquired cases) having a research of urinary *Legionella* (conceiving with severity factors). So it could conduce to an improved adjustment of the starting antimicrobial therapy of hospitalised adult patients with PNM.

R2185 Invasive procedures and aetiological diagnosis in severe pneumonia. A case study of 20 patients

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Objectives: The aim of this study was to improve the diagnostic accuracy in patients with severe pneumonia using an invasive procedure.
Methods: Over a 10-month period, 20 patients (ages 22–85 years) were studied. These patients were admitted to the Pneumology Department, the ICU or to pulmonary rehabilitation with clinical findings of severe community or hospital acquired pneumonia (CAP) (HAP). In conjunction to serological and microbiological diagnostic tests on the blood, urine, sputum (if available) broncoalveolar lavage (BAL) and/or transthoracic fine needle agoaspiration (TTFNA) were performed to identify the pathogens. Granulocytes >50% and a bacterial culture cut-off >104 CFU/mL was accepted in BAL to define infectious pneumonia.

Results: Aetiological diagnosis was possible in eleven of the twenty cases; three polymicrobial pneumonia and eight monomicrobial pneumonia. In four other patients the final diagnosis was noninfectious disease. In the remaining five patients determined as having infectious pneumonia the pathogens were unidentified. The noninvasive procedures were only successful in diagnosing two cases; *Legionella Pneumophila* detected by means of urinary antigen, and CMV disease by means of early viral antigens. Twelve of the 16 BAL and one of the three TTFNA provided diagnostic results.
Conclusions: Despite the low number of patients in the study, the results showed that invasive procedures, in particular BAL was very effective in confirming infectious pneumonia and improving diagnostic specificity.

R2186 Comparison of the BACTEC 9240 automated system and Isolator 10 blood culture tube for the recovery of *Brucella* species from blood cultures

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Objectives: Culture of blood for *Brucella* spp. has historically been performed by broth-based methods and has required prolonged incubation and the use of blind subcultures. Currently available methods that have been advocated include both isolator blood cultures and the use of automated, continuous monitoring blood culture instrumentation. The sensitivity and time to detection of *Brucella* spp. by the BACTEC 9240 (Becton Dickinson) and the Isolator 10 blood culture system (Oxoid) were compared in a prospective study.
Methods: The present study was prospectively carried out in the Ataturk University Medical Faculty Hospital, Department of Clinical Bacteriology and Infectious Diseases, between April 2001 and October 2003. Thirty-seven patients with suspected brucellosis, on the basis of clinical and epidemiological features or serology, were included in this study. The blood specimen was inoculated into a BACTEC 9240 (aerobic blood culture bottle and one was inoculated into an Isolator 10 Microbial Tube. The bottles and tubes were sent to the Clinical Microbiology Laboratories. Bactec cultures were incubated for 7 days; those negative at 7 days were incubated for two additional weeks the blood lysate was dispersed on the surface of trypticase soya agar medium with 5% sheep blood and on chocolate agar plates. When a positive bottle or isolator blood lysate were detected, identification was carried out by conventional methods for *Brucella* spp.
Results: The BACTEC 9240 system detected 21 (56.8%) whereas in the Isolator blood culture system detected only 18 (48.6%) positive cultures. Blood cultures were recovered within 2–4 days (mean time 3.2) in BACTEC 9240 blood culture system, while within 2–5 days (mean time 3.5) in isolator 10 culture system. The two systems were not superior to each other in that of recovering time ($P > 0.05$).
Conclusion: We concluded that BACTEC 9240 blood culture system could isolate *Brucella* spp. little more rapid than The Isolator 10 blood culture tube.

R2187 Evolution of antibodies against LPS from *B. melitensis* 16M and cytosolic proteins from *B. melitensis* B115 in patients with acute brucellosis

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Objetive: To study the serological evolution of specific inmunoglobulins against LPS from *B. melitensis* 16M and cytosolic proteins from *B. melitensis* B115 in patients with acute brucellosis.
Methods: It has been studied sera from 51 patients with brucellosis. The diagnosis of brucellosis was based on CDC criterions. These subjects were followed clinically and serologically for up to 10 months. Sera were taken at the moment of first clinical consultant (baseline sera) and in the 2nd, 4th, 6th, 8th and 10th month after have begun treatment (evolutive sera). All sera were tested by two ELISAs developed by us. One of the ELISA detected specific antibodies against LPS from smooth *B. melitensis* 16M and the other one detected antibodies against cytosolic proteins from rough *B. melitensis* B115.
Results: Each immunoglobulin against LPS showed a different profile (Fig. 1). From the beginning, IgG evolutive curve increased progressively and reached values a 20% higher than baseline sera during controlled period. Evolutive curve of IgM maintained a descendent course along the ten months studied. IgA levels decreased in the first four months and then they maintained them. The evolution of antibodies against cytosolic proteins was similar between the three classes of immunoglobulins (Fig. 2). Along the 10 months of follow-up they obtained values similar to the baseline sera. The curve of evolution of IgG decreased its value until a 20% of baseline sera along 10 months of follow-up.
Conclusions: IgM and IgA levels decreased progressively after have begun treatment while the evolution of IgG against LPS maintained high values during all period of study. This behaviour shows that IgG antibodies are independent of clinical outcome. Antibodies against cytosolic proteins showed little differences between them. Behaviour of IgG against cytosolic proteins was very different to observe against LPS because decreased slightly along the 10 months of follow-up.

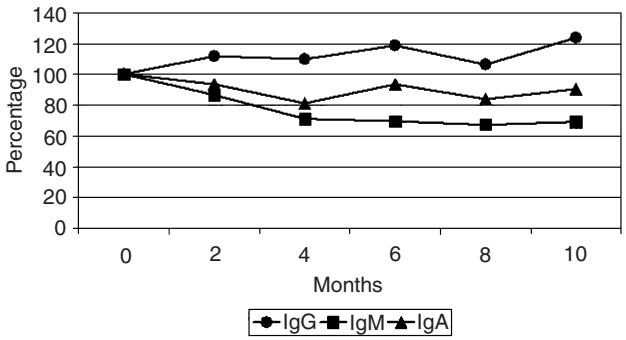

Figure 1: Evolution of antibodies against LPS in patients with acute Brucellosis.

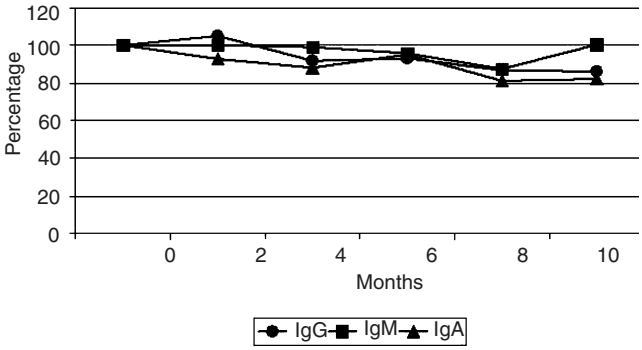

Figure 2: Evolution of antibodies against cytosolic protein in patients with acute Brucellosis.

R2188 The efficacy of urine Gram stain microscopy in predicting urine culture results

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Objectives: The purpose of this study was to compare the accuracy of the microscopic examination of uncentrifuged urine in predicting culture results in a group patient who had urinary tract infection symptoms.
Methods: A total of 200 urine cultures to diagnose or exclude urinary tract infections were included in this study. Urine Gram-stain microscopy was performed using one drop of well-mixed uncentrifuged urine applied to a slide. Gram-stained preparation was examined for at least 20 oil immersion fields with the positive smear having two or more organisms. Urine cultures were performed by inoculating 10 μL of uncentrifuged and well-mixed urine onto blood agar and EMB plates and incubating aerobically at 37°C overnight. Growth of $\geq$100 000 CFU/mL was considered as positive.
Results: Of the 200 urine cultures, 128 were negative, 37 were contaminated, and 35 were positive. Presence of bacteria on Gram-stain predicted 30 of 35 (85.7%) positive cultures. It was also found 56 of 128 (43.7%) negative cultures. The predictive value of a positive test of Gram-stain microscopy of uncentrifuged urine was found 34.88%, the predictive value of a negative test was 93.5%, and the efficiency was 62.6%.
Conclusions: These results suggest that positive cultures cannot be accurately predicted by only Gram-stain microscopy of uncentrifuged urine and that culturing should be performed.

R2189 Designing a new programme for calibration bacteriological loop

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Introductions: Several methods for calibration of Microbiology Loops are available, but colorimetric methods are the most common. The basis of the system consists in the absorbance of light by the dye solution prepared by the loop from a previous dye solution, which is used to delineate a calibration curve. Despite the recommendation of Evans Blue dye for calibration, currently, other dyes for instance methylene blue or crystal violet are being used for this purpose. Therefore, we decided to design a computer program to avoid the time consuming process and the difficulty of calculation and curve delineation; for this reason we made a comparison among Evans blue, methylene blue and crystal violet; the aim was to study the results of the experiments and choose the most appropriate dye for this loop calibration method by using a computer program.
Methods: The first step was to prepare a stock solution of each dye and the second step was to prepare different dilutions from the stock solutions according to the references, and the following step was to evaluate the light absorbance of each dilution in proper wavelengths. Then, we used a disposable and a reusable loop to prepare a final solution from the diluted solutions. Finally, we compared the light absorbance of the final solutions with the light absorbance of a solution made by two standard class A volumetric pipettes (0.01 and 0.001 mL). Evans blue (0.75% stock solution – 600 nm wave length) Methylene blue (0.5% stock solution – 700 nm wave length) Crystal violet (0.05% stock solution – 590 nm wave length)
Results: In comparison with experiments with methylene blue and crystal violet, in the experiment using Evans blue, the light absorbance of final dilutions and standard dilutions showed higher similarity. Furthermore, compared with crystal violet, the results of metheylene blue were more acceptable.
Discussion: Concerning the importance and the frequency of the use of calibrated loop by clinical microbiologists, implementing a computer program will facilitate the calibration process including calculation and curve delineation. Moreover, in comparison with methylene blue and crystal violet, Evans blue is the most appropriate dye for the colorimetric method for calibration of microbiology loop.

R2190 Comparison of stool test (HpSA) with rapid urease test and histology in the diagnosis of *Helicobacter pylori* infection

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Objectives: *Helicobacter pylori* is a curved Gram-negative bacillus colonising gastric epithelial cells. *Helicobacter pylori* is often associated with chronic gastritis and peptic ulcers but besides this its role in the aetiopathogenesis of gastric cancer and primary gastric MALT (mucosa-associated lymphoid tissue) lymphoma makes it more important. Invasive such as demonstration of the bacillus by cytological, histological methods, culture growth, rapid urease test and noninvasive tests such as urea breath test, serology are the methods used for the detection of *Helicobacter pylori* infection. Recently a new noninvasive test detecting *Helicobacter pylori* antigens in the stool samples by EIA was developed. We aimed to determine the sensitivity and specificity of stool test (HpSA) in the diagnosis of *Helicobacter pylori* infection.
Methods: Between May 2001 and October 2003 in the Department of Gastroenterology of Sisli Etfal Training and Research Hospital gastroscopy was performed to previously untreated 102 patients with dyspeptic complaints and the sensitivity and specificity of HpSA (Premier Plarinum HpSATM, *H. pylori* Stool Antigen, Meridian Diagnostic, USA) test was calculated by comparison with rapid urease test and histology.
Results: In our study the sensitivity of HpSA was found 92.2%, specificity 91.2%, positive predictive value 95.2%, negative predictive value 86.1%.
Conclusions: It seems that as the sensitivity and specificity of HpSA was found to be high and it is accurate, simple, noninvasive it has the potential to become the preferred diagnostic tool for *Helicobacter pylori* infections.

R2191 *Helicobacter pylori* infection: comparison of methods of diagnosis

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Objectives: The purpose of our study was to correlate the histological tests for *Helicobacter pylori* (HP) with (a) culture for HP, (b) rapid urease test (CLO-test) and (c) values of anti-Cag A antibodies as well as to correlate culture for HP with (a) CLO-test, (b) treatment with proton-pump inhibitors (PPI) and (c) treatment with nonsteroidal anti-inflammatory drugs (NSADS).
Methods: From June 2001 to June 2002 we studied 67 patients, 37 men and 30 women with median age 57 years old (range 20–84). All our patients underwent upper gastrointestinal gastroscopy and had up to three gastric biopsy specimens taken for culture, histological examination for HP and CLO-test. Also blood samples were taken for the estimation of anti-Cag A. Personal medical history was filled concerning cardiovascular disease, diabetes mellitus, peptic ulcer disease, alcohol consumption, smoking habit, PPI and NSADS intake. All cultures were incubated at 37°C under microaerophilic conditions and high humidity for up to 4 days in Skirrow agar base. Anti-CagA was estimated using ELISA (RADIM, normal value <15 RU/mL). Statistical analysis was performed by SPSS 10.0 program and methods used were: ANOVA and Fischer exact test.
Results: Eighteen of 67 patients were positive for HP in the histological test and 49 were negative, 21 were smokers, 18 were treated with PPI, 24 were treated with NSADS, 24 cultures were positive for HP and 42 were negative, 23 CLO-tests were positive and 44 negative while the mean value of anti-CagA was 22.28 (range 0–260).The following statistically important correlations

were found: (a) anti-CagA values with the histological result for HP($P = 0.007$), (b) histological findings for HP with the culture ($P < 0.001$), (c) histological findings for HP with CLO-test ($P < 0.001$), (d) culture for HP with CLO-test ($P < 0.001$), and (e) culture for HP with NSADS intake ($P = 0.003$). There was no statistically important correlation between the culture for HP and PPI intake ($P = 0.169$).
Conclusions: HP culture (a)is an economic and easy method for the diagnosis of HP infection giving results in accordance with histological examination, CLO-test and values of anti-CagA antibodies, (b) is statistically important correlating with NSADS intake, (c) can play a significant and useful role in the treatment of HP infection if it is followed by an antibiogram, as resistance of HP seems to increase observing therapeutical failures.

R2192 Comparison of identification of staphylococci in BD Phoenix™ and Vitek

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Objectives: Staphylococci are well documented as opportunistic human pathogens. The primary coagulase-positive species, *S. aureus*, has been implicated in numerous and widespread nosocomial infections and has been a major cause of morbidity and mortality. More recently, a growing number of species of the coagulase-negative staphyococci (CoNS) have also been associated with nosocomial infection. These infections are due, in large part, to the increased use of prosthetic and indwelling devices, as well as the growing number of immunocompromised patients in hospitals. Accurate and timely identification of staphylococci is important for a correct depiction of the clinical disease produced by these bacteria. This study examines two automated microbiology systems: BD Phoenix™ Automated Microbiology System (BD Diagnostics), and Vitek (BioMerieux) for their ability to identify staphylococci.
Methods: A total of 100 *S. aureus* and 195 non- *S. aureus* (mostly CoNS) clinical isolates were tested in Phoenix and Vitek I. The reference identification was conventional testing according to the methodology of Dr Kloos. Each system was set up and run according to their respective manufacturer's directions, including off-line testing (catalase and coagulase) for the Vitek 1.
Results: Identification accuracy for all 295 isolates was: Phoenix = 93.6% (276/295), and Vitek 1 = 90.8% (268/295). Each system correctly identified all 100 isolates of *S. aureus* with average time to results (TTR) of: Phoenix = 2.6 h, and Vitek 1 = 5.3 h. The non- *S. aureus* identification accuracy was: Phoenix = 90.3% (176/195), and Vitek 1 = 86.2% (168/195). Average time to results for these isolates came out as Phoenix = 7.0 h, and Vitek = 12.7 h. In this study supplemental testing (to get a single final ID) was not required for any of the *S. aureus* isolates for either system. For the CoNS strains the Phoenix system did not require any supplemental testing, while the Vitek system required supplemental testing for 116 isolates (59.5%).
Conclusion: Overall identification of staphylococci was good in both systems, while Phoenix provided an edge in the identification of non- *S. aureus* species. Time to results and need for supplemental testing were also favourable to Phoenix.

R2193 Detection of fosfomycin resistance in Gram-negative and Gram-positive organisms using BD Phoenix Automated Microbiology System

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Objectives: Fosfomycin (FF), a single-dose orally administrated antibiotic, has been used in therapy for various systematic infections, as well as uncomplicated urinary tract infection. *In vitro* antibiotic susceptibility testing (AST) of FF is limited to agar dilution method with addition of glucose-6-phosphate (G6P) in the agar medium. This study evaluated the BD Phoenix® Automated Microbiology System for the detection of FF resistance among Gram-Negative (GN) or Gram-positive (GP) organisms.
Methods: A total of 173 Gram-negative and 164 Gram-positive clinical isolates were tested. Test battery included species that are clinically commonly encountered. All testing was performed in parallel using the Phoenix System and the NCCLS recommended agar dilution method, Mueller–Hinton agar supplemented with 25 μg/mL of G6P (AD). AD plates were prepared in-house and used within 5 days. Same inoculum of each test strain was used for both Phoenix and AD testing. The results from Phoenix and AD were analysed for Essential Accord (EA) and Categorical Agreement (CA) based on the NCCLS recommended breakpoints (M100-S13) or CA-SFM (Report 2003).
Results: EA and CA for the organism groups tested is listed in the Table below:

Table .

Gram-negative	EA (%)	CA (%)
Citrobacter freundii	100	100
Enterobacter aerogenes	88	88
Escherichia coli	93	97
Klebsiella species	85	96
Miscellaneous enteric	100	100
Proteus species	88	100
Pseudomonas aeruginosa	86	86
Serratia species	100	100
Overall	91	96
Gram-Positive	EA%	CA%
Enterococcus faecalis	94	94
Enterococcus faecium	88	88
Staphylococcus aureus	96	100
Staphylococcus epidermidis	74	100
Overall	91	96

There were two isolates (*K. pneumoniae* and *P. aeruginosa*), which showed very major errors (VME) among GN isolates tested. No VME was observed among GP isolates evaluated. Only 1 isolate *P. aeruginosa* and *E. faecalis* demonstrated major errors.
Conclusions: This study indicates that the Phoenix System provides rapid, reliable AST results for FF against a broad selection of GN or GP organisms.

R2194 Detection of resistance to cefepime in Gram-negative bacterial isolates using the BD Phoenix Automated Microbiology System

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Objectives: Increasing resistance to extended-spectrum cephalosporins is a significant threat to patient care because of limited therapeutic options. The ability to detect resistance in enteric gram-negative bacilli (GNB) to Cefepime, a fourth-generation cephalosporin, using the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, Maryland, USA), a rapid automated ID/AST system, was evaluated in this study. Though the current NCCLS recommendation is to force all *invitro* cephalosporin results to resistant for ESBL-producing strains, the Phoenix MIC results were evaluated for these isolates with and without the BDXpert™ System modification.
Methods: A total of 265 Gram-negative clinical isolates (227 in the family Enterobacteriaceae and 38 glucose nonfermenters), including 123 cefepime-resistant strains, were included in the evaluation. Among these, 50 were ESBL-producing strains based on the NCCLS confirmatory method. Each strain was tested simultaneously in the Phoenix System and the NCCLS-recommended standard broth microdilution (SBM) reference method. Inocula

densities were adjusted to the equivalent of a 0.5 McFarland standard, and then inoculated into both panel types. Phoenix panels were placed into the BD Phoenix instrument for incubation and automated reading to completion. SBM panels were incubated at 35°C for 18–20 h in ambient air and read manually for MIC endpoint determination. Cefepime breakpoints and QC strains were based on those recommended in the current NCCLS standard (M100-S13).
Results: The essential agreement (EA) between Phoenix and the SBM for cefepime was 92.1%, and categorical agreement (CA) was 97.0%. The very major error (VME) and major (ME) rates were 3.3% (4/123) and 2.6% (3/114), respectively. When ESBL-producing *E. coli* and *Klebsiella* species were forced to be resistant by the BDXpert System per the current NCCLS recommendation, the CA increased to 98%. The EA also increased slightly to 94%, and the VME rate was reduced to 1.6%.
Conclusions: The BD Phoenix System provides an acceptable level of agreement for Cefepime results to the reference method, and is effective for use in the detection of resistance to cefepime in Gram-negative bacterial isolates.

Fungal infection

R2195 Iron acquisition from bacterial, fungal and human siderophores by *Candida albicans*

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Efficient acquisition and sequestration of iron bound to bacterial or human siderophores by *Candida albicans* could provide an important mechanism of pathogenicity. A new developed blue agar technique was used for cloning four nonpathogenic mutants of clinically isolated *C. albicans*, which were tested by that technique for their ability to produce siderophore and/or acquisition of iron from other types of siderophore. Only one mutant (M3) of *C. albicans* lacks both the high-affinity and low-affinity transport systems of iron. The nonpathogenic mutant (M3) of *C. albicans* can acquire iron from bacterial, fungal as well as human siderophores. The mechanism of iron uptake by various siderophores by nonpathogenic mutant (M3) of *C. albicans* was investigated. Iron acquisition from bacterial siderophores was glucose independent but concentration contingent, this indicates that simple diffusion is the underlying principle of bacterial siderophore (enterobacterin and pyoverdin) transport. Whereas, the uptake of fungal siderophores (ferricrocin, coprogen), as well as human siderophore (transferrin, lactoferrin, and ferritin) was glucose-dependent indicating they were transported via a high-affinity energy-dependent mechanism. Also the kinetic studies prove that, the affinity of the yeast cells to the bacterial siderophore is considerably lower than that to fungal and human siderophore. However, the rate of uptake of bacterial siderophore is much higher than the rate of uptake of fungal or human siderophore. The evidence suggested that the uptake of hydroxamates-type and ferrichrome-type siderophores could involve specific permease(s).

R2196 The susceptibility of clinically significant *Candida* isolates to fluconazole in the European part of Russia

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Objectives: To assess the susceptibility of clinically significant Candida isolates to fluconazole (FLU).
Methods: These data are interim data of the global antifungal surveillance program ARTEMIS Disk 2003, sponsored by Pfizer, Inc. A total of eight centres participate in Russia with four of them located in the European part of the country. *Candida* isolates were tested using disk diffusion method (DDM) according M44-P NCCLS guidelines. The results were assessed with automated plate reading system (BIOMIC (R)).
Results: From July 2003 through October 2003, a total of 290 strains were tested in the four centres. The material was taking from genitalia (32.4%), upper respiratory tract (19.3%), lower respiratory tract (17.9%), lower gastrointestinal tract (12.0%), urinary tract (6.5%), blood (2.8%). The main locations were: medical/general (35.8%), obstetrics/gynaecology (31.4%), those defined as 'other' (18.3%), medical ICU (6.5%), haematology/oncology (5.5%). Among all isolates the predominant species were *C. albicans* (73.8%), *C. glabrata* (8.6%), *C. parapsilosis* (4.1%), *C. krusei* (3.8%), *C. tropicalis* (2.8%). Other species counted for <1–2%: *C. kefyr*, *C. lusitaniae*, *C. norvegensis*, *C. guilliermondii* and *Candida* spp. The susceptibility data are summarised in Table 1.

Table 1.

	FLU					
Organism	% S	% SDD	% R	MIC_{50} (mg/ml)	MIC (mg/ml)	*n*
C. albicans	98.6	–	1.4	0.57	1.63	214
C. glabrata	72	12	16	5.66	84.45	25
C. parapsibsis	100	–	–	1.2	5.66	12
C. krusei	–	45.5	54.5	36.76	128	11
C. tropicalis	100	–	–	0.59	3.03	8

Conclusions: FLU remains active in vitro against all *Candida* species except for *C. glabrata* and especially for *C. krusei*. The surveillance programs such as ARTEMIS Disk 2003 are useful in determining epidemiology and susceptibility of different types of micro-organisms including fungi.

R2197 Assessment of species distribution and antifungal susceptibility of yeasts and moulds isolated in a dept. of microbiology and clinical microbiology deep mycoses laboratory, Cerrahpasa: increase of non-*albicans Candida* incidence over a 5-year period

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Objectives: The aim of this study is to characterise the species distribution and antifungal susceptibility patterns of yeasts and moulds isolated at a Turkish University Hospital deep mycoses laboratory between 02 January and 18 December 03 and to detect trends in species distribution by assessing the annual frequency in comparison with the findings since 1999 to date.
Methods: Totally 272 specimens (8 CSF, 6 corneal, 3 ear, 2 nose, 59 sputum, 109 BAL, 6 pleural, 8 oral, 1 gastric juice, 1 eusophageal, 4 feces, 10 urea, 5 vaginal, 1 pericardium, 11 hemoculture, 12 blood, 6 pus, 20 tissue biopsy) submitted from different units were studied. Yeast and moulds were identified by classical mycological methods. Significance of the isolates was assessed according to EORTC criteria. Antifungal susceptibility tests were done by NCCLS M27-A and M38-P methods.
Results: A total of 43 yeast and four moulds were isolated. *C. albicans* strains were 30.2% (13/43), non- *albicans Candida* species were 67.4% (29/43) and non- *Candida* yeast 2.3% (1/43). The moulds isolated were *Aspergillus niger* 50% (2/4), *Geotrichum capitatum* 25% (1/4), hyalohyphomycete 25% (1/4). No growth was observed in one antifungal receiving patient's material despite of microscopical positivity of fungal elements. *Aspergillus* latex aglu-

tination (LA) test was found positive in four patients and *Candida* LA in one patient. *G. capitatum* was isolated from a case of spondylodischitis. MICs of fluconazole (FLZ) were found mostly (63.6%) in S-DD cathegory. *In vitro* resistance to itraconazole (ITZ) (MIC higher than or equal to 1 μg/mL) as per NCCLS criteria was observed in five *C.tropicalis* (11.9%), resistance to amphotericin B (AMB) (MIC higher than or equal to 1 μg/mL) in three *C. tropicalis* (7.1%) isolates. Both *A. niger* srains showed high MIC values to azoles. *Invitro* MICs of *G. capitatum* isolate was found as 0.25 for AMB, 32 for FLZ, 8 for ITZ, 4 for ketaconazole and 0.5 for terbinafine.

Conclusions: Non-*albicans Candida* spp. increased from 29.5% (in 1999–2002) to 86% (in 2002) and maintained to show high prevalence in 2003. Rare opportunistic moulds other than *Aspergillus* are commonly encountered in deep mycoses suspected patients' routine materials as previously shown in our lab. A casual link between azole resistance and increase of non- *albicans Candida* spp has been suggested. Our five years data could guide and serve as a baseline for future choice of treatments and/or prophylaxis in our hospital.

R2198 *Candida* Non-*albicans* oral isolates susceptibility patterns to various antifungal agents

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Purpose: To evaluate the incidence and susceptibility to antifungals of other species of *Candida* than *Candida albicans*, recovered from Romanian population.

Materials and methods: Fifty-three oropharyngeal samples were included in the study. For identification, the colony appearance on Chromagar *Candida* medium, chlamidospores production and substrate assimilation profiles with API 20 C aux system were used. The fluconazole (FL) susceptibility assessment was conducted using Etest method and ATB Fungus (bio Merieux, France), was used to assess for susceptibility to six antifungals: nistatine (NIS), miconazole (MIC), econazole (ECO), ketoconazole (KET), amphotericine B (AMB) and 5-flucitosyne (5FC).

Results: From 53 oral samples included in this study 65 *Candida* spp. isolates were recovered: 41 (63%) *C. albicans* and 24 (37%) other species. nine (13,8%) *C. krusei*, 4 (6.1%) *C. kefyr*, 3 *C. glabrata*, 2 *C. dubliniensis*, 2 *C. norvegensis*, 1 *C. inconspicua*,1 *C. lusitaniae*, 1 *C. magnoliae*, 1 *C. tropicalis*. From all non-*albicans Candida* isolates 12(50%) were recovered in mixture with *Candida albicans*. The antifungal susceptibility patterns for fluconazole were 54.1% (13) susceptible. 29.1% (7) S-DD, and 16.6% (4) resistent 9 all *C. krusei* isolates). 91.6% from all non-*albicans Candida* isolates were susceptible to ketoconazole, 75% to miconazole, 66.6% to econazole, 62.5% to 5-flucitosyne, 50% to nystatine and 45.8% to amphotericine B. The rest of isolates presented intermediate susceptibility to these agents. No isolates were resistant.

Conclusions: The co-existence of *C. albicans* with other species of *Candida* is quite important (12.3% from all samples), and some problems in therapy could arise. Ketoconazole followed by fluconazole seem to be the most reliable antifungals from all drugs tested against non- *albicans Candida* species recovered from Romanian population.

R2199 *Paecilomyces variotii* fungaemia in a patient with multiple myeloma, case report and literature review

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Background: Infectious complications are common in patients with multiple myeloma. However *Paecilomyces variotii*, a common saprophytic fungus, rarely causes human infection. We report the first case of *P. variotii* fungaemia in this illness with good response with adapted antimycotic treatment. A 67-year-old woman with stage IIIA IgA*f* myeloma was admitted at hospital in March 2002 for fever, weight loss and anorexia. Myeloma with high tumour burden (increased *f*2 microglobulinaemia level, many and significant lytic bone lesions) was diagnosed in January 2001. In March 2002, she presented fever for a long time and chills. Cultures of blood samples from central catheter and peripheral veins were positive for *Paecilomyces variotii*. No infection localisation was found. A treatment by liposomal amphotericin B was performed during six weeks with good response. Unfortunately, in May 2002, his myeloma relapsed fulminantly with many and progressive plasmacytomas with cutaneous, facial and vertebral localisation but without increase in bone marrow plasmacytosis. Despite chemotherapy by melphalan plus dexamethasone and radiotherapy, her disease progressed and she died 3 months later. Paecilomyces is a cosmopolitan filamentous fungus, which inhabits the soil, decaying plants, and food products. Some species of *Paecilomyces* are isolated from insects. *Paecilomyces* species can cause various infections in humans. These infections are occasionally referred to as paecilomycosis. Corneal ulcer, keratitis, and endophthalmitis due to *Paecilomyces* may develop following prolonged contact lens use or ocular surgery. *Paecilomyces* is among the emerging causative agents of opportunistic mycoses in immunocompromised hosts. Direct cutaneous inoculation may lead to these infections. A review of the literature suggests that infection with this fungus can cause substantial morbidity and is probably best treated with aggressive antifungal therapy and vigilant observation for complications. However despite adapted antimycotic therapy, the infection can be rapidly fatal

R2200 Catheter-associated funguria: identification and antifungal susceptibility

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Objectives: Recently, fungal urinary tract infection (UTI) represents a high-risk event. In the current study we aimed to determine the incidence of fungal infection associated with indwelling urinary catheters and to assess yeasts profiles to antifungal agents.

Material and methods: Urine specimens from 105 adult inpatients with indwelling urinary catheters (group I: 45 intensive care unit (ICU) patients, group II: 60 patients admitted to urology department) were examined. Isolation of yeast strains was done by culturing urine specimens on conventional media. Identification of isolates was performed by using CHROM agar *Candida* medium and API20 C AUX Kit. Susceptibility of different yeast species to different antifungal agents had been evaluated using candifast test and *E*-test.

Results: The overall prevalence of fungal infections was 24% with highest incidence among ICU patients (40%). *Candida albicans* (*C. albicans*) (33.3%) was the most commonly isolated species among ICU patients, while *C. glabrata* (28.5%) was mostly isolated in urology department. *Candida* species represented a higher isolation rate of 78.5% than other species of *Trichosporon* (14.3%) and *Cryptococcus* (7.2%). Amphotericin B proved to be an effective agent to most of yeast isolates with an overall sensitivity (93%) by candifast test and with MIC less than or equal 2 μg/mL by *E*-test. Seventy one per cent of isolated *C. albicans* strains were resistant to fluconazole with high value of MIC90.

Conclusion: In conclusion, *Candida* species, especially non-*albican* species and other yeasts were prominent causes of funguria associated with indwelling catheters. By virtue of recorded resistance to fluconazole, correction of iatrogenic risk factors, such as removing of unnecessary antibacterials and changing or removing indwelling catheters with alkalanisation of urine may constitute an initial treatment of fungal UTIs. Periodic repeated studies may establish identification of emerging species and their antifungal susceptibility to minimise the risk of antifungal resistance and accordingly, achieve better control of fungal infections.

R2201 Investigation of fungi in bronchoscopically acquired specimens of patients with pulmonary disorders

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Objectives: The aim of our study was to determine the incidence of fungi in bronchoscopic samples of patients with pulmonary disorders
Methods: Between June 2001 and 30 April 2003 we obtained 162 bronchial lavage (BL) specimens from totally 157 patients. Fungi were identified according to classical mycological methods and assessed by EORTC criteria.
Results: Underlying disease and predisposing factors were lung and organ cancers (99), past or active tuberculosis (20), pneumonia (19), interstitial lung disease (10), bronchiectasis (7), Hodgkin lymphoma (5), sarcoidosis (3), rheumatoid arthritis (2), KML (1), Behcet's disease (1), *Pamphigus vulgaris* (1), others (7), long-term antibiotic usage (37), corticosteroids (13). Age range was 16–85 years. Fungi isolated were 8 *C. albicans* and 16 non-albicans *Candida* (5 *C. tropicalis*, 8 *C. parapsilosis*, 3 *C. kefyr*), 1 *Trichosporon* sp, 1 *Aspergillus niger*, 1 *A. versicolor*. Of these patients one with previous tuberculosis story was diagnosed as aspergilloma due to *A. niger* with clinical and radiological findings and was proven by culture of operation material. In one symptomatic patient three yeast strains were isolated together as *C. albicans*, *C. tropicalis* and *Trichosporon* sp. suggesting a mixed infection. Other *Candida* isolates were assessed as oral flora contamination or bronchial colonisation mostly due to previous antibiotic usage, because of the lack of evidence of pulmonary candidiasis.
Conclusions: Reported estimates of diagnostic utility of BL have varied because of differences in patient populations, types of mycoses, diagnostic criteria and study methods. *Aspergillus* and *Candida* were frequently identified in BL specimens but were eventually proved to be pathogens in approximately 25 and 0% of cases, respectively. In our study *Candida* were assessed as possible pathogen in 1.3% (2/157) of cases. Previous studies that included neutropenic patients showed that *Candida* sp. isolation from BL samples can represent an infection on some occasions. The outstanding finding of our study was that *Candida* sp. isolation from respiratory samples obtained through broncoscopy in non-neutropenic patients is of no clinical relevance in most cases however saprophytic fungi can be isolated as causative agents. The combined evaluation of clinical information, radiographical findings and mycological examination of BL samples offers a useful approach to the diagnosis of respiratory mycoses due to opportunistic fungi.

R2202 Antifungal susceptibilities and 25S intron genotypes of *Candida albicans* pharyngeal isolates of oncology patients

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Objectives: *Candida albicans* is the leading aetiological fungal agent in immune compromised hosts. *C. albicans* strains are divided into three genotypes (A, B, and C) by 25S intron analysis on the basis of the presence of a transposable group-I intron on the gene coding for the 25S rRNA. Different genotypes are shown to have different susceptibilities to antifungal agents, especially to flucytosine.
Methods: In this study, we determined the antifungal susceptibilities and 25S intron genotypes of 46 *C. albicans* strains isolated from the pharyngeal specimens of oncology patients with solid tumours. Antifungal susceptibilities were determined by microdilution method and genotypes were determined by PCR using the primers CA-INT-F (5′-ATAAGGGAAGTCGGCAAAATAGATCCGTAA-3′) and CA-INT-R (5′-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3′).
Results: A total of 21 (45.7%) of the 46 isolates were found to be genotype A, 6 (13%) genotype B and 19 (41.3%) genotype C. Results of antifungal susceptibilities are shown in table. No significant relationship was observed between the genotypes and antifungal susceptibilities.

Antifungal Agent	Genotype	S	I	R
Ketakonazole	A	21	–	–
	B	6	–	–
	C	19	–	–
Flukanazole	A	21	–	–
	B	6	–	–
	C	19	–	–
Itrakonazole	A	10	7	**4**
	B	4	2	–
	C	12	6	**1**
Amphotericine B	A	20	1	–
	B	6	–	–
	C	19	–	–
Flucytosine	A	18	3	–
	B	6	–	–
	C	17	2	–

Conclusions: Genotype C was found at a higher frequency among these isolates. Our previous studies showed that among colonising isolates genotype C was found at a higher frequency. Although in previous studies genotype A was found to be more resistant to flucytosine, in our study group we found no significant difference.

R2203 The first isolation and identification of clinical isolate *Candida dubliniensis* in routine microbiological laboratory in Slovakia

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Objectives: This study presents the first isolation and identification of *Candida dubliniensis* in Slovakia. This *Candida* was isolated from 24-year-old Slovak woman with inflammation of women pan organs caused by mix-infection with *Candida albicans* and *Candida glabrata*. The next inquiry after 3 months was positive for *Streptococcus agalactiae* and *C. albicans*. In July 2003, this woman was positive for *Escherichia coli*, *S. agalactiae* and *Candida* sp. The dark and light green colonies on CHROMagar *Candida* and the different morphology of chlamydospores indicated the presence of two different *Candida* species.
Methods: Clinical *Candida* isolates were cultivated on CHROMagar *Candida* (Becton Dickinson, UK). Identification was performed with commercial biochemical set API 20C AUX (bioMérieux, France), germ-tube test in human serum, growth at 42 and 45°C on Sabouraud-dextrose agar, assimilation of sugars (D-xylose and alpha-methyl-D-glucoside) performed by glass-tube test and the production of chlamydospores. These tests were completed by PCR assay using set primer pair (Cd-oligo2/F and Cd-oligo2/R) specific only for *C. dubliniensis*. Standard *C. albicans* CCY 29-3-112 and *C.dubliniensis* CBS 7987 were used as control strains. Susceptibility to antifungal drugs clotrimazole, fluconazole, nystatin, miconazole, pimaricin-natamycin and itraconazole was tested by disc diffusion test.
Results: The combination of the mentioned tests confirmed infection of this woman with both *C. dubliniensis* and *C. albicans*. The patient was treated with the combination of miconazole/metronidazole. After 4 months, when the first isolation of *C. dubliniensis* was examined, the inquiry for *Candida* presence was repeated. The vaginal swab was positive for *E. coli*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *S. agalactiae* and *C. albicans*, but it was negative for *C. dubliniensis*. All clinical *Candida* isolates were susceptible to clotrimazole, fluconazole, nystatin, miconazole, pimaricin-natamycin and itraconazole.
Conclusion: We report the first isolation of *Candida dubliniensis* from patient in the Slovak Republic.

R2204 Investigation of reidentified *Trichoderma longibrachiatum* strains

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Objectives: *Trichoderma* species are common soil-borne fungi. Although they are mainly saprophytic organisms, they have been reported to cause opportunistic infections in immunocompromised humans with increasing frequency. The most of these clinical isolates are belonging to the *T. longibrachiatum* species. To gain information about their biochemical properties and to detect variability among them we collected data about the available *T. longibrachiatum* strains. This study was designed to supplement these data with the examination of three further clinical isolates, reidentified with molecular methods as *T.longibrachiatum* species.
Methods: The influence of temperature and pH on growth was studied on minimal agar medium. The utilisation of compounds as carbon and nitrogen sources was investigated on microtitre plates. Haemolytic abilities were detected in blood agar. The extracellular protease activity profiles of the strains were obtained by *p*-nitroanilide substrates after column chromatography. *In vitro* antifungal susceptibilities were determined by *E*-test method.
Results: All the strains were able to grow on minimal media at temperatures ranging from 10 to 40°C, with an optimum at 30 or 35°C. The pH ranging from 3.0 to 7.0 supported the growth, with an optimum at pH 4.0. From the investigated compounds some served both as nitrogen and carbon sources. The test in blood containing agar revealed that all the strains secrete haemolysing enzymes. Both trypsin- and chymotrypsin-like protease activity profiles suggest the presence of several isoenzymes. The *in vitro* antifungal susceptibilities of the isolates were as follows (in μg/mL): 2.0–3.0 to amphotericin B, 64–256 to fluconazole, 2–32 to itraconazole and 0.25–2.0 to ketoconazole.
Conclusion: The aims of the investigation were to gain information about the properties of clinical *T. longibrachiatum* strains. As it was revealed, all the three reidentified *T. longibrachiatum* strains have the ability to grow at elevated temperatures, to tolerate neutral pH, to utilise compounds both as carbon and nitrogen sources and to produce extracellular proteases and haemolytic enzymes. All of these features can promote their growth as facultative human pathogens; moreover their low susceptibility to some antifungal drugs may cause difficulties in the medical treatment. The work was supported by grant F037663 of the Hungarian Scientific Research Fund.

R2205 Fluconazole susceptibility in yeasts and nosocomial candidaemia: a south-west England tertiary hospital experience

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Background: Nosocomial candidaemia is a significant cause of morbidity and mortality in the hospitalised critically ill patients. Reduced susceptibility of some *Candida* species to fluconazole is widely reported. A 3-year clinical audit was conducted across two tertiary care hospitals (Bristol Royal Infirmary and Bristol Children's Hospital) in southwest England to study the distribution of *Candida* species and antifungal susceptibility profiles (AFST).
Methods: Blood culture yeast isolates and antifungal susceptibility profiles over 3-years.
Results: A total of 39 yeast isolates in first blood culture over 3-years. *Candida albicans* (51.28%), *C. parapsilosis* (28.2%), *C. glabrata* (15.38%), *C. guilliermondii* and *C. lusitaniae* (2.56% each). All isolates were sensitive (*in vitro*) to amphotericin B, fluconazole and flucytosine. 16.6% of *C. glabrata* were susceptible to itraconazole. 70% of yeast isolates were obtained in patients from intensive care units.
Conclusion: *Candida. albicans* was the commonest cause of nosocomial candidaemia. All isolates were uniformly susceptible to amphotericin B, fluconazole and flucytosine (except reduced susceptibility of *C.glabrata* to itraconazole). No reduced susceptibility to fluconazole was evident. Amphotericin B and fluconazole are the current therapeutic options for newly diagnosed nosocomial candidaemia.

R2206 Fungal contamination of soft contact lens and conjunctivitis

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Caracas, VE

Objectives: Fungal contamination of contact lenses is a common occurrence, but *Aspergillus fumigatus* is not a frequent identified aetiological agent in these cases. For this reason we report a case of fungal contamination of soft contact lens and bilateral conjunctivitis due to *A. fumigatus* in a immunocompetent patient.
Methods: Case report and review of literature. Patient was ophthalmologically evaluated. Cultures were made from conjunctival fluid and from contact lens surface and liquid. Bacteriological and mycological cultures were realised.
Results: A male patient, 20 years old, with long-time bilateral myopia, using soft contact lens for 8 years, referred 3 weeks ago inflammation and secretion in both eyes. Clinically impress as a bacterial acute conjunctivitis. Samples were taken and initial treatment with topic tobramycin and dexametasone was prompted. Discontinuation on use of contact lens was advised for a couple of weeks. Contact lens cultures on Saboraud were positive for identification of *A. fumigatus* (both lens). Due to clinical patient improvement and negative results for cultures of conjunctival fluid no additional treatment was provided.
Conclusion: There are few articles in literature about contamination and infection related to contact lens use due to *Aspergillus fumigatus*. This patient had no apparently additional risk factors for this infection, he is immunocompetent and he is not working in a risky environmental area. We concluded that probably contact lens liquid was not appropriated in regard to its antimicrobial activity. However, for an appropriate potential therapy we have not commercially available any topic ocular antifungal drug. This report also emphasise the importance of contact lens products containing preservatives for lasting protection from micro-organisms, especially those that could potentially produce even keratoconjunctivitis and other systemic complications.

R2207 Fungal infection of the bile from the patients undergoing bile ductus surgery in a department of general and liver surgery, Warsaw

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Objectives: The study comprised the patients undergoing surgery in Department of general and liver Surgery, Medical University of Warsaw during 3 years (2000–2002) due to the gall bladder and bile ductus pathology.
Methods: Fungal strain were cultured from the following clinical specimens: bile and swabs from the drains of the bile ductus, taken before and after operation. Samples were inoculated into Sabouraud's medium with chloramphenicol and gentamicin. Cultured isolates were identified using Candi Select (Biorad) media and ID32C and API *Candida* tests (bioMerieux). Susceptibility to antifungal agents was tested using an *E*-test system (AB Biodisk).
Result: In total 81 specimens were cultured (in 2000 – 11; in 2001 – 31; in 2002 – 39). Of them 87 strains of yeast-like fungi were isolated. *C. albicans* was the most commonly isolated species: 42 strains (48.4%), followed by *C. glabrata* – 13 strains (15%) and *C. inconspicua* – 13 strains (15%), *C. tropicalis* – 7 (8.1%), *C. krusei* – 4 (4.7%), *C. parapsilosis* - 1 (1.1%), *C. guilliermondi* – 1, *C. valida* – 1, *C. holmii* – 1, *Zygosaccharomyces* spp. – 1, *C. famata* – 1, *C. sake* – 1, *Saccharomyces cerevisiae* – 1. *C. albicans* strains were sensitive to

amphotericin B and fluconazole. All strains of *C. glabrata* and *C. inconspicua* were susceptible to amphotricin B and itraconazole.
Conclusion: *C. albicans* was the fungal species mast frequently isolated from the bile of surgical patients. *C. glabrata* and *C. inconspicua* were isolated with a high frequency (15%).

R2208 *Candida* spp. in urine specimens of a general hospital's patients

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Purpose: To study the presence of *Candida* spp. in the urine sediment of patients treated in a general hospital.
Methods: During a 6-month period (May–October 2003), 3520 urine samples from patients equal in number, 1882 men and 1638 women, were examined in the microbiological laboratory of our hospital. From them 80.7% were hospitalised patients and 19.3% outpatients. Chemical analysis and microscopic examination of fresh preparation of urine sediment was performed in all specimens. Quantitative counting of white blood cells (WBC) and blastoconidia as well as search of existence of pseudohyphae characteristic of *Candida* spp. was done in the urine sediment.
Results: Of the 3520 patients examined, presence of *Candida* spp. was observed in the urine sediment of 213 patients (6.05%). The percentage of men with *Candida* was 4.8% and women 7.5%. The bigger percentage of patients with *Candida* spp. were treated in the Intensive Care Unit (13.7% of patients in ICU had *Candida* spp.) and followed cardiologic clinic (8.4%), pathological (8.0%), surgical (6.2%), nephrologic (4.2%), urologic (4.0%) while the 1.9% of exterior patients had *Candida* spp. in the urine sediment. From the 213 patients with *Candida* spp., the 42.7% had <5 WBC per high power field (hpf), while the 57.3% had >5 WBC per hpf. In addition 33.8% had >15 WBC per hpf, while 17.4% had >50 WBC per hpf. In the 25.8% of patients with *Candida* spp., casts were observed in the sediment of urine. From the 123 women with *Candida* spp., the 65.9% had in the examined samples seldom squamous epithelial cells while the 34.1% had a lot of squamous epithelial cells, indicating vaginal contamination.
Conclusion: Urine sediment with pseudohyphae and blastoconidia of *Candida* spp. is a frequent problem of hospitalised patients. A majority of them present infection from *Candida* spp. as this appears from the intense pyuria and presence of pseudohyphae. To reduce this problem, restriction of abuse of antibiotics is recommended and to receive concern for improvement of conditions of individual hygiene of hospitalised patients.

R2209 *Rhodotorula rubra* fungaemia in an immunocompetent patient successfully treated with fluconazole

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Introduction: *Rhodotorula* is an ubiquitous yeast of the family Cryptococcaceae. The genus contains eight species, with *Rhodotorula rubra* the most common cause of human disease. Rhodotorula infection, although rare, can be serious and usually occur in immunocompromised hosts. The yeast is usually resistant to fluconazole and is associated with considerable. A very limited number of Rhodotorula rubra infections has been reported in immunocompetent hosts.
Objectives: To describe a rare case of *Rhodotorula rubra* fungaemia in an immunocompetent patient successfully treated with fluconazole.
Case Report: A 64-year-old man was admitted to the hospital because of altered consciousness and disorientation. The patient suffered of hypertension and coronary heart disease and had a history of COPD. His condition initially was evaluated as a case of diuretic therapy induced electrolyte disturbances and dehydration. He was afebrile. Treatment with normal saline infusion with potassium replacement was started. He regained consciousness on day 3 of hospitalisation. On day six blood cultures yielded a methicilin-resistant *Staphylococcus aureus* and the patient was treated with i.v. vancomycin 1 g b.i.d. for 4 weeks. On day 12 of hospitalisation the patient's temperature rose again to 38.5°C. New blood cultures yielded *Acinetobacter baumani* and imipenem was added to regimen. He became afebrile on day 8 of imipenem treatment. On day 25 of hospitalisation his temperature rose again to 39°C and new blood cultures yielded *Rhodotorulla rubra* sensitive to fluconazole. The patient was put on i.v. fluconazole and responded on day 2 of fluconazole treatment he continued to improve and became afebrile on day 5 of fluconazole. Blood cultures remained negative. The patient continued to receive oral fluconazole 200 mg bid at home for 1 month. He was seen in the outpatient clinic 1 and 2 months later in excellent condition without any evidence of infection.
Conclusions: *Rhodotorulla rubra* infection, although rare, can be seen in immunocompetent patients. Prolonged treatment with broad spectrum antibiotics can be the predisposing factor.

R2210 Epidemiology of *Candida* strains in a Greek university infectious diseases (ID) centre

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Objectives: *Candida* sp. is emerging as an important pathogen or coloniser in hospital settings. An alarming shift towards non-albicans strains non susceptible to fluconazole has been noted in several studies complicating the selection of empirical antifungal treatment. Aim of the study was to survey the epidemiology of *Candida* strains isolated from various body sites in a Greek tertiary University ID Centre.
Methods: Strains isolated and recultured on Sabouraud dextrose 2% agar underwent an identification procedure including the combination of: germ tube test, API TEST, inoculation on mycosel agar and study of colony morphology cultured on corn-meal agar. Strains were isolated either from hospitalised patients or from patients referred to our ID outpatient clinic.
Results: Between 2001 and 2003, 145 *Candida* strains were isolated from various body sites (27% vaginal, 23% oral, 23% urine, 9% sputum, 3% bood, 5% pus specimens). Sixty-two per cent were *C. albicans*. The most common non-albicans strain was *C. glabrata* (20%). Isolation per specimen showed that *C. albicans* was isolated from 61% of vaginal, 58% of sputum, 29% of pus, 88% of oral, 48% of urine, 75% of blood and 38% of other (mostly tissue) specimens, respectively.
Conclusion: Non-albicans strains are emerging as common *Candida* isolates, especially among isolates of nosocomial origin or from recurrent vaginitis specimens. Identification to the species level must always be performed and concern should be raised about the overuse of antifungal agents both in hospitals and the community.

R2211 Melanin production in *Cryptococcus neoformans* strains isolated from patients with AIDS

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Objectives: *Cryptococcus neoformans* is an encapsulated yeast which causes infection predominantly in immunocompromised individuals. Cryptococcosis occurs in 6–8% of patients with AIDS. The unique predilection of *C. neoformans* for the brain has been attributed to the presence of high concentrations of catechol precursors that can serve as substrate for the fungus laccase enzyme. The laccase (CNLAC1) catalyses the synthesis of polymerised melanin *in vitro* when the cells are grown in the presence of phenolic sub-

strates. The ability of *C. neoformans* to synthesise melanin is associated with virulence. Melanin is deposited in the fungal cell wall, where it could provide cell wall support and integrity. Melanised *C. neoformans* cells are less susceptible to nitrogen- and oxygen-derived oxidants, microbicidal peptides, and macrophage-mediated phagocytosis than nonmelanised cells. It is now proved that *C. neoformans* is melanised in human brain tissue by using the melanin-binding monoclonal antibody 11B11. We investigated the melanin production of 43 *C. neoformans* v. *neoformans* strains isolated from cerebrospinal fluid and blood of AIDS patients.
Methods: Melanin production was tested, after 6 days, at 37°C on niger seed agar and minimal defined medium containing l-dopa. Melanin production was scored as follows according to the colour intensity of the medium: 3+ high activity, 2+ medium activity, 1+ low activity, and 0 no activity.
Results: The high pigment production was detected in 12/43 (27.9%) strains, medium activity was detected in 21/43 (48.8%) and the low pigment production in 10/43 strains (23.2%). The strains grown on the niger seed agar produced brown pigment and intensely black colonies on the medium containing l-dopa were observed.
Conclusion: The majority of tested strains had the medium pigment production. There were no significant difference in melanin production on both media up to 7 days, but the activity produced on medium containing l-dopa was more rapid.

R2212 The production of hydrolytic enzymes by pathogenic *Candida* non-albicans species

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Objectives: Yeasts of genus *Candida* are human commensals and they can cause superficial and deep-seated candidosis. Some *Candida* non-albicans species are able to cause damage to host cells too, usually in immunocompromissed patients, but the hydrolytic enzyme production of other *Candida* sp. is not well studied. We have tested the *in vitro* production of acid proteinase, phospholipase and acid phosphatase enzymes in different non-albicans species.
Methods: A total of 97 strains of diferent *Candida* non-albicans species (*C. tropicalis* 20, *C. parapsilosis* 17, *C. kefyr* 16, *C. krusei* 17, *C. guillermondii* five, *C. glabrata* 18, *C. lusitaniae* one, *C. incospicua* one, *C. humicola* one and *C. famata* one) were isolated from different clinical specimens (blood, faeces, urine, sputum, vaginal swabs, throat swabs) from neutropenic and other patients and healthy persons. Isolated strains were identified by standard methods and assimilation test API 20C AUX. Acid proteolytic activity was assayed on a solid medium containing bovine haemoglobin as substrate, the phospholipase activity was determined by plate assay using phosphatidylcholine as substrate and acid phosphatase activity by liquid medium using phenophtaleine diphostate.
Results: It was noticed that enzyme activities were a strain characteristic, but strains isolated from pathological material were more productive than isolates recovered from carriers. No enzyme activity could be demonstrated for examined strains of *C. lusitaniae*, *C.incospicua*, *C. humicola* and *C. famata*. The proteinase activity was positive in 24.71% (23/97) of strains and phospholipase activity in 35.05% (34/97) non-albicans strains. Acid phosphatase activity was detected in 51.55% (50/97) tested strains of *Candida* sp.
Conclusions: In this study strains of several non-albicans species isolated from pathogen samples were tested for hydrolityc enzyme production. The difference in proteinase, phosfolipase and phosphatase secretion between the isolates from the tested groups (infection and colonisation) demonstrates the existence of a correlation between hydrolytic enzyme production and infection. The high proteolitic and phospholipase activity were detected only in *C. tropicalis* and *C. parapsilosis* strains. These results indicate that candidal extracelular hydrolytic enzymes, like in *C. albicans*, seem to play a complex role in human candidosis.

R2213 Virulence factors in dermatophytes: screening of SUB genes encoding subtilisin-like proteases

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Dermatophytes are a group of closely related filamentous fungi capable to invade keratinised tissues (skin, hair and nails) of humans and animals. Belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*, these fungi are usually grouped in three categories based on their host preference and natural habitat. Members of the anthropophilic (human), zoophilic (animal) and geophilic (soil) ecological groups are potentially able to cause human infections. The colonisation process is assisted by the production of various hydrolytic enzymes, namely subtilisin-like proteases, which are encoded by a family of three genes (SUB1, SUB2 and SUB3). Since their expression is induced during the infection process, they are considered potential virulence factors. The presence of SUB genes was assayed by PCR with specific primers in a collection of 209 human clinical dermatophytes, representing ten species previously identified phenotypically and genotypically, and 30 reference strains. The results obtained showed that SUB genes are poorly represented among the analysed dermatophytes. SUB1 was only detected for 22 of 29 (75%) *M. canis* and 22 of 23 (91%) *M. audouinii* isolates and 1 of 88 (1%) *T. mentagrophytes* isolates. SUB2 was found in 23 of 29 (79%) isolates of *M. canis*, 20 of 23 (87%) *M. audouinii* and 1 of 7 (14%) of *M. gypseum* isolates. Concerning SUB3 gene, positive results were obtained for two of three (67%) isolates of *E. floccosum*, 19 of 29 (66%) *M. canis*, 20 of 23 (87%) *M. audouinii*, one of seven (14%) *M. gypseum*, as well as for two of 88 (2%) *T. mentagrophytes* and two of 46 (4%) *T. rubrum* isolates. The chi-square analysis of contingency tables was used to test statistical independence among occurrence of virulence traits and origin and species allocation of isolates. Significant associations ($P < 0.05$) were found between the presence of SUB genes and isolates of the species *M. canis* and *M. audouinii*, showing that these virulence factors may have an important role in the infection process for theses two species and a reduced one for the other species.

R2214 Epidemiological surveillance of *Candida* species isolated from patients hospitalised in an intensive care unit

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Objectives: The aim of this study was to determine the epidemiological surveillance of *Candida* infections in the anaesthesiology intensive care unit (ICU) of Dokuz Eylul University hospital and to evaluate the relation between colonisation and infection by these species.
Methods: Weekly surveillance cultures of the oral and anal cavities of the patients who admitted to ICU between 18/02/2002 and 31/03/2003 were obtained. Thirty-nine *Candida* species isolated from these cultures and 28 *Candida* strains isolated from various clinical specimens of the same patients were included for the analysis. The strains were identified by germ tube test, morphology on CHROMagar *Candida* and corn meal Tween 80 agar and API 20C AUX system. The susceptibility of the strains against amphotericin B and fluconazole were determined by microdilution method according to NCCLS M27-A2 standards. Epidemiological surveillance was carried out by resistotyping which included eleven inhibitory substances and randomly amplified polymorphic DNA (RAPD) PCR. Three primers were used for RAPD analysis of *Candida albicans* and *C. tropicalis* and four primers for *C. glabrata*. Band patterns were evaluated visually and coefficient of Dice was calculated.
Results: Thirty four of the isolates were determined to be *C. albicans*, 21 and 12 were identified as *C. glabrata* and *C. tropicalis*, respectively. There were 13 patients who was infected with *C. albicans*. 9 and 3 of the patients were infected with *C. glabrata* and

C. tropicalis strains, respectively. Most of the colonising and infecting strains of the same patient had MIC values within twofold dilutions. 22, 7 and 1 resistotypes were recognised for *C. albicans*, *C. glabrata* and *C. tropicalis* isolates, respectively. 10–24 band patterns were detected for *C. albicans* strains while 3–5 and 6–10 patterns were observed for *C. glabrata* and *C. tropicalis* isolates. The number of patients whose colonising and infecting pathogen showed the same or related pattern by resistotyping changed between 3 and 8 according to *Candida* species. This number was found to be 7–9 for *C. albicans*, 6–9 for *C. glabrata* and 2–3 for *C. tropicalis* infected patients with different primers.
Conclusion: A substantial risk of colonisation by *Candida* species lead to infection in ICU. *Candida* infections in the ICU of our hospital mostly originate endogenously.

R2215 Prevalence of fungal peritonitis in patients undergoing peritoneal dialysis

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The patients undergoing peritoneal dialysis treatment are the risk group of patients for appearance of fungal peritonitis. The aim of the study was to establish prevalence of fungal peritonitis in patients undergoing peritoneal disease. According to the results of the conducted sanitary control of the peritoneal dialysis department a possible correlation between the appearance of fungal infections and the presence of fungi in the smears of work surfaces, and in the air in this department was established.
Material and method: 968 peritoneal fluids of patients undergoing peritoneal dialysis were examined from 1.1.1997. to 1.7.2003. The diagnosis of fungal peritonitis was established both on the basis of clinical findings and on two consecutive positive mycological examinations of peritoneal fluids without finding any pathogenic bacteria. Hygienic-epidemiological control of peritoneal dialysis department in the above-mentioned period of time included sanitary examinations of the air (23) and work surfaces (615).
Results: In 54 (5.58%) patients the presence of fungi was established, in 37 (3.82%) patients *Aspergillus* spp. were detected, in 12 patients *Candida* spp. were detected, and in only 5 patients (0.52%) the finding of *Penicillium* spp. was detected. The important prevalence of fungal peritonitis was recorded in 1997 (14.59%) whereas the percentage of positive samples was significantly reduced (0.62% – 1998, 1999, 2000; 0.52% – 2001; 0.41% – 2002; 0.00% – 2003) during the rest of the time. A significant number of air inspections (all the 23) in the peritoneal dialysis department was done in the period of time from 1997–1999 when the prevalence of fungal peritonitis was the highest. *Candida* spp. was detected in only three of air samples by sanitary control in the peritoneal dialysis department. Only one positive finding of *Candida* spp. was established by sanitary control of work surfaces in the peritoneal dialysis department. There is no correlation in prevalence of fungal peritonitis in patients undergoing peritoneal dialysis with the findings of fungi in the smears both of work surfaces from and from the smears of air in the rooms where dialysis is performed.

R2216 Genomic diversity of *Candida albicans* and *Trichosporon* spp. based on the analysis of the ribosomal DNA

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Candida species are the most common fungi causing hospital fungal infection and *Trichosporon* species are emerging as opportunistic agents that can cause a variety of diseases in hospitalised and immunocompromised patients. Molecular typing characterisation of *C. albicans* using subgrouping based in the analysis of the site of transposable intron (intron site) of the 25S rDNA has been applied and the analysis of the ribosomal DNA intergenic spacer 1 (IGS) region of *Trichosporon* species showed variation among *T. asahii* clinical isolates.
Methods: Thirty clinical isolates of *C. albicans* were analysed according to introns site region of the 25S rDNA using the pair of primers: CA-INT-L: ATA AGG GAA GTC GGC AAA ATA CAT CCG TAA and CA-INT-R: CA-INT-R: CCT TGG CTG TGG TTT CGC TAG ATA CTA CAT. For the 7 isolates of *T. asahii* the sequence analysis of IGS region was studied with the following pair of primers: 26SF: ATC CTT TGC AGA CGA CTT GA and 5SR: AGC TTG ACT TCG CAG ATC GG.
Results: *C. albicans* isolates showed three groups: genotype A – 19 isolates, genotype B – nine isolates and genotype C – two isolates. No correlation was observed among the three genotypes, source of isolation and medical ward. For *T. asahii*, four types were identified. Types 1 and 4 were present only in urine specimen (4 specimens) and types 3 and 5 in oropharyngeal secretion (3 specimens). All patients were hospitalised in different wards. Interestingly, the urine and oropharyngeal specimens had specific types of *T. asahii* and that the four genotypes were identified in this collection.
Conclusions: Genotype A was the most prevalent in this collection of *C. albicans* and these results are concordant with previous ones. For *T. asahii* more data are necessary to conclude if there is a correlation between types and specimen

R2217 The synergic antifungal effect of a monocloneal antibody fragment with amphotricin B

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Objectives: Systemic candidiasis prevalence is increasing as a result of increasing in immnocompromised patients. Resistance to current antifungal is another problem, but new molecular methods for production of recombinant antibodies made new hopes for these patients.
Methods: The broth microdilution test was used in current study. 50 μl of a twofold dilution (40 to 0.02 μg/mL) of amphotricin B in RPMI broth and 100 μl suspensions of the inoculum's strains were added to the sterile flat-bottomed microtittre plate. 50 μL of the antibody (50 and 5 μg/mL) were added to each wells. Final volume of 200 μL was made by RPMI. In the negative control 50 μL of RPMI was used instead of antibody. The plates were incubated at 37°C overnight and the minimum inhibitory concentration was defined by the lower concentration inhibiting growth. The colony count was conducted for each well, where there was seen a visual reduction in the yeast growth. Results were represented as the colony forming units in 1 mL of broth and analysed by SPSS software.
Results: The amphotericin B MIC was reduced six folds against *C. tropicalis* and *C. parapsilosis* ($P = 0.001$) by this antibody. This reduction is four fold for *C. albicans* outbreak ($P = 0.032$), *C. albicans* fluconazol resistant ($P = 0.001$) and *C. krusei* ($P = 0.008$). The lowest effect of antibody was seen against *T. glabrata* that showed only twofold reduction for amphotericin B MIC. The greatest effect of recombinant antibody was seen against *C. tropicalis* and *C. parapsilosis*. *C. albicans* outbreak, *C. albicans* fluconazole resistance, and *C. krusei* were the next sensitive strains whereas *C. glabrata* showed the lowest sensitivity (only twofold reduction) to this antibody. There wasn't seen any growth in wells incubated with 55 μg/mL antibody but 9.4×10 CFU/mL was seen in wells without antibody.
Conclusion: As there is seen an increasing change of systemic candidiasis from albicans to non-albicans *Candida* strains recently and in respect to resistance of theses strains to commonly used antifungal agents, combination of them with newly designed recombinant antibody can be increased their efficiency.

R2218 Fungal infections in cases admitted to Yazd central laboratory, from autumn 2000 to spring 2003

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Objectives: Cutaneous fungal infections are among the relatively common mycoses, comprising a considerable percentage of admissions to physicians, especially dermatologists. Knowing the frequency, distribution, and geographic status of these diseases can be helpful for planning for control and prevention of them by the health system managers. The objective of this descriptive, retrospective study was to determine the relative frequencies of various fungal diseases in persons admitted to Yazd central laboratory.
Methods: From autumn of 2000 to spring of 2003. Sampling had been performed from the patients referred to this laboratory by the physicians. After clearing the specimen in 10–20% KOH and lactophenol, microscopic exam of direct smears, and if requested by the physician, culture on Sabouraud's dextrose agar and Mycosel agar (SC and SCC) had been done, and the results were written in laboratory work lists. The demographic data and results of tests on 3744 such cases were evaluated and analysed by SPSS Win software.
Results: From total 3744 cases, 1508 cases (40%) were positive for superficial and cutaneous fungal agents, including 855 males and 653 females. There was a statistical significant ($P = 0.000$) difference between 2 sexes regarding the relative frequencies of infections. Dermatophytes (43.6%) and malassezia furfur (33.4%) were the most common offenders, and erythrasma, candidiasis, other yeasts, and saprophytes comprised other infections in decreasing order of frequencies ($P = 0.001$). The most commonly afflicted age groups were 20–29 years (26.7%) and 10–19 years (26%). The least commonly infected persons belonged to 60 years and more age group. Upper extremities (17.8%) and axilla (1.6%) were the most and least commonly infected areas, respectively. Only 169 cases (17%) of 966 requested cultures were positive, and the zoophilic fungus *Trichophyton verrucosum* (26.6%) was the most frequently isolated organism. *Trichophyton schoenleinii* (1.2%) had the least frequency.
Conclusion: It seems that tinea versicolor and the contagious dermatophytosis with zoophilic agents have higher prevalence in this area and more education and health care programmes are needed.

R2219 *In vitro* activity of MICAFUNGIN (FK-463) against *Candida* spp.

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Objectives: The increasing incidence of life-threatening fungal infections has driven the search for new, broad-spectrum fungicidal agents that can be used for treatment and prophylaxis in immunocompromised patients. Micafungin (FK463) is a new echinocandin with *in vivo* an *in vitro* antifungal activity against *Candida* spp. The aim of this study was determinate the susceptibility of *Candida* spp. to Micafunfin.
Methods: A total number of 98 *Candida* spp. (13 *C. albicans* fluconazole resistant doses dependent, 45 *C. albicans* fluconazole susceptibles, 21 *C. glabrata*, 11 *C. krusei*, 6 *C. tropicalis*, and 2 *C. parapsilosis*.) isolated from different clinical specimens were included in this study. We determined minimal inhibitory concentration (MIC) of Micafungin by following the NCCLS M27A-2 broth microdilution method. Endpoints were defined as the lowest concentration of drug resulting in prominent inhibition (MIC80) and total inhibition (MIC100) of visual growth compared with the growth in the control wells containing no antifungal. After MIC was measured, 20 μL were subcultured from each well that showed completed inhibition, from the last positive well, and from the growth control, onto Sabouraud dextrose agar plates. The plates were incubated at 35°C until growth was seen in the growth control subculture (24 h). The minimum fungicidal concentration (MFC) was the lowest drug concentration that showed either no growth or fewer than three colonies to obtain approximately 99–99.5% killing activity.
Results: The MIC90–50 determined at 24 and 48 h were as follows: *C. tropicalis* (6): MIC range: 0.5 to >4 mcg/mL. *C. parapsilosis* (2): MIC range: 4. In these species MIC90 and MIC50 were not determined because no enough isolates. *SDD: Susceptible doses dependent, R: Resistant and S: Susceptible.

Isolates	MIC24 h (100/80%) mcg/mL	MIC48 h (100/80%) mcg/mL	MFC
C. albicans	MC_{90}1/0.25	MC_{90}4/0.5	$MFC_{90} > 4$
SCD* and R* (13)	MC_{60}0.5/0.25	MC_{60}1/0.25	MFC_{60}1
C. albicans S*	MC_{90}0.25/0.12	MC_{90}0.5/0.25	MFC_{90}0.25
(45)	MC_{60}0.25/0.06	MC_{60}0.12/0.06	MFC_{60}0.12
C. glabrata	MC_{90}4/4	$MC_{90} > 4/ > 4$	$MFC_{90} > 4$
(21)	MC_{60}0.06/0.06	MC_{60}0.06/0.06	MFC_{60}0.06
C. krusei	MC_{90}1/0.5	MC_{90}1/0.5	MFC_{90}1
(11)	MC_{60}0.5/0.5	MC_{60}0.5/0.5	MFC_{60}0.5

Conclusion: These data suggest that Micafungin shows a good *in vitro* activity against all *Candida* spp. except against *C. glagrata* and *C. parapsilosis*. The value of these *in vitro* results as predictors of therapeutic outcome is to be established in clinical trials

R2220 Protease activity in *Candida* spp. isolates from patients with otitis externa

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Objectives: *Candida* spp. have emerged as important opportunistic pathogens in immunocompromised patients. They have the ability to produce a variety of hydrolytic enzymes, such as proteases, phospholipases and phosphatases. There are some evidence for a role of extracellular protease activity and their ability to cause damage to host cells *in vivo*. The aim of this study was to determine *in vitro* protease activity of twelve strains of *Candida* spp. isolated from external ear in patients with otomycoses.
Methods: The protease production was determined using the test medium consisted of agar plates containing bovine serum albumin (BSA), 60 mL of a solution containing 0.04 g MgSO4.7H2O, 0.5 g K2HPO4, 1 g NaCl, 0.2 g dried yeast extract, 4 g glucose and 0.5 g BSA. The solution was sterilised by filtration, mixed with 140 mL of melted agar and poured into Petri dishes. The inoculum of 106 CFU/mL was prepared in normal saline and 10 μL of suspension of each strain was inoculated in triplicate. After the incubation at 370C for 7 days the diameter of the zones around the colonies was considered as a measure of protease production. The protease activity (Pz) was measured in terms of the ratio of the diameter of the colony plus the precipitation zone. Low Pz signified a high production of the enzyme, i.e. high virulence, while a high Pz induced low production of the enzyme, i.e. low virulence. The average Pz value was obtained with three separate samples of each strain.
Results: Eleven of 12 *Candida* spp. strains have shown a positive protease activity and the Pz values ranged between 0.61 and 0.78 (Pz average 0.69). The protease activity of *Candida* spp. strains was observed three days after inoculation.
Conclusions: The majority of tested *Candida* spp. had a protease activity. Proteinase production is considered to enhance the organisms' ability to colonise and penetrate host tissues, which could be important in establishing the infection of the external ear. It is documented that protease enzymes are able to degrade a number of proteins important in host defence such as immunoglobulins, complement and cytokines.

R2221 Voriconazole activity against clinical isolates of *Candida*, focusing on fluconazole-resistant strains

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Madrid, E

Background: Antifungal resistance among invasive isolates of *Candida* is a source of concern. *Candida kruesi* and *C. glabrata*. are known to express intrinsic (*C. krusei*) or acquired (*C. glabrata*) resistance to fluconazole and may also show decreased susceptibility to amphotericin B. Data regarding the activity of voriconazole against *Candida* species expressing resistance to fluconazole are limited.
Objective: To determine the *in vitro* activity of voriconazole against *Candida* spp. isolated in our hospital from blood, sterile, mucocutaneous and respiratory sites during the last 2 years.
Methods: The *in vitro* activities of fluconazole (FZ), itraconazole (IZ), ketoconazole (KZ), and voriconazole (VZ) were determined by the microdilution method following NCCLS criteria. MICs were visually determined after 24 h incubation at 35°C. Interpretative criteria have not yet been defined for voriconazole; however, for purposes of comparison, we have employed a susceptible breakpoint of $\leq$1 mg/L.
Results: We tested 218 clinical isolates of *Candida* obtained from 164 patients. Overall, the frequency of FZ-resistance ($\leq$64 mg/L) and intermediate resistance (16–32 mg/L) was respectively: 7.8% (17) and 16% (35). Of these, 98.6% (49) were inhibited by voriconazole at an MIC of $\leq$1 mg/L., and three strains (*C. albicans*, *C. parapsilosis*, *C. krusei*) were resistant to voriconazole (MICs $\leq$ 4 mg/L were observed). globally, the rate of voriconazole resistance was 1.4%.
Conclusion: Voriconazole was very active against all clinical isolates of *Candida* (99% of MICs were $\leq$1 mg/mL) and showed a excellent potency *in vitro* against fluconazole-resistant *Candida* strains.

R2222 Frequency of high-level fluconazole resistance in *Candida* isolates in a general hospital

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Background: It is now known that there are isolates of *Candida* exhibiting two types of azole resistance. The phenotype RR: high-level resistance to both fluconazole (MIC, $\geq$64 mg/L) and itraconazole (MIC, $\geq$1 mg/L) and the phenotype RS: high-level resistance to fluconazole (MIC, $\geq$64 mg/L) and susceptibility to itraconazole (MIC, $\leq$ 0.125 mg/L). Reports on the frequency of these phenotypes in a clinical setting are scarce.
Objective: To evaluate the frequency of the two different phenotypes of azole resistance (RR and RS) in 712 *Candida* clinical isolates from blood and other sterile, mucocutaneous and respiratory sites during a 6-year period.
Methods: From 2001 to 2003, 712 isolates of *Candida* spp. from 516 patients were recovered. The species distribution identified by ATB 32C was: 343 *C. albicans*, 127 *C. glabrata*, 121 *C. parapsilosis*, 50 *C. tropicalis*, 34 *C. krusei* and 37 others. The *in vitro* activities of amphotericin B (AB), fluconazole (FZ), itraconazole (IZ), ketoconazole (KZ) and flucytosine (FC) were determined by the microdilution method following NCCLS criteria.
Results: The percentages of resistance of *Candida* isolates were: AB 1.8%, FZ 8.7%, KZ 12%, IZ 16.6% and FC 2.1%. The frequency of phenotype RR was 8.7% (62 isolates). The species distribution of isolates RR was: 34 *C. krusei*, 18 *C. glabrata*, 5 *C. albicans*, 3 *C. parapsilosis*, 1 *Trichosporon* and 1 *Zygosaccharomyces*. No isolates with phenotype RS were observed.
Conclusion: Overall, resistance of *Candida* isolates in our institution is 8.7% to FZ and 16.6% to IZ. In all isolates the high-resistance phenotype (RR) was detected.

R2223 Dermayophytoses in Erzurum, Eastern Anatolia

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Objective: To determine the incidence of causative pathogens responsible for the dermatophyte infections in Eastern Anatolia, Erzurum.
Methods: In this study, hair, nail, and skin samples obtained from 503 patients, suspected with dermatophytoses, were studied. Distributions of patients were tinea pedis (n = 141, 28.3%), tinea corporis (n = 121), tinea ungium (n = 78), tinea ingiunalis (n = 73) Tinae manum (n = 70), tinea capitis (n = 20). First, native preparations were done for these clinical samples by using 20% KOH and then stained with lactofenol cotton blue for direct microscopic examination. In order to cultivate, isolate and identify, the dermatophytoses, each sample was inoculated on saboraud dextrose agar, mycobiotic dextrose agar and potato dextrose agar as duplicate, and then incubated for up to 4 weeks at 37o C and room temperature. At the end of the incubation period, cultures were examined with macroscopic and microscopic.
Results: Diagnosis of the patients as dermathoytoses were performed as to laboratory findings, and distributions of dermathophytoses were tinea pedis (65 in 141 samples, 46.1%), tinea corporis (n = 12 in 121, 9.9%), tinea ungium (n = 27 in 78, 34.6%), tinea ingiunalis (n = 17 in 73, 23.3%), tinea manum (n = 7 in 70, 10.0%), tinea capitis (n = 11 in 20, 55%). Dispersions of dermathophytes isolated from patients' samples were *Trichophyton rubrum* 72.5%, *Trichophyton mentagrophytes* 16.8%, *Trichophyton schoenleinii* 3.6%, *Trichophyton tonsurans* 1.4%, *Microsporum canis* 1.4% and *Epidermophyton floccosum* 0.7%. *Trichophyton rubrum* was the most frequent one in all forms of dermatophytoses except tinea capitis. *Trichophyton schoenleinii* was the most frequent dermatophyte (45.4%) in tinea capitis.
Conclusion: As tinea pedis was the most frequent clinical form; *Trichophyton rubrum* was the most frequently isolated one. When we compare our results with the other studies performed in other regions of Anatolia, on contrary to expectations due to geographical distinction, there were no differences.

R2224 Candidaemia at intensive care units in Saint-Petersburg, Russia

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Objective: To determine epidemiology, risk factors, clinical symptoms, treatment and outcomes of candidemia in ICU in Saint-Petersburg.
Methods: We have analysed prospectively demographic, clinical and microbiology data for 100 patients with candidemia at ICU in 19 hospitals in Saint-Petersburg, during the years of 1996–2003.
Results: Prospectively 985 patients at high risk for candidemia have been observed. *Candida* spp. (110 strains) were isolated in blood cultures from 100 patients (10%). Eighteen per cent of these patients were children <15 years of age. The most common species was *C. albicans* (28%), followed by *C. parapsilosis* (27%), *C. guilliermondii* (13%), *C. tropicalis* (7%), *C. krusei* (4%), *C. guilliermondii* (4%), *C. lipolytica* (2%), *C. zeylanoides* (2%), *C. rugosa* (1%), *C. lusitaniae* (1%), *C. kefyr* (1%), *C. famata* (1%) and *Candida* sp. (9%). Two and more different *Candida* spp. were present in blood of 10% of patients. Concomitant non- *Candida* fungaemia was present in 3% of patients (*Geotrichum candidum*, *Trichosporon* spp.). Concomitant bacteraemia (*Ps. aeruginosa*, *Klebsiella* spp. and other) was present in 22% of patients. Associated risk factors for candidemia included broad spectrum antibiotics (95%), central lines (92%), extensive surgery (32%) and underlying disease (cancer – 29%, trauma – 14%, burns – 12% and diabetes – 5%). At time of diagnosis the majority of patients presented with non-specific symptoms. Fever >38C was found in 88% of patients, whereas hypotension occurred in 19% patients. Leucocytosis and leucopenia were observed in 21 and 16% of patients respectively. A focal

site of infection was detected in 31% of cases, with lungs being the most common target organ (28%). Other sites of infection included kidneys, peritoneum, heart, liver, CNS, spleen, eyes, skin and oesophagus. Seventy-four per cent of patients received antimycotic therapy: fluconazole (55%), amphotericin B (31%), caspofungin (7%), Ambisome (5%), itraconazole (1%), ketoconazole (1%). In 63% of patients central lines were removed. Overall mortality rate was 45% (28% in children and 49% in adults).
Conclusion: Candidemia is a nosocomial infection associated with high mortality rate. Non-albicans *Candida* spp. predominated as etiologic agents of candidemia. Effective antifungal treatment, removal of contaminated central lines are important in the management of these infections.

R2225 *Trichophyton tonsurans* in Aragon (Spain)

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Objective: The aim of this study is to examine the evolution of *T. tonsurans* between 1987 and 2003 in Aragón (Northeast of Spain).
Methods: Skin scrapings and hair samples were obtained after cleaning the lesions with alcohol at 70%. The material collected was microscopically examined in 40% KOH/Parker Quink ink (v/v) stain for the presence of fungal hyphae and arthrospores, and inoculated on Sabouraud's dextrose agar with antibiotic, mycobiotic agar and dermatophyte test medium. Identification of fungal cultures was made on the basis of both macroscopic and microscopic appearances. Slide cultures and other confirmatory tests such as *Trichophyton* agar slants, Christiansen's urea agar slant, potato dextrose agar, corn meal and *in vitro* hair perforation test were performed when necessary (1).
Results: 74 patients had dermatophytosis caused by *T. tonsurans* which was collected from the patients respectively in four Spanish hospitals that were located in the same geographical area. Of these 74 cases, only 25 were born in Spain of Spanish parents and 2 were born in Italy of Italian parents. But between 2000 and 2003, 53 cases (71.6%) were diagnosed and of these 53, 45 (84.9%), were immigrants or had immigrant parents.
Conclusion: Dermatophytoses caused by *T. tonsurans* it is increasing in Aragón (Spain). Most of the cases come from immigrant families that originate in Africa. The rapid increase of immigration in our country and the easy transmission of this antropophilic dermatophyte are probably the main reasons for this increase of *T. tonsurans* in our area.
Reference:
1. Rebell, G. & Taplin, D. Dermatophytes their recognition and identification. 2nd Ed. 1974. University of Miami Press.

Tropical and parasitic diseases

R2226

Abstract withdrawn.

R2227 Study of *Enterobius vermicularis* infection among kindergarten children in Ardabil, Iran

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Objectives: *Enterobius vermicularis* is a common helminthic infection, affecting almost 1 billion people worldwide from all socioeconomic classes. In Iran, its prevalence is 25–92%, with a high prevalence among children. Aim of this study was to determine the prevalence of this parasite in Ardabil kindergartens.
Methods: 400 children <7 years old from 10 kindergartens were evaluated by Graham's scotch adhesive tape technique in Ardabil, Iran, 2003.
Results: The prevalence of enterobiasis in the studied children was 18.3%. The highest rate (21.9%) was observed in children 4–5 years old and the lowest rate (14.3%) was seen in children 1–2 years old. The infection rate of males and females was 16.4 and 21.2%, respectively ($P > 0.05$). Prevalence of enterobiasis in children of illiterate mothers (54.5%) was higher than those of literate mothers (12.2%) ($P < 0.05$).
Conclusion: Therefore for prevention of this infection, health education, especially for illiterate or low literacy parents is necessary.

R2228 Seroepidemiology of toxoplasmosis in women referred to Ardabil laboratory health centre for medical examination before marriage, Iran, 2002

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Objectives: Infection with *Toxoplasma gondii* can cause sever illness when the organism is transmitted to fetus or when it is reactivated in immune-suppressed persons. The aim of this study was to determine the antibody prevalence of toxoplasmosis in women referred to laboratory of health center for medical examines before marriage.
Methods: This cross-sectional study was performed on 504 sera collected from women in Arabia city, Iran, in 2002. The samples were studied by indirect immunofluorescent assay (IFA) for determination of IgG and IgM antibodies to toxoplasma.
Results: The seroprevalence of IgG antibody at a titre of ^{3}1:20 was 34.7%. There was the most of antibody titre frequency in 1:20 titre (11.7%) and the least of them in 1:3200 (0.4%) and 1:6400 (0.4%) titres. Only 20 persons (4%) showed IgM antibody against *Toxoplama gondii*. No statistically significant differences in the levels of antibodies were observed in relation to age.
Conclusion: As 65.3% of these women in Ardabil city were seronegative, health education to omit its risk factors, especially during the pregnancy is necessary.

R2229 Diseases imported from tropical regions in own material

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Szczecin, PL

Objectives: The development of tourism, business trips and migrations have resulted in a real possibility of importing tropical diseases to Poland. The aim of study was epidemiological analysis of the diseases imported from tropics according to the regions of the world in which they were acquired and methods of prevention.
Methods: The study was based on own material accumulated in the Department of Infectious Diseases of Pomeranian Medical University in 1990–2000. The diagnosis of imported diseases was based on clinical presentation, epidemiological history and accessory investigations such as search for malaria plasmodium in peripheral blood smears, examination of faeces for amoebic cysts or eggs of trematodes and of urine sediments for schistosoma eggs. Additionally, immunoserological tests were performed in case of malaria, amoebic liver abscesses and schistosomiasis, typhoid fever, as well as blood and faeces cultures, essential laboratory tests and imaging-ultrasonography and computed tomography.
Results: Between 1990 and 2000.54 patients diagnosed with diseases imported from tropics were hospitalised in the Departmen, including 45 with malaria, 5 with amoebiasis, 2 with trematodiasis and 1 female patient with typhoid fever. Tropical malaria due to *Pl. falciparum* invasion was diagnosed in 31 cases, in 12 tertian malaria (*Pl. vivax*), in 2 double infections (*Pl. falciparum + Pl. vivax* and *Pl. falciparum + Pl. ovale*).Tropical malaria was imported from equatorial Africa, tertian malaria from India and double infections from West Africa. Amoebiasis due to *Entamoeba histolytica* was diagnosed in 5 patients-in one case intestinal amoebiasis and in four amoebic liver abscesses. The disease was associated with stay in India, Syria or Sudan. Among two patients with trematodiasis contracted in Africa, was one with schistosomiasis (*Schistosoma haematobium*) and another-fascioliasis (*Fasciola hepatica*).The only case of typhoid fever was diagnosed in a young woman who had previously visited Nigeria. Less than half of patients used prophylactic media against tropical diseases.
Conclusions: The knowledge of the principles of prevention of tropical diseases is insufficient among persons travelling to tropical countries. Malaria was the most common cause of imported diseases; the minority of travellers use pharmacological prophylaxis of the disease. Amoebic liver abscesses were found exclusively in men. Trematoid invasions and typhoid fever were relatively rare.

R2230 Parasitic infestation of the ceacum and acute appendicitis. Case reports

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Pireus, GR

Objectives: Parasites such as *Enterobius vermicularis* may have a causal role in appendicular pain and chronic inflammation. Nevertheless it is rarely associated with histological changes of acute appendicitis. The relationship between the parasite and acute appendicitis is rare and much disputed. The incidence of acute appendicitis and helminthic infestation in children is higher than in adults. The highest incidence occurs in the range of 6 to 15-year-old patients, while infestation is more frequent in female than in male. Two interesting cases of patients older than 15 years with acute appendicitis caused by *Enterobius vermicularis*, are presented in this study.
Methods: From December 2000 to December 2003 three patients were admitted to our department with the clinical presentation of acute appendicitis. Tow female patients, 15 and 19 years old, respectively, and one male patient 16 years old were concerned. Emergency appendectomy was performed.
Results: The histopathological study showed the presence of *Enterobius vermicularis* and acute inflammatory changes in the appendix, in all of them. Laboratory examinations for *Enterobius vermicularis* infestation of the bowel were followed.
Conclusion: The clinical presentation imitates an attack of acute appendicitis, but the true nature of the disease is diagnosed only through a histological examination. Appendectomy eliminates the complication but not the cause of intestinal disease.

R2231 A decade of malaria in Yazd Province (near to endemic areas of Iran)

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Introduction and objectives: Despite vast efforts for eradicating and controlling of malaria, this infection is known as one of infections with high incidence and mortality especially in endemic parts of the world. In few cities of Iran like Yazd, that hosted many Afghan immigrants, who came from endemic areas caused importing cases of malaria. The general purpose of this study was to determine the demographic parameter of people, who infected with malaria for handling and treatment of them.
Methods: A descriptive cross-sectional study was conducted on all patients with malaria, who had been referred to Yazd health centres from 1990 to 2000. In this decade totally 3193 cases were been diagnosed with positive blood smear. The demographic parameter of patients was prepared using SPSS software.
Results: The maximum and minimum incidence of malaria was seen in 1993 (847 cases) and 1997 (114 cases) respectively. The most prevalent species' was *Plasmodium vivax* (83%) but *P. malariae* was detected only in 0.1% of cases. Majority of infected patients were Afghan immigrants and fortunately no drug resistant case was seen in this study.
Conclusion: According our results, we suggested that due to suitable climate conditions for various species of anopheline mosquitoes in Yazd province and also increasing the frequency of Afghan immigrants entered to this province, there is a very high risk of malaria transmission in this area and it is necessary to have more attention for controlling of this infection.

R2232 Status of cutaneous leishmaniasis in Taft from 1997 to 2002

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Introduction and objectives: Leishmaniasis is one of the most important tropical and subtropical infections throughout of our world. The visceral and cutaneous forms of this disease (both anthropophilic and zoonitic) are endemic in Iran. In recent years, several Yazd cities have been highly infected with cutaneous leishmaniasis. The general purpose of this study was to determine the disease profiles in the city of Taft.
Methods: This survey was a descriptive cross-sectional study, which was conducted on coetaneous leishmaniasis patients, who referred to Taft health centres from 1997 to 2002. Their infection was diagnosed with laboratory tests and their demographic parameters were collected by a designed questionnaire. Finally data was collected and analysed using SPSS software.
Results: From 278 detected cases including 76% male and 24% female, in 1997 a peak of infection incidence was seen as most of cases were diagnosed (112 cases) in this year, whereas the minimum rate of infection was seen in 2001 (only 12 cases). 67.3% of cases were seen in 11–20 years age group and in different closed rural areas Kahdouieh village was determined as the highest infected area in Taft.
Conclusion: The incidence rate of cutaneous leishmaniasis in Taft is lower than closed cities like Yazd. Since there are potential vectors, sensitive humans and suitable environmental conditions in this area, it is necessary to promote permanent controlling and

surveillance programs to eliminate this infection, otherwise there is the risk of spreading of this infection in Taft.

R2233 Malaria in university hospital patients in Oman, 1996–2001

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Objectives: Malaria used to be endemic in Oman, in 1990, there were over 32 000 cases but with the introduction of malaria control in 1992, the incidence fell to 1091 cases in 1998. 70% of cases were caused by PF usually chloroquine-resistant, and many imported cases were in visitors to Zanzibar which used to be a colony of Oman. By 2001, all but 2 of 635 cases diagnosed in Oman were imported. To document the changing clinic-epidemiology of malaria in hospital patients we started a prospective study in 1996.
Methods: All patients with malaria admitted to the Unversity Hospital from 1996 to 2001 were studied.
Results: 45 patients aged 2–58 years (mean 22 years), were included. Malaria was imported in 32 cases, 19 in returning visitors to Zanzibar. Only eight patients had taken prophylaxis. Malaria was caused by PF in 33 patients. Three patients had severe malaria, but none died. Early diagnosis and treatment of PF cases with quinine was invariable. The numbers of malaria cases declined to only 5 in 2001.
Conclusions: Early diagnosis and appropriate treatment of malaria in the University hospital has resulted in early recovery and no deaths during the period. Most cases have been in visitors to Zanzibar. The declining numbers of cases may delay diagnosis and appropriate treatment in the future.

R2234 Case report: broad ligament hydatic cyst

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Objective: To present an unusual presentation of an infectious disease such as hydatic cyst in the endemic area.
Case Report: Is a 49-year-old housewife woman complaining of left lower quadrant pain, pelvic heavyness and irrigular vaginal bleeding from 3 months before admission. In the genital exam there was vaginal bleeding and a mass about $9 \times 8 \times 10$ cm in the left adenex.
Lab. exam: CBC = WBC = 7800/Hb = 11/2 mg. ESR 36. Sonography = endometrial diameter was 8 mm and a cystic mass without echo about $98 \times 86 \times 90$ mm in the left adenex. Diagnostic dilatation and curretage were done. Its pathologic result showed proliferative endometrium with diagnosis of ovarian cyst cystectomy was done. The pathologic result of the cyst showed broad ligament hydatic cyst.
Conclusion: Hydatic cyst usually involve lung and liver but it can infect other organs. Sometimes it has unusual presentation. Therefore, in the endemic area every patient with a mass without specific cause hydatic cyst must be considered.

R2235 Myopericarditis as a manifestation of primo-infection by *Toxoplasma gondii* in an immunocompetent patient: a case report

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Toxoplasma gondii is an intracellular parasite producing heart disease in immunodeficient patients as well as in immunocompetent individuals. We report the case of a previous healthy 23-year-old male seen the Emergency Department of our hospital suffering from a 2-day thoracic pain. The patient presented a permanent pleuritic retroesternal pain, irradiated to the right hemithorax, worsening with respiratory movements. He did not refer nausea, vomiting or sweating as well as previous trauma or intense effort, or dyspnoea. The patient had not had any infectious disease in the previous days. At the physical examination the patient showed a blood pressure of 130/80 mmHg, 61 heart beats/min, and a temperature of 36°C. Blood oxygen saturation was 98%. He was eupneic with normal cardiac and pulmonary sounds. No relevant alterations were seen in the chest radiography.
Blood chemistry findings were: RBC 4.98×106/mL, haemoglobin 16.2 g/dL, haematocrit 47.9%, leucocytes 10 180/mL (82.8% neutrophils, 7.5% lymphocytes, 6.9% monocytes) with some atypical lymphocytes. The haemostatic values were normal. A CK of 1032 IU/L with a CK-MB of 108 IU/L was found. The ECG showed an ST segment elevation in V1–V3, with decreasing T wave in the lateral leads. The patient was admitted with the clinical diagnosis of myopericarditis and treated with anti-inflammatories (lysine aspirin). At day seven after admission the patient was discharged asymptomatic with a normal ECG. During admission, serum test for influenza A and B virus, adenovirus, respiratory syncytial virus, *Chlamydia*, *Legionella*, *Mycoplasma*, *Coxiella*, *Borrelia burgdorferi* and HIV were all negative. ELISA test for *Toxoplasma* was positive with an IgG of 9.24 IU (positive >3) and IgM 4.9 EU (positive >1 EU). Although primary infection with *T. gondii* is usually asymptomatic in inmunocompetent individuals, a small percentage can suffer myopericarditis, although this manifestation is exceptional. Treatment is aimed to prevent complications.

R2236 Evaluation of non-microscopic tests in malaria diagnosis

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Objectives: The need of new rapid and easy to develop diagnostic techniques such as dipsticks acquire a very important role in the management and control of malaria. This disease is responsible for more than 1 million of deaths each year, and the estimated prevalence worldwide exceeds 300 million of cases. The severity of this illness is closely related to the delayed laboratory results; consequently inmunochromatographic tests (ICTs) can be considered a very useful tool in the diagnosis due to it's easy performance that do not require highly trained personal, and short duration to develop such tests. In this study dipsticks sensitivity

% infected RBC	Microscopic examination (*n*)	ICT (*n*)	PCR (*n*)
P. falciparum			
Positive			
> 1%	12	12	12
0.1–1%	28	28	28
< 0.1%	21	20	21
Negative	15	2	0
P. vivex			
Positive			
> 1%	1	1	1
0.1–1%	3	3	1
< 0.1%	7	4	7
P. ovale			
Positive			
0.1–1%	2	1	2
< 0.1%	3	1	3
P. malariae			
Positive			
0.1–1	1	1	1
Negative	1	1	0

and specificity were evaluated versus microscopic diagnosis and results were compared with PCR.
Methods: ICTs were evaluated with EDTA venous blood samples from symptomatic patients either with history of travel or living in endemic malaria countries. Direct examination included both thick and thin blood films with Field's stain. Finally all the samples were compared with PCR to assess differences between both techniques. ICT studied was NOW Malaria (ICT-Amrad, Sydney, Australia) that uses monoclonal *P. falciparum* antibodies against HRP-2 and a panspecific *Plasmodium* spp. aldolase.
Results: ICT has a high sensitivity for *P. falciparum* including samples with low parasitaemia concentrations. There were 15 false-negative microscopic examinations; all had positive PCR results and finally ICT detected 13 cases. For other *Plasmodium* species the sensitivity of the test was significantly lower.
Conclusions: ICTs offers useful information in the rapid diagnosis of *P. falciparum* infections with excellent specificity and sensitivity; consequently these tests could be implemented in health centers devoid of facilities for more complex laboratory tests. With the rest of *Plasmodium* species the test lacks specificity due to the monoclonal panspecific antibody used. Sensitivity decreases significantly when parasitaemia falls below 0.1% of infected red blood cells.

Sexually transmitted diseases (except HIV)

R2237 PCR detection of *Chlamydia trachomatis* from endocervical smears

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Objectives: *Chlamydia trachomatis* is one of the most common aetiological agents of sexually transmitted diseases worldwide. *Chlamydia* infection is largely asymptomatic, but if it is left untreated, it can have particularly severe long term consequences for women. These sequelae include pelvic inflammatory disease, ectopic pregnancy and infertility.
Methods: To evaluate the performances of the Amplicor CT PCR assay (Roche Diagnostics) on endocervical smears for the detection of *Chlamydia trachomatis*. A population of 171 women attending the gynaecology clinic of Kosice entered the study. Endocervical smears specimens were processed according to the manufacturer's instructions. The majority of the patients presented with discharge and other symptoms of a genital infection. The women were 17–45 years old.
Results: An overall prevalence of 18.7% (32/171) was observed for *Chlamydia trachomatis* infection.
Conclusions: PCR is proved to represent a fast and precise method of *Chlamydia trachomatis* evidence in smears from uterine cervix. This work was supported by grant VEGA MS SR No. 1/9279/02.

R2238 Markers of HBV, HCV and HIV infections among persons with multiple sexual partners

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Objectives: To investigate the prevalence of HIV, HBV and HCV markers among persons with multiple sexual partners.
Material and methods: This is a prospective study including persons with more than 5 sexual partners for the period of 6 months grouped by sex, age, education, nationality and sexual practice. The investigation was made for HCV using ELISA kits: ORTHO HCV 3.0 ELISA, ABBOTT HCV EIA 2nd GENERATION, Murex anti HCV/Version III and UBI HCV 3.0 ELISA and confirmed by Immunoblot Assay Chiron RIBA HCV 3.0 Strip Immunoblot Assay and HCV RNA, and genotypes were determined with RT/PCR using Amplicor (Hoffman La Roche). HBV and HIV infections were checked using Behring ELISA kits and Enzygnost anti HIV 1/2 Plus. Lia Tek HIV 1 + 2, Western blot Kits and Chiron RIBA HIV1/HIV2 SIA.
Results: Tested were 200 persons with multiple sexual partners at the age from 17 to 70 years. We verified present or past HCV and HBV infections in 142 (71%) patients: 29 (14.5%) had verified HBsAg, 41 (20.5%) anti HBs, 72 (36%) anti HCV and 21 (10.5%) had been with HIV infections. 100 of them noted that they had been drug users more than 1 year before this examination without informing their partners. By monitoring the group of nondrug users (100) we verified anti HCV in 13 (13%), HBsAg in 13 (13%), anti-HBs in 22 (22%) and HIV infection in 21 (21%).
Conclusions: Sexual transmission of HBV, HCV and HIV among persons with multiple sexual partners is intensive and among them, drug users, present or past, may be one of the most important links of the transmission chain. Education and vaccination for HBV is our duty in order to prevent these infections in our country.

R2239 PolA PCR in syphilis diagnostic

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Objectives: *Treponema pallidum*, causative agent of syphilis, cannot be cultivated *in vitro*. Although serology is a valid tool for syphilis diagnosis in most cases, there are still some situations in which the determination of the disease is very difficult, e.g. at the beginning of infection or in reinfection. Polymerase chain reaction (PCR) as a direct detection method is very useful in such circumstances. However, serum samples taken for routine serological diagnostic were found to be less suitable for PCR, since spirochetes are trapped in cloths.
Method: We put into practice polA PCR for DNA detection of *T. pallidum* and examined 52 routine clinical specimens (49 serum samples, 2 lesion swabs and 1 liquor) from persons in different phases of syphilis.
Results: *T. pallidum* DNA was detected in three serum samples (one *syphilis primaria*, two *syphilis secundaria*) and in one lesion swab.
Conclusions: These data suggest that treponemal DNA is detectable not only in blood (as indicated by previous studies) but in serum as well. Moreover, we report an interesting case of DNA positivity in an adequately treated; patient 3 months after the treatment in our study.

R2240 Amoxicillin-clavulanic acid in treatment of urethral infections

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Objectives: Urethral infections are very frequent in young population. We present our results with one drug treatment.
Materials and Methods: 62 male patients, aged among 16–24 years. No fever, with disuric symptoms, Le<10 × 10/9/L, piuria, urethral secretion. In neither of them, therapy was not given prior to infection. Treatment was with amoxicillin–clavulanic acid oral, 1 g/bid, for 10 days. We performed urethral samples in all of them three times: in the stage of disease, immediately after treatment and 1 month after.

Results: In first group of 75% patients we isolated *Streptococus faecalis* and *Escherichia coli*. Second group of 15% was with *Escherichia coli*, while third group of 10% was with *Streptococus faecalis*. Amoxicillin–clavulanic acid was sensitive in all group of patients. Follow up urethral samples showed complete healing in 69% patients in first group, while in second and third group success was almost 95%.
Conclusion: Our results point toward very efficient treatment with amoxicillin–clavulanic acid in urethral infections. Recommended dosage is 1 g/bid for 10 days.

R2241 Microscopic examination of vaginal discharge specimens for *Trichomonas vaginalis* and other micro-organisms in 18–45 age group women

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Objectives: *Trichomonas vaginalis* is a protozoon that causes trichomoniasis which characterised with a foamy yellowish odorous discharge and superficial defects and necrotic ulcers in vaginal mucosa. *Trichomoniasis* is transmitted from human to human with sexual contact and can be seen in almost every part of the world. The aim of this study was to determine incidence of *Trichomonas vaginalis* in 18–45 years age group women with vaginal discharge. Seventy vaginal discharge specimens obtained from women who applied to gynaecology Outpatient Clinic of Social Insurance Institution Hospital with vaginal discharge complaints in duration of September 1 to December 15 2003.
Methods: Samples were taken from posterior forninx of vagen with the help of a speculum and sterile cotton swabs. All the samples examined by wet mount preparations, gram staining and giemsa staining method under the light microscope. Wet mount preparation were examined with $\times 20$ and $\times 40$ magnification, the stained preparations were examined with $\times 100$ magnification.
Results: *Trichomonas vaginalis* was seen in 6 (9%) of 70 specimens; five of them were detected by three methods but one of them detected by only wet mount preperation. *Trichomonas vaginalis* most frequently observed between 25 and 35 years old women. Of seventy samples 9 (13%) were positive for *Gardnerella vaginalis*, only one was positive with *Mobiluncus* spp. and 11 (16%) were positive for *Candida* spp. With Gram staining method.
Conclusion: On the basis of our study, it is possible to say that in spite of definite diagnosis of *trichomoniasis* made by cultivation method, examining vaginal smear with direct microscope also has an important role in the diagnosis of this infection. Fascilating and shortening the time needed for the diagnosis of trichomoniasis with direct microscopic examination will help to decide and begin the treatment of this infection.

R2242 Features of vaginal and intestinal microbiocenosis of young women with menstrual function disturbances

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Objectives: To study the vaginal and intestinal microbiocenosis status in patients with menstrual function disturbances in childbearing age.
Methods: There were 57 women of childbearing age under supervision: 15 – with normal menstruations (I group), 22 patients – with hypomenstrual syndrome (II) and 20 – with hypermenstrual syndrome (III). Estimation of vagina and intestinal microbiocenosis status was carried out with the use of cytomorphological and bacteriological methods.
Results: According to the results of cytomorphological analysis of vaginal smears, in II and III groups of supervision, inflammation changes with predominance of *coccus flora* are registered in 50% of cases. Indications of bacterial vaginosis in patients of these groups are found in 3.5–4 times more often ($P < 0.01$), than in I group. It is necessary to emphasise, that the mixed-infections are most typical for the women with hypomenstrual syndrome: combination of cytological features of bacterial vaginosis with morphological features of chlamydiasis and DNA-virus infections (HPV and herpes simplex). Thus contamination of women by *Chlamydia* and DNA-viruses was registered in 100% of cases. During the study of vaginal microbiocenosis in II and III groups of patients, disbiothical disturbances which deal with the presence of *Enterobacteriaceae* sp. and Candida sp. were most frequently ($P < 0.01$) marked. It is typical that the concentration of *Lactobacillus* sp. in III group of women was much lower ($P < 0.01$) than those in I group. The degree of weight of intestinal microbiocenosis changes was less expressed. However, disbiothical changes of intestinal biotope was met twice more often in II group of patients in comparison with I. In this case disbiothical shifts are caused by increase frequency presence of *E. coli* (lac−) and (haem+).
Conclusion: Hipomenstrual syndrome is characterised by disbiothical disturbances in the structure of vaginal and intestinal biotops in combination with Chlamydia sp. and DNA-virus genital infections. Disbiothical changes typical for the hypermenstrual syndrome abounded by the vaginal biotop.

R2243 *Chlamydia trachomatis* infections in a Northwestern Greece population

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Objectives: Worldwide, *Chlamydia trachomatis* is one of the most common sexually transmitted bacterial pathogens. The aim of the present study was to determine the prevalence of *C. trachomatis* in adult women and men in Northwestern Greece using a highly sensitive PCR-based diagnostic assay.
Methods: 2672 symptomatic and asymptomatic women aged 18–55 years and 214 symptomatic men aged 18–65 years not attending sexually transmitted disease (STD) clinic were enrolled in this study from November 1998 to December 2003. Two types of specimens were collected from each patient: cervical swab and first-void urine (FVU) from women, urethral swab and FVU from men. All these specimens were examined with the automated amplification system: PCR Cobas Amplicor, CT-PCR test (Roche Diagnostic Systems). Also, laboratory diagnosis for common STDs, and collection of demographic information and behavioural data were performed. According to our results, *C. trachomatis* was detected in 46 women (1.72%) and 10 men (4.67%). As regard female population, 65% of them were married, 64% did not use condom, 12% were pregnant. Most commonly identified sexually transmitted pathogens were *Gardnerella vaginalis* (45%), and *Candida* (14%), while *Trichomonas vaginalis* infection was found in 3%, and HPV in 0.5% of the women tested. As regard male population, 51% of them were married, 73% did not use condom, and one man had coinfection with *N. gonorrhoeae*. Among patients with *C. trachomatis* infection, the majority of them (88%) were young (under 30 years), 67% of them reported more than one sexual partner within the last year, and 94% of the women had urogenital symptoms. As regard the type of specimen, cervical swab specimens were slightly more sensitive than FVU for the detection of *C. trachomatis* infection in women, and FVU compared with urethral swab in men. In case of couples tested, screening males alone detected 80% of the infected couples. After appropriate treatment, all *C. trachomatis* infected patients had negative result in the follow-up visit. Repeated infections with *C. trachomatis* were not detected. In conclusion, the prevalence of *C. trachomatis* infection in our geographic area is low. In standard clinical practice, there is need to screen both males and females, and test two types of specimens, urogenital swab and urine.

R2244 Occurrence of bacterial vaginosis, *Mycoplasma hominis* and *Ureaplasma urealyticum* among women infected with *Chlamydia trachomatis*

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Objectives: We compared two groups of women infected and non-infected with *Chlamydia trachomatis* to evaluate the frequency of bacterial vaginosis (BV), *Mycoplasma hominis* and *Mycoplasma urealyticum*.
Methods: Under investigation were 55 sexually active nonpregnant women aged from 18 to 53 years old (the average age = 29). Vaginal swabs were collected for the diagnosis of BV, using Amsels' criteria: – presence of 'clue cells' (vaginal epithelial cells so overlaid with bacteria that cell border was obscured) – amine 'fishy' odour upon mixing alkalinising vaginal fluid with one drop of 10% KOH solution – pH greater than 4.5 – thin, homogenous, milky vaginal discharge Endocervical swabs were collected for *C. trachomatis* and urogenital mycoplasmas detection using *Chlamydia* Direct IF assay (bioMerieux, France) and *Mycoplasma* IS-2 assay (bioMerieux, France), respectively.
Results: Significantly more cases of BV and urogenital mycoplasmas were found among women infected with *C. trachomatis* compared with non-infected group.
Conclusion: Infection with *C. trachomatis* appears to be a risk factor for occurrence of BV and urogenital mycoplasmas among women in reproductive age. This suggests the necessity of using of appropriate diagnostic procedures. BV is associated with different complications among pregnant and also non-pregnant women in reproductive age. Amsels' criteria are useful for rapid diagnosis and appropriate treatment of BV.

R2245 Micro-organisms associated with bacterial vaginosis and vaginal flora changes in part of the population of adolescent and adult women

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Objectives: Evaluation of associated micro-organisms isolated from the lower genital tract in part of the population of adolescent and adult women in gynaecological ambulances.
Methods: Samples from vaginal and endocervical flora of 383 women in the age from 17 to 58 years were diagnosed applying direct detection method (*Chlamydia trachomatis* RNA detection), nonspecific and specific culture isolation (*Trichomonas vaginalis*, *Neisseria gonorrhoeae*, nonspecific aerobic and anaerobic flora, *Lactobacillus* sp., *Gardnerella vaginalis*, detection and identification of *Candida* sp., culture of *Mycoplasma hominis* and *Ureaplasma urealyticum*.
Results: 37 adolescents (17–19 years) and 346 adult women (20–58 years) were studied in the year 2003. Most frequently isolated micro-organisms were *U. urealyticum* (22.3%), both *M. hominis* and *U. urealyticum* (15.0%) followed by *Candida* sp. (12.7%). In 7.3% were isolated both *U. urealyticum* and *Candida* sp. *Mobiluncus* sp. was detected in direct smears in 3.0%. Anaerobes were isolated in 8.3%, *Gardnerella vaginalis* in 4.3%. *C. trachomatis* RNA was detected only in 9 cases (2.3%). Typical sexually transmitted patogens (*N. gonorrhoeae* and *T. vaginalis*) were isolated only in 1 case (0.03%).
Conclusions: A high prevalence of *M. hominis* and *U. urealyticum* is remarkable, but their pathogenic role is not clear. In 52.3% samples with *U. urealyticum* was isolated *Lactobacillus* sp., which is considered as a normal vaginal flora. Vaginal candidosis is also important, 6 species of *Candida* was detected, of which *Candida albicans* is dominant (81.6%). Low prevalence of *T. vaginalis*, *N. gonorrhoeae*, *C. trachomatis* and *G. vaginalis* is remarkable. Wide analysis of all potentional pathogens is useful, all correlations can be considered and interpretated succesfully.

Molecular virology (incl. diagnostics)

R2246 HBV genotypes in a selected group of patients hospitalised in provincial hospitals in Lublin, Poland

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Objective: The aim of present study was to determine the viral genotypes in the chosen group of patients in different hepatitis B stages.
Materials and methods: There were sera samples collected from 20 patients (3 women and 17 men) hospitalised in province hospitals in Lublin. The patients' age ranged from 10 to 75 years. Firstly, HBV DNA level was examined in each sample by using commercial molecular hybridisation kit (Digene Hybrid Capture System HBV assay). Then the viral genotypes were detected according to Inno-LiPA HBV DR Amplification and Inno-LiPA HBV genotyping (Innogenetics) manufacturer's instructions. QIAamp DNA Mini Kit (Qiagen) was used for isolation viral DNA from the sera samples.
Results: All studied sera were positive for HBV DNA. HBV genotype A was the most common in studied samples. It was determined in 16 (80%) patients. There was genotype D detected in 3 (15%) samples and both genotypes A and D were present in 1 (5%) serum.
Conclusions: Our results are similar to these presented by other authors maintaining that the genotype A is the most common in north – west of Europe.

R2247 Detection of enteroviruses, adenoviruses and rotaviruses in sewage with nested RT- PCR

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Objectives: Enteroviruses, adenoviruses and rotaviruses are incriminated for a lot of epidemics of gastroenteritis constituting a significant problem for the public health. The viruses resist in all the common methods of sewage treatment that are applied in the most biological treatment plants. Aim of present work is the detection of these viruses in sewage of Attica and Achaia regions. The sampling was performed in the period between July 2001 and January 2003 from the entries of the biological treatment plants of Psitallia and Patras.
Methods: For the detection of these viruses, molecular techniques such as RT-PCR and nested PCR were used, that have significant sensitivity and specificity. Initially, the condensation of the sample was performed with centrifugations and ultracentrifugation, in order to isolate the viruses. Followed, isolation of RNA of enteroviruses and DNA of adenoviruses with adsorption of nucleic acids in aluminium particles. For the isolation of rotaviruses, a specific RNA isolation kit was used (QIAGEN). For the detection of viruses, RT-PCR and nested PCR was used with the use of suitable primers. The size of the 1st PCR products was 301 bp for adenoviruses, 540 for enteroviruses, 1062 for rotaviruses, while in

the nested PCR the sizes were 143 bp for adenoviruses, 123 bp for enteroviruses and 189 bp for rotaviruses. For the evaluation of reliability of the method positive markers were used supplied by CEFAS, Weymouth, UK. For rotaviruses, positive samples were from patients with gastroenteritis were used. In the end, PCR products were visualised by electrophoresis in agarose gel 2%.
Results: 50 samples of untreated sewage were processed. All three viruses were detected with the methodology used. Concretely, 19 samples were found positive for enteroviruses (38%), 42 samples were found positive for adenoviruses (84%) and 19 samples were found positive for rotaviruses (38%). The positive samples for adenoviruses were typed and found to be all adenoviruses 40 and 41.
Conclusion: Increased isolation of adenoviruses in the untreated sewage strengthens the opinion for their utilisation as indicators of virological pollution of environment. It was the first time to detect rotaviruses in sewage.

Viral diseases

R2248 Clinical complications of norovirus infections in patients with severe underlying disease: a new syndrome of norovirus infection?

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Introduction: The effects of Norovirusinfections have been described as a short self-limiting disease. An investigation of a Norovirus outbreak in an universitiy hospital provided evidence for severe complications in patients (pts) with severe underlying diseases.
Methods: Attack rates were determined. Clinical outcomes were defined: >1 day diarrhoea, >1 day vomit, creatinine rises >10%, potassium decrease >20%, CRP >56 mg/mL. Risk factor analyses regarding underlying diseases and medication have been performed using multivariate analyses. A norovirus infection syndrome was defined using the sum of possible clinical outcomes as a score for severity.
Results: Five wards experienced NV outbreaks in the period from November 1, 2002 to January 31, 2003. These wards belonged to the following departments: psychiatry, nephrology, gastroenterology, cardiology and traumatology. In 5 outbreak wards 84 patients and 60 nurses had been infected and revealed an overall attack rate of 32% (pts) and 76% (nurses), respectively ($P < 0.0001$). 71 patients acquired nosocomial infections. 23 pts showed clinical symptoms longer than 48 h, 14 pts developed relapses, 3 pts needed haemodialysis, 1 pt needed cardioversion for three times, creatinine increases >10% were seen in 22 pts, potassium losses >20% in 7 pts, observed CRP levels were high (median 58 mg/mL). Age >65 years (OR 12.6, CI95 2.0–243 for prolonged course), immunosuppressive therapy (OR 22.7, CI95 3.7–449 for at least 4 possible positive outcomes), cardiovascular disease (OR 17, CI95 2.2–401 for potassium decrease >20%), renal transplant (OR 13.8, CI951.7–298 for potassium decrease >20%) had been determined as risk factors for above defined outcomes.
Conclusion: The high attack rates of Norovirus infections demonstrates a high contagiosity of this virus-variant. In contrast to healthy people Norovirus infections in hospitalised patients may lead to severe consequences forming a certain new syndrome. Therefore strict infection control measures should be applied.

R2249 Q-fever in Bulgaria in contemporary conditions

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Q-fever is most important rickettsiosis in Bulgaria. Observed are a large number of sporadic cases and many epidemics covering from several tenths to hundreds of sick persons. The rickettsiosis is established on the territory of the whole country. A great number of epizootics among the domestic animals were described. In humans Q – fever is observed in two essential forms: acute – mainly atypical pneumoniae and chronic – Q-rickettsial endocardites. During 1993–2000 years 14 353 sera from patients were tasted for antibodies against *C. burnetii* II phase. The positive reactions were 14.97%. In most of the sick persons no risk element was found. A process of decrease of cases of Q-rickettsial pneumoniae is observed. During the same period are discovered and traced 22 patients with Q-rickettsial endocardites (1.02% of the acute forms). During the last years is obvious a tendency to decrease the number of the seropositive against *C. burnetii* domestic animals. In 1989–2002, 226 085 sera from cattle, sheep and goats were investigated and the seropositive were 5.17%. Highest is the percentage of positive goats – 7.19%. For comparison – during 1950–1976 years the seropositive animals were 17.56% (23 259 samples tested) and in 1977–1988 the positive were 14.79% (38 470 tested). Those are periods of reforms in the Bulgarian agriculture.

R2250 Measles virus genotypes in Belarus

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Objectives: The European Regional Office of WHO has targeted measles for elimination from the region in 2007. In view of the goal of measles elimination, it is of great importance to assess the circulation of wild-type measles viruses (MV). However, there is no available information on measles virus genotypes circulating in Republic of Belarus. Measles cases admitted to the Hospital of Infectious Diseases of Minsk (Belarus) within 2002–2003 have been prospectively analysed. Clinical, routine laboratory, as well as serological and virological examination of 13 patients hospitalised with the diagnosis of measles infection has been carried out. Patients, 16–25 years of age, had different history of vaccination against measles. The work has been done in the framework of a grant BTEP ID#16, 2002.
Methods: Patients' clinical and routine laboratory data were collected prospectively. Paired serum specimens obtained from these patients were examined at the SRC VB 'Vector' (Russia). Serum specimens were assayed for the presence of MV-specific antibodies using commercial test kits. Differential analysis with rubella was carried out. Measles virus infection was confirmed by RT-PCR. Two primers specified for the hyper variable part of the N gene were used for RT-PCR. The nucleotide sequence analysis (Beckman Coulter XL-2000 DNA Analysis System) was carried out.
Results: All 13 measles cases described here were laboratory confirmed. Sequence analysis revealed that genotype A was mostly present in 2002. Isolates belonged to MV genotype D6 and D7 were identified through 2003. Retrospective analysis of clinical manifestations showed measles infection caused by D6 and D7 virus genotypes had a more severe and prolonged course of the disease in comparison with measles infection caused by A virus genotype. Serological investigation revealed only D6 and D7 genotypes caused MV-specific IgM appearance and four-fold MV-specific IgG growth in paired serum samples. The clinical cases of A genotype MV infection revealed no IgM appearance within the initial course of the disease, and IgG growth no more than 2–3-fold.

Conclusion: D6 and D7 MV genotypes have been registered in Minsk (Belarus). These MV genotypes caused more severe disease with the manifested antibody response, while A genotype caused a milder disease with a weaker antibody response.

R2251 Heterologous expression of the HPV16.E6 increases the resistance to stress

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The malignant phenotype of high-risk types human papillomavirus depends mainly on the expression of two viral genes, E6 and E7. If control of E6 is lost, this protein can trigger immortalisation of cells and cancer. The aim of this study is to determine if E6 protein changes the response of yeast to stress, as a model of eukaryotic cell immortalisation. In the present work we expressed HPV16 E6 (pYES2.E6) in *S. cerevisae* in order to study the response to thermal stress, to pheromone recovery, and to caffeine treatment. The heterologous expression of E6 in yeast results in a higher resistance towards caffeine, but there are no changes when the cells are incubated in high temperatures. In what regards pheromone treatment, E6 expressing cells are able to overcome the cell cycle arrest, albeit exhibiting the morphogenetic alterations due to the presence of pheromone. Since the protein kinase C is a central protein in the general response to stress a deleted mutant in PKC1 was transformed with pYES2.E6, in order to study the suppression of the increased resistance to stress. The subcellular localisation of the protein E6, under stress conditions, is studied by co-localisation of the fusion protein E6-GFP, using an inducible (pYES2) and a constitutive (pUG35) system. The results obtained points to the conclusion that the heterologous expression of E6 in the yeast *S. cerevisiae* leads to an increased resistance to stress, showing that E6 interacts with specific components of the biochemical machinery of yeast cells.

R2252 Evaluation of thyroid functions during interferon-alpha treatment of patients with chronic viral hepatitis

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Objectives: Recombinant interferon-alpha (IFN-alpha) is used in the treatment of chronic viral hepatitis. During IFN-alpha treatment thyroiditis, Graves' disease, permanent hypothyroidism can develop. This retrospective study is designed to determine the frequency of thyroid disease that is seen during IFN- alpha treatment of patients with chronic hepatitis.
Material and Methods: We investigated the frequency of thyroid functions in 182 patients with chronic viral hepatitis treated with IFN-alpha in Department of Clinical Microbiology and Infectious Diseases. 90 (49.6%) patients had chronic hepatitis B (CHB) and 92 (50.9%) of them had chronic hepatitis C (CHC). Thyroid hormones levels were determined before, during (3 and 6 months) and after (6 months) treatment.
Results: 62 (68.9%) of CHB patients were male and 28 (31.1%) were female. The median age was 34 $\pm$ 11 years (range 17–62). 37 (40%) of CHC patients were male, 55 (60%) were female and the median age was 49 $\pm$ 11 years (range 19–65). Thyroid dysfunction was found in 10% of all patients. The incidence of thyroid dysfunction was 6.7% in CHB whereas 15.2% in patients with CHC ($P = 0.1$). Among these cases, 16 of 20 were females (80%) and 4 were males (20%) ($P < 0.02$. OR = 5.6). In 12 patients hyperthyroidism was detected and the others had hypothyroidism. In two patients, hyperthyroidism antithyroid treatment was required after the cessation of IFN-alpha treatment. Among the patients with hypothyroidism, only one case necessitated hormonal replacement. Spontaneous remission was observed in other patients.
Conclusions: Thyroid dysfunction is commonly seen among patients with chronic viral hepatitis who receive IFN-alpha; therefore, close follow-up of these patients should be recommended.

R2253 Determination between recent and past cytomegalovirus infection

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Introduction: The detection of virus-specific IgG and IgM antibodies is of great value in the diagnosis of acute/primary CMV infection in women during their reproductive period. If both anti CMV IgG and IgM are detected in first monitoring, it is not possible to clearly define whether or not infection event is going in the patient as IgM may last for months from primary infection. Antibodies with low avidity are detectable at a very early stage of infection whereas high avidity antibodies indicate past infection.
Material and methods: A total of 51 sera from patients with diagnosis 'spontaneus abortion' were examined with ImmunoLISA CMV IgG, IgM, and CMV IgG Avidity (Orgenics, Israel). This tests is assigned for simultaneus detection of specific, IgG and IgM antibodies for CMV. Anti CMV antibodies are treated with a denaturating agent, able to dissociate low affinity bound IgG antibodies from the coated antigen.
Results: Specific anti CMV antibodies were detected in a total of 51 examined sera. Positve for specific IgM antibodies were only 2 sera(4%). As for the avidity of detected IgG antibodies in 49 (96%) sera the value was high, while in only 2(4%) the value was low, indicating possible resent infection. Simultaneous presence of both classes of antibodies could indicate anamnestic information of previous infection, where IgM antibodies are residual or ongoing acute/primary infection.
Conclusion: The analysis of gained data, showed that positivity to specific CMV IgG antibodies is frequent. Due to the rare detection of low avidity index of these IgG positive determinated sera, we agree with the recommendations for implication of this avidity test mostly in the individuals belonging to the groups: pregnant women and transplantated patients.

AIDS and HIV infection

R2254 Gynaecomastia in a vertically-infected adolescent treated since birth

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Objective: gynaecomastia is an emerging untoward event of HIV infection treated with antiretrovirals. Although first related to protease inhibitors (PI) and eventual associated dysmetabolism, it has been recently reported also in patients (p) who abandoned PI-based HAART or never received PI.

Case report: At the age of 13.5 years, a p with congenital HIV infection developed a true, sonography-confirmed bilateral gynaecomastia associated with local paresthesia. Developmental milestones and pubertal stage were within normal limits. Chronic kidney-liver disease and administration of drugs potentially related to gynaecomastia were carefully excluded. An endocrinological workout did not detect any gonadal, hypophyseal and thyroid abnormality and prolactin proved normal. Notwithstanding the absence of HIV-related disorders, at the age of 5 our p started anti-HIV therapy due to increasing viremia. During the 8.5-year follow-up, 5 different lines were administered including HAART

regimens based on ritonavir, nelfinavir and lopinavir/r. Stavudine (61 months) was the nucleoside analogue (NA) administered for the longest time. At the time of onset of gynaecomastia (8 months ago), a negligible viremia was retrieved (280 HIV-RNA copies/mL) while CD4+ count was favourable (848 cells/μL). Clinical alterations included in the definition of the lipodistrophy syndrome and abnormalities of lipid-glucose metabolism were never detected. gynaecomastia did not show significant modifications during the 8-month monitoring.
Discussion: Our case report is exceptional, since among ~80 literature cases of gynaecomastia described in HIV-infected p, none emerged in paediatric–adolescental age. Both pathogenesis and evolution of gynaecomastia are increasingly investigated and special attention is deserved to relations with dysmetabolism and the fat redistribution syndrome (all absent in our report), as well as antiretroviral agents. In the reported p, NA were administered during 8.5 years, and PI during 7 years while non-NA reverse trascriptase inhibitors were never used. While all PI were involved in the described cases of gynaecomastia, among NA stavudine seems to be the most frequent drug although biases due to the common resort to this last compound in HAART combinations, cannot be excluded. Our p represents the first case of true gynaecomastia reported in pediatric HIV disease. The absence of dysmetabolic and lipodistrophy alterations makes the pathogenesis uncertain

R2255 HIV-Infected patients: detection of HIV-1 drug resistance-associated mutations

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Madrid, E

Objectives: Drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) Reverse transcriptase and protease genes lead to lower sensitivity to antiretroviral agents and are an important cause of drug failure. The aim of this study was evaluating the presence of mutations in antiretroviral treated patients.
Methods: The plasma of 314 HIV-1 infected patients were analysed. Plasma of patients were collected for genotyping analysis to identify resistance mutations in reverse transcriptase and Protease regions using ABI ViroSeq HIV-1 genotyping system. Changes in susceptibility were assessed by a computer program that interprets drug resistance: Standford database (http//:/hivdb.standford.edu/hiv/).
Results: On the HIV-1 Reverse Transcriptase region 1097 mutations were detected, 717 (65.36%) of them were primary mutations correlated with high level of drug resistance. On the Protease gene 1091 mutations were found, 749 (68.65%) of them were secondary mutations. Wild codons were determined in 9.23% (29/314) on the Reverse Transcriptase region and 7.32% (23/314) on the Protease gene. Genotyping was not available because not enough cDNA were obtained in 22 (7%) samples. Presence of mutations related with high-level of resistance were detected: 141 to Zidovudine, 128 to Lamivudine, 183 to Nonucleoside RT inhibitors and 223 to Protease inhibitors.
Conclusion: The detection of key mutations associated with HIV-1 antiretroviral resistance is very useful because it enhances clinical management, but further studies and more data are needed to find more relevant clinical procedures.

R2256 Evolution of HIV-1 resistance mutations in patients on antiretroviral therapy

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Objectives: Antiviral drug resistance testing is being incorporated increasingly into daily clinical management of HIV-1 infected patients since the emergence of drug resistance is an important limitation of antiretroviral therapy.
Methods: We examined the evolution of drug resistance mutations in 11 therapy-experienced patients who had three resistance test separated by a mean of 12 months (range 4–15). Plasma of patients was collected for gentyping analysis to identify resistance mutations in Reverse Transcriptase and Protease regions using the ABI ViroSeq HIV-1 genotyping System with interpretation of antiretroviral susceptibility by the Standford database.
Results: The median viral load (copies/mL) and mean number of mutation/patient for the first, second and third time-points were 204 000, 5.5; 122 000, 6.1 and 211 000, 8.1, respectively. On the HIV-1 Reverse Transcriptase region 202 mutations were detected, 84 of them were primary mutations correlated with high level of resistance. Most common mutations observed were on codons 67 and 215 (22) and 181 and 190 (14). On the *Protease* region 222 mutations were found, 166 of them were secondary mutations. Prevalent mutations involved substitutions at codons 10 (21), 63 (31), 71, 26 and 90 (23). High prevalence of nucleoside inhibitors resistance-associated mutations was determined in eight of the 11 patients continuosly. Multinonucleoside-resistance was present in every patient during all the study. Eight of the studied patients had mutations associated with Protease inhibitors resistance from the beginning.
Conclusion: The use of resistance test may significantly improve the selection of optimal treatment regimens for patients after failure of previous therapies.

R2257 Nodular regenerative hyperplasia, new HIV-associated disease?

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Many liver diseases have been described in HIV patients, some of them are due to HIV treatment. We report one case of liver disease not recognised as drug side effect. A 59-year-old woman, known to be infected with HIV infection presents a chlostatic hepatitis compatible with the diagnosis of nodular regenerative hyperplasia. Nodular regenerative hyperplasia (NRH) of the liver is well described and characterised grossly by diffusely nodular liver resembling micronodular cirrhosis. Nevirapine-induced hepatitis occurrs shortly after drug initiation in patients with and without pre-existing liver disease. Significant elevations in liver enzyme levels occurr but resolve promptly in most cases with discontinuation of the nevirapine. NRH has never been reported previously in HIV infection and at the moment there are no NRH associated with nevirapine side effect.

R2258 Immune reconstitution without antiretroviral therapy in patient with AIDS and tuberculosis

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Objectives: Tuberculosis has a negative influence on HIV infection, enhancing the viral replication and accelerate the progression of HIV disease. We report a case of immune reconstitution under antituberculous therapy in a patient with AIDS and lymph nodes tuberculosis, without HAART.
Methods and results: A 29 years old male, heterosexual, non-drugs user was admitted in our hospital for fever, lymphadenopathy and loss of weight. He presented: fever, dry skin, submaxillary and laterocervical lymphadenopathy (6/4 cm), left axillary lymph nodes, tachycardia (120/min), TA = 120/80 mmHg. Also he had inflammatory syndrome (ESR = 120–130mm/h, leukocytosis = 10100/mm^3, CRP = 3.65 mg/dL), blood cultures were negative for usual strains (BacT/Alert). Positive ELISA for HIV was confirmed by Western Blot and CD4 count was 102/mm^3. Cervical ultrasonography examination showed many right cervical

lymph nodes disposed in cluster with medium enhanced echogenity. The abdominal ultrasonography examination showed lymphadenopathy in the liver's hila above 1.6 cm and retropancreatic. Lymphoma was excluded by haematological examination. Lymph nodes histopathology showed small granuloma containing macrophage cells with epithelioid character and 1–2 giant multinucleated Langerhans cells, suggestive for lymph node tuberculosis. Tuberculosis has confirmed by histopathology. We couldn't do blood cultures for mycobacterium and the cultures from lymphadenopathy biopsy remained negative. The patient was under antituberculous therapy in association (isoniazid + rifampin + ethambutol + pyrazinamide); he didn't receive any antiretroviral therapy. After 2 months of antituberculous therapy, lymphadenopathy and ESR decreased, leukocyte was 6800/mm^3 (PMN = 71%, lymphocytes = 27%, monocytes = 2%) and CD4 count increased 263/mm^3. After 7 months of antituberculous therapy, re-evaluation showed: lymphadenopathy disappeared, ESR = 30–50 mm/h, leucocytes = 4500/mm^3 (PMN=50%, lymphocytes = 40%, monocytes = 5%, eosinophiles = 5%) and CD4 count was 241/mm^3. Ultrasonographic, lymph nodes with vascularisation, necrosis and calcification were described.
Conclusions: Despite the severe immunosupresion, probably the lack of other coinfections produces a satisfying immune response after 7 months of antituberculous therapy. Therapy of tuberculosis as associated infection in AIDS patients can produce immune reconstitution even in the absence of antiretroviral therapy

R2259 HIV-related pulmonary hypertension. A case report and literature review

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Background: Isolated pulmonary hypertension is a rare manifestation of HIV infection, with a reported frequency of 0.5% among HIV infected patients. To date more than 200 symptomatic cases have been reported. Diagnosis is not related to CD4 count or HIV-RNA levels, prognosis is unfavourable and the mean survival is 6 months. Standard therapy includes oxygen administration and vasodilating agents. Sildenafil has also been used since 1998. The effect of HAART is unknown.
Purpose: To report a new case of HIV-related pulmonary hypertension in an asymptomatic patient presenting with low oxygen saturation.
Case presentation: 36-year-old male, heavy smoker (60 pack/years), diagnosed as HIV-positive 5 years ago (CDC stage C3), with poor compliance to antiretroviral treatment. He presented in the outpatient HIV clinic totally asymptomatic. His vital signs were normal except for low O2 saturation (85–88%). Clinical findings were unremarkable, and his laboratory tests and electrocardiogram normal. His CD4 count was 24/mm^3 and HIV-RNA 1 500 000 copies/mL. The high-resolution chest CT showed emphysematic lungs and spirometry revealed final bronchiolar disease without obstructive pulmonary disease. The transthoracic cardiac ultrasound documented severe pulmonary hypertension (systolic pulmonary arterial pressure ~63 mmHg). He refused to undergo pulmonary catheterisation. All risk factors for secondary pulmonary hypertension were excluded in this patient (addiction to iv drugs, interstitial pulmonary disease, chronic obstructive disease or acute infection) and the diagnosis of HIV-related pulmonary hypertension was established. Diltiazem and HAART regimen were administered, but, once discharged from the unit, he did not adhere to the treatment. Eight months later, he is hospitalised due to worsening dyspnoea.
Conclusion: HIV-Pulmonary Hypertension should be included in the differential diagnosis in patients with pulmonary hypertension and respiratory failure, despite the absence of typical symptoms.

R2260 Invasive infections in Romanian HIV patients

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Objectives: To monitor the frequency of bacteraemia and fungaemia as well as spectrum of pathogens isolated in blood cultures of HIV infected patients admitted in our clinic.
Methods: We evaluated the results of blood cultures using BACT/ALERT automated systems and the resistance of pathogens isolated from HIV infected teenagers and adults, during 06/2002–12/2003. Antimicrobial susceptibility tests were performed by the disk diffusion methods according to the NCCLS standards.
Results: Of the total of 457 blood cultures, 30 were positive (6.56%), isolated from 29 patients Three pathogens were isolated only in one patient (*Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphyloccocus aureus*). The mean age of teenagers was 15 years and of adults were 34 years, 23 men and 10 females. gram-negative micro-organisms consisted 50% of pathogens and the most of them were *Salmonella* spp. (23.33%), the second being *Haemophilus Influenzae* (13.33%). *Bacteroides fragilis* was isolated only from one patient. The others were *E. coli*, *Shigella* spp., *Klebsiella pneumoniae*, each of them 3.33%. *Cryptococcus neoformans* (26.67%) has been isolated in patients suffering from meningitis or only fungaemia (3.33%). gram-positive micro-organisms (30%), in particular *Staphylococcus* spp. (26.67%) were the other pathogens. All the patients suffered from severe immunodeficiency (value of CD4 lymphocites count was <200/mmc). No patients received antiretroviral therapy. Carbapenems, vancomycin, teicoplanin, linesolid were the most sensitive drugs. Trimethoprim/sulphamethoxazole, a prophylactic drug was resistant for most of micro-organismes. The fungal isolates were sensitive to all antifungal drugs, except flucytosine. Sepsis and fungal meningitis were the most frequent cause of death, 69% of patients being still alive.
Conclusions: Gram-negative bacteria (especially *Salmonella* spp. and *H. Influenzae*) and *Cryptococcus neoformans* are isolated from HIV infected pacients in most of cases. The high resistance rates were found in *Pseudomonas aeruginosa* and some *Staphylococcus* spp. All *Cryptococcus neoformans* were resistant to flucytosine, two of them becoming resistant to fluconazole during the treatment. HAART therapy and Hib vaccinations can improve the prognosis of severe immunocompromised HIV patients.

R2261 Efficacy and safety of the combination of tenofovir and didanosine as a backbone regimen

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Background: Tenofovir, as a Nucleotide analog Reverse Transcriptase Inhibitor, is one of the new antiretrovirals used in HAART. Since Tenofovir (TFV) combinations have only recently been in use, there is a lack of clinical data regarding to the co-administration of didanosine (ddI) and TFV.
Objective: To record the results of an antiretroviral regimen containing ddI+ TFV as backbone in terms of HIV-RNA levels and CD4 cell count and safety
Methods: Eleven (11) HIV (+) patients (10 males), with a mean age of 43.9 years (range 30–72) were studied retrospectively. A combination of TV (245 mg) and ddI (250 mg) was administered with either a PI (most commonly lopinavir/ritonavir) or efavirenz.
Results: Among the 11 patients, only 1 was nave, 8 were classified as CDC stage A, 1 stage B and 2 stage C. The mean baseline values for CD4 and viral load were 283 c/mm^3 and 375 172 c/mL (4.34 logs), respectively. After a mean time of 18.5 weeks (range 8–36) under the new regimen the patients had mean CD cell count 373 c/mm^3 and HIV-RNA mean value of 14326 c/mL (2.36 logs). Fasting administration of ddI was always followed. One patient discontinued medication due to gastrointestinal adverse events

and a second one performed poor compliance. Only one patient presented mild hypophosphotemia.
Conclusions: Co-administration of TFV and ddI (250 mg) seems to be effective in experienced HIV(+) individuals. The use of didanosine at reduced dose was not associated with adverse events or significant toxicity.

R2262 C-reactive protein: which role today in infectious diseases?

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Objectives: C-reactive protein (CRP) is a marker of acute or chronic inflammation, routinely used in the clinical practice; it represents a more specific and precocious value of diseases activity if related to erythrocytes sedimentation rate. PCR is significantly higher in certain diseases as sepsis, pneumonia, osteomielytis and prosthetic joint infections and is also related to the severity of illness but results lower in immunocompromised patients (pts).
Methods: We prospectively analysed a group of patients admitted to our department in the period January 2002–October 2003; we included the following conditions: pneumonia, sepsis, meningitis, soft tissue infections, orthopaedic infections and HIV+ pts with concomitant infections. We considered the following parameters: gender, age, cause of admission, CRP at the admission and serological status for HIV. For our study we used a multi-way analysis of variance.
Results: We considered 125 pts (50 females), mean age 48 years (range 16–85), sorted in six different diseases: 32 pneumonia (13 HIV+), seven sepsis (1 HIV+), 18 meningitis (all HIV−), nine soft tissues infections (all HIV−), 15 orthopaedic infections (one HIV+) and 44 pts HIV+ with diseases others than the previous. CRP mean value at admission for each pathology was: 75.5 mg/L in pneumonia (99.3 in HIV− and 44.2 in HIV+), 195.7 mg/L in sepsis (158.7 HIV− and 418 HIV+), 66.5 mg/L in meningitis, 130.4 mg/L in soft tissues infections, 80.8 mg/L in orthopaedic infections (86.3 in HIV− and 4.2 in HIV+) and 37.7 mg/L for HIV+ with other pathologies. The mean CRP value for HIV− pts was 102.0 mg/L significantly higher then the mean CRP value (38.6 mg/L) observed in HIV+ pts ($P < 0.05$). We found a significant correlation between CRP and diseases analysed; there were higher values in sepsis, soft tissues infections and pneumonia.
Conclusions: Our study confirms the importance of CRP in monitoring infectious diseases, especially in sepsis, soft-tissue infections and pneumonia, where the CRP is significantly higher than in others infections. Besides we found relevantly lower the CRP value in HIV infections: this confirms the literature's data about the role of immunodepression and hepatic comorbidities in lowering CRP. However, if we compare the causes of access in hospital in HIV+ and HIV− pts, we have to consider that in the first group there are more cases which could be cured as outpatients, and that could partially explain this great difference in PCR value.

R2263 Role of *Chlamydia pneumoniae* in atherosclerotic plaques seen in HIV-positive patients treated with different antiretroviral regimens

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Vascular complications have been described in HIV-positive patients treated with highly active antiretroviral regimens (HAART). Protease inhibitors (PI) have been associated with the onset of metabolic disturbances, including lipodystrophy and it has been suggested that may play a role in determining an atherosclerotic damage of the vascular wall. Recent appreciation of atherosclerosis as a chronic, inflammatory disease has rekindled efforts to examine the role that infectious agents may play in atherogenesis. Several infectious agents have been implicated as likely culprits of atherosclerosis, being *Chlamydia pneumoniae* the most suspected. To evaluate the possible role of *C. pneumoniae* in the pathogenesis of atherosclerotic plaques in HIV-1 positive individuals, we have detected the presence of antibodies to *C. pneumoniae* in 59 HIV-infected patients: 30 (group A) with carotid plaques and 29 (group B) without carotid lesion. Sixty-two healthy individuals were the control group. Regarding the antiretroviral treatment, six patients of group A were naives to antiretroviral therapy, three were treated with NNRTI based regimens, 21 with PI based regimens. Among group B, 10 patients were naives, two were treated with two NRTI, two with three NRTI, nine were treated with NNRTI-based regimens, five with PI-based regimens. All patients in groups A and B treated with antiretrovirals were in stable therapy from at least 12 months. The patients were evaluated also for the presence of independent risk factors, such as cigarette smoking, alcohol consumption, cholesterolaemia and triglyceridaemia. No significant correlation in the *C. pneumoniae* seroprevalence and titer (IgG and IgA) was observed between group A and group B and between these two groups of patients and the control group. Patients using PI seem to be more prone to have atheroma in comparison with the HIV-positive patients treated with NNRTI ($P < 0.0001$). In conclusion, in HIV-positive patients, the serological status for *C. pneumoniae* is not associated to development of atherosclerosis. Analysing the role of therapy with PI and other well known risk factors for the development of cardiovascular disease, it seem that the antiretroviral therapy may play a pivotal role in the development of cardiovascular diseases involving, in our study, the epiaortic vessels confirming similar evidences reported in the literature.

R2264 Response of HIV-positive patients to the long-term salvage therapy by LPV/r

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Objectives: The cohort of 19 protease inhibitor (PI) experienced patients was followed for the period of up to 37.5 months, during the years 2000–2003. Changes of HIV-1 RNA plasma level and CD4+ count were monitored and evaluated with regard to the baseline characteristics.
Methods: The HIV-1 RNA plasma level was determined by a quantitative RT-PCR; the CD4+ cell count was determined by FACS. Analysis of the protease-coding region was performed by direct sequencing of the PCR product (derived from the patient's peripheral blood mononuclear cells or plasma).
Results: First virologic and immunologic values were determined after 3 months of therapy. Nine patients achieved complete suppression of viral load, five patients showed only partial supression (at least by 1 order of magnitude) and five patiens did not respond at all. The increase of CD4+ count closely corresponded to decrease of viral load. Median increase by 100 cells/mL was detected for patients with undetectable viral loads, 40 cells/mL for patients with partial suppression and median decrease by 40 cells/mL in cases of virologic failure. Further analysis of patients' response lead to redistribution of patients to two groups: 'responders' – nine patients who exhibited complete suppression of viral replication for the period of up to 37.5 months and 'non-responders' – 10 patients who experienced virologic failure on LPV/r treatment. The high incidence of negative predictive factors such as lopinavir mutation score higher than five, prior use of more than 3 PIs and occurrence of I54V mutation correlated with long-term virologic failure. The distribution of these factors was not significant from the short-term point of view. On the contrary, the correlation of positive factors (low lopinavir mutation score, association with efavirenz and occurence of V77I mutation) with decrease of viral load was more signifficant in the short-term analysis.
Conclusion: The results of LPV/r salvage therapy showed to be encouraging. 47% of patients from our study achieved stable suppression of viral replication accompanied by significant improve-

ment of CD4+ count for 31 months on average. Furthermore, the LPV/r proved to be potent inhibitor despite unfavourable prognosis, even though the effect lasted only few months.

R2265 The occurrence of CCR5 del32 and CCR2B alleles in HIV-1 positive persons in the Czech Republic

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Objectives: Chemokine receptors CCR5 and CCR2 are important surface coreceptor structures, which are reported to influence the course of HIV-1 infection. It is known that people homozygous for mutated CCR5 gene are HIV-1 resistant due to nonexpression of CCR5, and the course of infection is delayed in heterozygotes. However, 64I mutation of CCR2 gene (CCR2B) was found to delay disease progression but not to reduce infection risk. Our study was aimed to detect the frequency of both CCR5 del32 and CCR2B alleles in the HIV-1 positive persons in the Czech Republic.
Methods: Genomic DNA samples from peripheral blood of 158 HIV-positive patients from five AIDS centres were analysed by PCR and PCR-RFLP method, as we previously reported, for the presence of CCR5 del32 and CCR2B alleles.
Results: CCR5 32-bp heterozygous deletion was present in 17.7% samples, homozygous genotype was not found. Of 26 samples tested for the presence of CCR2B allele two samples were found to be heterozygous and one homozygous. The analyses of additional samples are continuing.
Conclusion: Our data on CCR5 del32 are similar to data from other Western Europe HIV-1 positive populations. Our preliminary findings on CCR2B suggest lower frequency than previously published. We suggest examination of all HIV-positive patients for the presence of CCR5 del32 and CCR2B alleles before the start of the treatment with C–C antagonists in the future. Our work was supported by grants of Ministry of Health 96/55 and by Charles University VZ 111 4 0000 5.

R2266 Infections (non-AIDS) in a HIV-positive population

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Apart from the opportunistic infections that define AIDS, group HIV shows a greater incidence of certain concomitant infections than the general population, in relation to the route of infection. We studied the existence of syphilis, hepatitis C virus (HCV), cytomegalovirus (CMV) and herpes simplex virus type 2 (HSV-2) in a group of 764 carriers of our sanitary area (311.720 people, prevalence of 2451/100 000 inhabitants). The analytical techniques of FTA-ABS, EIA, W-B, LIPA, etc., have been the habituals ones of laboratory. Among HIV positive group, 93 cases (12.2%) of syphilis have been diagnosed (six with positive serology also in CSF), predominating in men (77.4%) with average age of 38.7 years old (24–64 rank), on women (average age 34.0 years, rank: 21–43 years). In the infection by HCV, the co-infection is of 151 cases (19.8%), fundamentally genotype 1 (54.3%), 3 (23.8%) and 4 (19.2%), corresponding the 82.8% to men with average age of 37.9 years old, without difference of age withing the female gender. In 17 cases (2.2%) there were coinfection by both virus, mainly in men (76.5%). It was detected CMV in 416 carriers (54.5%), mainly in men (75.5%), with an average age of 37.1 years old, against 31.4 years old in women. The infection by HSV-2 was present in 35.4% of the infected ones, being men 65.7% with an average age higher than the women (40.8 and 33.3%, respectively). Our conclusion is that there are a high rate of prevalence in the infection by HIV among ower population, and coinfection, fundamentally with CMV, HSV-2 and HCV, emphasising the last one because of it aggravates the prognosis of the infection by HIV, interfering in the treatment and increasing its mortality remarkably.

R2267 Experience with lopinavir: solution vs. pills

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Objective: Lopinavir (LPV) is a protease inhibitor (PI) which is available both in solution (SOL) and pills (PL): 5 mL bid vs. three pills bid, so the solution seems to have a kinder adherence. We want to study the LPV treatments and compare the SOL and the PL treatments, considering indications, tolerability and efficacy.
Method: Retrospective study of all the LPV treatments from December 2001 to February 2003 in the HIV unit of the Costa del Sol Hospital, a general hospital in Andalucia (South of Spain) attending to 400 HIV-infected patients.
Results: A total of 59 patients have received LPV. Mean age: 42.9 years (30–59), 19 female (34%) and 40 male (71%). Mean time from HIV diagnosis: 11 years. Risk: 44 (74.7 %) drug-abusers, 11 (18.6%) heterosexuals and four (6.7%) homosexuals. Three patients consumed toxic actively. CDC stage: C3: 40, A3: 8, A2: 5, B2: 2, B3: 2 and C2: 2; so 50 (89%) <200 CD4/mm^3. Adherence: 18 (30.5%) <80%, 8 (13.5%) 80–90%, 16 (27%) 90–95% and 17 (28.8%) >95%. LPV indication: in >75% de patients it was up a second line of treatment (32% after simplification procedure) and only seven (12.5%) were naives. 34 weeks tend to treat analysis: 20 (33.9%) remain with viral load <50 copies/mL, nine (15%) virological failure, three (5%) have died, six lost of outcome, 2 (3.4%) needed therapy intensification, six (10%) repudiated the treatment, in 11 the outcome is not complete. We have indicated 39 LPV-SOL (66.1%) and 20 LPV-PL (33.8%). From 39 LPV-SOL, 16 (41% total SOL) must be stopped, switching to PL, mainly by gastrointestinal intolerance, and four of them left definitely. From 20 LPV-PL, two left the treatment and two were switched to SOL in order to improve the adherence, with good tolerance in both.
Conclusions: In our group LPV is used as treatment after a simplified regimen (32%) and in very hard conditions by adherence, immunological stage and previous treatment. Although it is few cases, we have found no differences in efficacy between SOL and PL. The SOL is in general worse tolerated than PL, but for many patients it used is more gratify (only 5-mL bid vs. three pills bid), so we think that it must be probe as initial way in-patients with adherence problems.

R2268 Long-term survival and intermittent interruption of antiretroviral therapy in a HIV-positive individual with HIV-related pulmonary hypertension

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Introduction: Pulmonary arterial hypertension related to HIV (PAHRH) is a rare complication of HIV infection. Treatment is difficult with a median survival of 6 months. Prospective studies have shown a benefit of antiretroviral therapy (ART). We report a patient with PAHRH with long-term survival, in whom lipodystrophy necessitated interruption of ART.
Case report: A 34-year-old HIV-positive male was diagnosed with PAHRH 10 years ago. Echocardiography revealed a right ventricular systolic pressure 59 mmHg over right atrial pressure (RV/RA-pressure). Therapy consisted of sorbidilat, digoxin and coumarin. At the same time, ART was started with AZT, then changed to 3TC, d4T and indinavir. He remained stable until 5 years later when he complained of worsening dyspnoea. Echocardiography revealed increased enlargement of the right ventricle (RV/RA-pressure 75 mmHg). An ACE inhibitor was added and ilumedin

with clinical improvement, but ilumedin was stopped after 6 months due to severe headaches. Clinically, he regained the level before deterioration; and the echocardiographic findings returned to the previous values. ART was successful with a CD4 cell count >1000 cells under a persistently nondetectable viral load. A significant worsening of the lipodystrophy was noted and therefore indinavir was switched to nevirapine. The lipodystrophy progressed further to the point where the patient was severely handicapped, not being able to hold his head upright due to a buffalo hump. Given the excellent CD4 values and the progressing lipodystrophy, ART was stopped. One month after discontinuation, the viral load had risen to $>2 \times 10^6$ copies/mL. The CD4 count had fallen from 1882 to 1215 cells. Dyspnoea remained stable, as did the 6 min walking test and the echocardiographic examination (RV/RA-pressure 24 mmHg). After 7 months, the patient wished to resume ART. Tenofovir, nevirapine and ddI were started, subsequent evaluation of PAHRH showed stable disease.
Conclusions: In this patient with long-term survival after diagnosis of PAHRH, interruption of ART did not result in worsening of PAHRH. Despite a rise in viral load and fall of CD4 cells, clinical and echocardiographic parameters remained stable for a follow-up of 7 months. If treatment interruption is considered a close follow-up is warranted to detect early signs of deterioration of PAHRH. Interruption should be reserved if no alternatives are available.

R2269 Mutational pattern in failing patients to antiretroviral regimen including tenofovir

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Objective: To evaluate the resistance mutational pattern in antiretroviral-experienced patients including tenofovir in their therapy regimens.

Material and methods: Twenty plasma samples from 20 HIV-1 infected patients were analysed for drug resistance. All the patients were followed at the La Paz University Hospital, Madrid, Spain. All the patients failed treatment with a mean viral load of 17 793 cop/mL (range 385–83 100). The CD4+ cell count ranged from 15 to 977/mL, with a mean of 422/mL. The disease stages CDC were as follows: 31.6% (C3), 21.1% (B3), 15.8% (C2) and 10.5% (A2, A3, B2). The 90% ($n = 18$) of the patients received a previous therapy regimens with zidovudine or stavudine. 16 patients received more than three therapy regimens. Only in two cases did not receive any nucleoside analogues reverse transcriptase inhibitor (NRTI). Sequencing of the protease gene and RT gene was performed by the TruGene HIV-1 assay (Visible Genetics, Toronto, Canada), following the manufacturer's recomendations. Sequences were compared with the HIV-1 LAV1 reference sequence. We analysed the selection of K65R mutation (selected by tenofovir in vitro) and the accumulations of nucleoside analogues mutations (NAMs).
Results: The frequency of the mutations were as follows: K65R in 45%, M41L in 40%, D67N in 30%, K70R in 15%, L210W in 30%, T215Y/F in 55% and K219Q/E in 25% of the patients. The data analysis are showed in Table 1.

NAM without K65R	11 patients (55%)
T215Y/F+K65R alone	0
K65R alone	9 patients (45%)

Conclusions: Clearly, there are two mutational patterns in failing patients to antiretroviral regimen including tenofovir: one with K65 R alone mutation and the other with NAMs without K65R. It's confirmed that K65R is negatively associated with the NAMs.

Hepatitis

R2270 Vertical transmission of hepatitis C virus in the Czech Republic

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Objectives: By the method of multicentre study to determine the incidence of vertical transmission of the hepatitis C virus in children who were born to mothers who suffer from hepatitis C.
Methods: From the year 2001 to 2003, we analysed a group of 73 children who were born to mothers who suffer from hepatitis C. Of the 73 children who made up the analysis group, a determination as to whether or not vertical transmission occurred could be made in 51 cases. Of the remaining 22 children, either they had not reached the age of 18 months when a determination as to vertical transmission could be made, or we were no longer able to continue follow-up testing as the mothers stopped attending our clinic. We collected blood samples from umbilical cord, and at the age of 3, 6, 12, 18, 24 months, and in the affected children at 3 and 4 years of age. All the children were tested for the presence of hepatitis C antibodies using the ELISA method, and when possible we tested the children for RNA HCV using the RT-PCR method. We evaluated vertical transmission positive if at least two blood samples were tested positive for RNA HCV or if anti-HCV antibodies persisted longer than 2 years of age.
Results: In our analysis we determined vertical transmission to have occurred in seven of 51 cases analysed. Four of these seven children were PCR-positive at the age of 2 years. The remaining two of these seven children continued to test positive for hepatitis C antibodies. One of the affected children lost viraemia and the antibodies at the age of 1 year.
Conclusions: Vertical transmission occurred in seven of 51 cases analysed (13.7%). We can state that the presence of RNA HCV in umbilical cord blood or in the blood of 3-month old children does not necessarily mean that vertical transmission has occurred. Furthermore we can state that testing positive for viraemia at the age of 6 months more than likely indicates the presence of vertical transmission as well as the presence of antibodies at the age of 18 months.

R2271 Genotype analysis of chronic hepatitis C patients in correlation with ways of contagion and patients' origins

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Our objective is the study of chronic hepatitis C genotype expression and its correlation to ways of contagion and the patients' origins. Our material consists of 64 patients of chronic hepatitis C (42 men, 22 women, average age 39 years old). An analysis of hepatitis C genotype was held and the correlation to the ways of contagion and the patients' origins was studied.
Results: See Tables
Conclusions: The main ways of contagion appears to be the intravenous administration of drugs and a considerable number of patients refers to sporadic cases. In the category of drug addicts, genotype 3 is prevailing, while in the category of the sporadic cases there is a greater extent of heterogeneity – thing which emphasises the heterogeneity of the group in itself. In the groups

Table 1.

	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Intravenous Drug Administration	5	0	23	0
Sexual intercourse	1		1	0
Blood transfusion	4	2	2	2
Nor determined contag on way	7	4	6	1
High risk groups	0	2	1	0

Table 2. Allotment according to genotype and origins

Genotypes	1	2	3	4
Greeks	13 (28.3%)	9 (19.0%)	22 (47.8%)	2 (4.35%)
Immigrants	6 (33.3%)	4 (22.2%)	7 (38.8%)	1 (5.6%)

of contagion through sexual intercourse and blood transfusion, there seems to be an equal allotment of genotypes. Finally, the group of the other ways of contagion appears to be influenced by the other groups and is allotted between genotypes 2 and 3, probably because the carrier transmitting the virus belongs to these groups. As regards the origins of the patients, and though the cases are not equally allotted, it appears that there is a differentiation between the Greeks and the immigrants. So the vast majority of the Greeks is allotted between genotypes 2 and 3, while the immigrants are allotted among genotypes 1, 2 and 3 (genotypes 1 and 3 showing a slightly higher percentage).

R2272 Relationship between the level of basal antigenaemia of HCV and hepatic affectation in HIV patients

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Objective: The aim of this work is to study the relationship between the antigenaemia (core Ag) of HCV and the rate of hepatic fibrosis in patients HCV–HIV coinfected prior the treatment.
Materials and methods: We have studied the levels of HCV antigen (HCV-Ag) in 22 sera from 22 patients with chronic hepatitis C by ELISA (Ortho trak C assay, Ortho Clinical diagnosis, Raritan, NJ, USA). The degree of hepatic fibrosis was studied by the histological activity rate (HAR-Metavir) in hepatic biopsy.
Results: Nine (40.9%) of 22 patients have antigenaemia levels <100 pg/mL. Seven (77.8%) of these patients have a severe hepatic affectation: five (55.6%) have a severe HAR-Metavir (degree 3) and two have a cirrhosis HAR-Metavir (degree 4); and the rest two patients have a moderate HAR-Metavir (degree 2). Six (27.1%) patients have antigenaemia levels from 101 to 175 pg/mL: three (50%) have a slight HAR-Metavir (1) and the three other a moderate HAR-Metavir (2). Seven (40.9%) patients have antigenemia levels higher than 175 pg/mL: one (14.3%) patient has a slight HAR-Metavir (1), and six patients have a moderate HAR-Metavir (2)

Table. Distribution of patients by HCV antigenaemia level and degree of hepatic affectation (HAR-Metavir)

	HAR-Metavir			
Ag (pg/mL)	Slight (1) No.	Moderate (2) No.	Severe (3) No.	Cirrhosis (4) No.
0–100	0	2	5	2
101–175	3	3	0	0
> 100	1	6	0	0

Spearman's Rho: −0.703.

Conclusion: There is a decrease of antigenaemia with the increase of hepatic affectation (HAR-Metavir) in HCV–HIV coinfected patients.

R2273 Persistence of HBV-DNA in children who cleared HBsAg after IFN treatment

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Objectives: The aim of this study was to assess the long-term clinical and virological response in children with chronic hepatitis B who cleared HBs antigen during IFN-α treatment.
Material and methods: Twenty-seven children who cleared HBsAg during IFN treatment were available for repeated clinical and virological evaluation on the average 8 years (range 7–10 years) after completion of therapy. Serological markers of HBV infection were assessed by use of commercial tests AxSYM (Abbott). HBV-DNA was detected in serum and immune complexes using HBV-Sharp Signal System (Digene).
Results: All children were in good clinical condition without any clinical or biochemical symptoms of liver disease. Two patients (7%) had detectable HBsAg in serum, and one of them was also positive for anti-HBs. The remaining 25 patients were HBsAg-negative, anti-HBs-positive. HBeAg was negative in all subjects, whereas anti-HBe antibodies were present in 16 children. HBV-DNA was detected in 11 (40%) children, mainly in a form of immune complexes.
Conclusions: Our data suggests that HBV replication may persist for years after antiviral therapy-induced clearance of HBsAg.

R2274 Seroprevalence of HBV markers in medical students

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Republic of Macedonia is a region with intermediary seroprevalence of HBV (2–4%) and interfamilial and sexual transmission as a dominant routine. Universal vaccination of newborns is the only cost-benefit measure for prevention. In Macedonia vaccination is not routinely undertaken due to economic reasons, although from 1990 we vaccinated a high risk groups such as health care workers and medical students. For them HBV infection is professional disease.
Objective: To present seroprevalence of HBV markers in medical students and determining additional risk factors.
Material: This study was performed in two phases: phase I included 110 medical students (80 female, 30 male) age range 22–27 (median 24.5), testing ALT and HBV markers (HBsAg, anti HBc total and antiHBs by ELISA, Behring Processor II.). If they had normal ALT levels and negative HBV markers entered phase II and 77 students were vaccinated on voluntary basis. Demographic and personal characteristics including anonymous survey for sexual contacts, were analysed for this group.
Results: From 110 medical students, eight (7.3%) had confirmed previous contact with HBV, three (2.7%) of them are HBsAg carriers. 37/77 (48.1%) have previous nurse training school and all of them performed bloody medical interventions while studying. 12/77 (15.5%) during clinical training as students also practiced medical interventions. 58/77 (75.3%) have regular sexual partner: 29/58 (50%) with safe sex practicing, 19/58 (32.8%) using intermittent and 10 (17.2%) do not use any kind of protection. 17/77 (22.1%) had irregular partners: 11/17 (64.7%) with constant, five of 17 (29.4%) occasional and one of 17 (5.9%) with no protection at all. Two of 77 (2.6%) without sexual contact. No drug users among analysed students, and only one with piercing and tattoo.
Conclusion: Starting 2004, routine vaccination for all newborns will take place in Macedonia and issue of additional group for vaccin-

ation and its timing is actualised. Higher prevalence among medical students suggests that vaccination should be initiated at the beginning of nurse high school (inexperience and frequent practicing of medical interventions) and before sexual activity, due to high per cent of unsafe sex practicing, attacking same age group.

R2275 Serum HBV-DNA levels, serum aminotransferases levels and serological markers in evaluation of chronic HBV infection

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The course and outcome of chronic HBV infection are variable. Chronic HBV infection is also associated with an increased risk of developing hepatic cirrhosis and hepatocellular carcinoma. The recognition and diagnosis of this disease are supported by the detection of HBV antigens and specific HBV antibodies. Quantitative determination of viraemia levels in sera contributes to the management of patients chronically infected with the HBV. The aim of this study was to investigate the clinical significance and the disease progression in chronic HBV infection by the quantitative determination of HBV-DNA, detection of serological markers and determination of aminotransferases levels.
Material and methods: Serum samples (320 samples in total) of 235 patients, 4–73 years old, (225 adults, 10 children) with different stages of chronic HBV infection were examined over a 2-year period, 2002–2003. Serum HBV-DNA was measured by a quantitative PCR (Amplicor HBV Monitor Test, Roche Diagnostic Systems). Also, in all samples detection of serological markers (HBsAg, anti-HBs, HBeAg, anti-HBe) and measurement of ALT/AST were performed. Serological markers were determined by an enzyme-linked immunoassay MEIA (AxSym3) and aminotransferases by kinetic method.
Results: According to the results of this study, 55 patients (50 adults, five children) were evaluated as chronic active hepatitis B cases, rate 23.4% (HBsAg+, HBV-DNA>10^5 copies/mL, persistent or intermittent elevation of ALT/AST levels). In 47 of them HBeAg− and anti-HBe+ were found, while eight patients had HBeAg+ and anti-HBe−. Another group of 151 patients (146 adults, five children) were evaluated as inactive HBsAg carriers, rate 64.3% (HBsAg+, HBeAg−, anti-HBe+, HBV-DNA<10^5 copies/ml and persistently normal ALT/AST levels). The remaining 29 adults patients were evaluated as resolved hepatitis B cases, rate 12.3% (HBsAg−, anti-HBs+, HBV-DNA<400 copies/mL and normal aminotransferases levels).
Conclusions: The combination of quantitative determination of HBV-DNA by polymerase chain reaction, detection of serological markers and aminotransferases levels contribute significantly to the management of chronic infection and evaluation of the efficacy of antiviral therapy. The majority of the patients with chronic active HBV infection (85.4%) were found to have HBeAg(−). This data coincides with other studies in Greece and Mediterranean area where viral mutations occur and might influence the expression of HBeAg.

R2276 Evolution of haematimetric parameters during the treatment of chronic virus C infection with interferon and ribavirine in HIV-coinfected patients

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Objective: Analysis of the evolution of the haematological series during the treatment of chronic hepatitis HCV with interferon alpha plus ribavirin in HIV-coinfected patients.
Methods: Twenty-one HCV and HIV-coinfected patients were treated with PEG-Interferon α-2a (11 patients) or standard interferon-α (10 patients), plus ribavirin. Patients with abnormal count of each of the haematological series were excluded. Hemoglobin concentration and leucocytes and platelets counts were analysed at 2, 4, 6, 8, 12, 18, 24 and 48 weeks after the beginning of the therapy, and 24 weeks after the end. The results are showed as median and interquartile range.
Results: At baseline, median haemoglobin concentration and lymphocytes, polymorphonuclear neutrophyles and platelets counts were 14.3 g/dL (13.50–15.50), 2280/mm^3 (interquartile range, 2080–2780), 2790/mm^3 (2260–3200), and 179660/mm^3 (126000–217000), respectively. A progressive decrease of every cell population was detected: polymorphonuclear neutrophiles attained the nadir at 4 weeks after the beginning of the therapy, with a median of 1400/mm^3 (990–2350), and a diminution of 45% (18–67%). The rest of parameters attained the nadir at 12 weeks after the beginning: haemoglobin concentration, 12.95 g/dL (12–13.58); platelet count, 121 000/mm^3 (95 000–151 250); lymphocyte count, 1230/mm^3. The respective diminutions of these values were 9% (5–16%), 31% (16–45%) and 50% (16–63%). The decline was independent of response to the treatment, but was higher in the PEG-interferon-α-2a group. There were not haemorrhagic or infectious complications. Cell counts and haemoglobin concentration returned at baseline levels after the end of treatment.
Conclusions: A significant decrease in all haematological series must be expected in HIV–HCV coinfected patients treated with interferon plus ribavirin, with a higher decrease in those treated with PEG-interferon. This diminution occurs in the first weeks of treatment, and return at baseline levels after the end of it.

R2277 Prevalence of lichen Planus in HCV-infected patients in Guilan Province (Iran), 2002

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Background: Hepatitis c virus account for over 90% transfusion – associated hepatitis, >50% likelihood of chronicity, leading to cirrhosis or hepatocellular carcinoma in 25%. With regard to the controversy in correlation between HCV infection and oral lichen planus in various geographic areas, this investigation was done to determine the prevalence of oral lichen planus in HCV positive patients in Guilan (Iran).
Materials and methods: In this cross- sectional study, 63 HCV infected patients in Guilan were examined. The authors made use of an enzyme-linked immunosorbent assay (ELISA ÍÍ) and positive samples rechecked with Western blot.
Results: In this study, there were 33 female and 30 male. The mean age was 43.8 ± 17.4 SD years. Three cases had reticular form of oral lichen planus (4.7%) that this prevalence rate is higher than normal population rate in this area (0.5–2%).
Conclusion: This study showed that oral lichen planus is prevalent in HCV-infected patients and diagnostic tests for HCV-antibodies may be requested in confirmed lichen planus cases.

R2278 Lamivudine treatment in renal transplant recipients with chronic hepatitis B

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Objectives: Lamivudine appears to be safe and effective in the treatment of chronic hepatitis B virus infection in patients with chronic renal failure, though experiences are still limited. We described our experiences with lamivudine treatment in six renal transplant recipients with chronic hepatitis B.
Methods: Six renal transplant recipients (three male, three female) aged 40–70 years suffered by chronic hepatitis B. All patients were HBsAg positive, three of them were HBeAg positive. All were HBV-DNA-positive, precore mutant was proved in three of them.

All patients had serum alanine transferase (ALT) levels 3–10 times the upper limit of normal prior to treatment. Four patients had anti-HCV antibodies, HCV RNA was positive in two of them. Blood samples were assessed monthly after initiation of lamivudine treatment and later every 3 months. Samples were tested by ELISA for HBsAg, HBeAg, anti-HBe and anti-HCV; by using PCR for HBV DNA and HCV RNA.
Results: Lamivudine treatment was administered in six patients for 15–55 months. Initial dose 100 mg/day was given in five patients, initial dose 35 mg/day was given in one patient with partial renal insufficiency. The lamivudine dose was reduced (10–50 mg/day) in four patients according to creatinine clearance. The graft of one patient was rejected and haemodialysis treatment was reinstituted. HBV DNA became undetectable in all patients within 1–6 months of initiation of lamivudine therapy. Serum HBeAg disappeared in three patients during 2–17 months, with the emergence of anti-HBe in all of them. No patient lost HBsAg. Serum ALT normalised in five patients within 1–9 months. Continuing elevation of ALT was observed in one patient with chronic hepatitis C and positive result of HCV RNA. Recurrence of chronic hepatitis B was observed in the oldest patient 7 months after initiation of lamivudine treatment. Lamivudine treatment continued, but the women died 8 months later for progression of liver cirrhosis. No side-effects of lamivudine treatment was observed during therapy.
Conclusion: Lamivudine treatment induced initial virological response in all 6 renal transplant recipients with chronic hepatitis B. Recurrence of hepatitis B with progression of liver cirrhosis till death was observed in one patient. No evidence of hepatitis B recurrence was proved in the other five patients with lamivudine therapy during 2–4 years monitoring.

R2279 Distribution of hepatitis B virus genotype in patients with chronic HBV infection

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Objectives: To study the distribution of HBV genotype in a group of chronic HBV infected patients.
Methods: HBV serological markers, HBsAg, antiHBc total or IgM, HBeAg or anti HBe were detected in sera of 36 chronic HBV-infected patients (28 men and eight women, range age 30–60 years) by enzyme immunoassay (MEIA Axsym Abbott) whereas HBV viral load was determined by a branched DNA method (HBV DNA 3.0 bDNA assay, Versant). HBV genotypes were determined by using the amplified HBV polymerase gene (HBV-pol gene), domains B to C in a reverse hybridisation assay, LiPA (INNO-LiPA HBV Genotype, Innogenetics). The amplification of HBV- pol gene, domain B to C was done in two steps (i) DNA was isolated from 500l of patients sera (QÉÁámp Ultrasens Virus Kit Qiagen) (ii) part of the extracted DNA was amplified by a nested polymerase chain reaction procedure, using primers derived from HBV DNA pol-gene. LiPA is a line probe assay capable to identify HBV Genotypes A to G.
Results: Three distinct HBV serological profiles were found among the 36 chronic HBV infected patients Twenty-eight of them had HBsAg (+) antiHBcIgG (+), IgM (−) and anti HBe (+) whereas another three patients had the same findings but anti HBc IgM (+). Five of the 36 patients had HBsAg(+) anti HBc IgG (+), IgM (−) and HBeAg (+). HBV DNA was positive in sera of all 36 patients and the viral load was ranged from 1120×10^3–$120\,000 \times 10^3$ copies/mL. Only genotypes D (97%) and F (3%) were found among the 36 chronic HBV infected patients.
Conclusions: HBV genotype D and F are prevalent among chronic HBV liver disease patients in our area. Thirty patients have HBV genotype D which is associated with the serological profile HbsAg (+), anti HBC IgG (+), anti HBe (+). This serological profile usually was found in HBV chronic infected patients who had HBV precore mutant infection in our area. Such mutants are thought to be associated with faster progression of chronic disease and even with fulminant hepatitis. Precore stop mutations seems to be associated with HBV genotype D.

R2280 Early use and long-term follow-up of interferon therapy in acute hepatitis C cases

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Introduction: Acute hepatitis C infection has a wild course, and patients rarely seek medical attention, therefore the diagnosis of the acute illness is difficult.
Case reports: Two patients attended to hospital with jaundice. Case 1 had been infected 6 weeks previously when he had a haircut. Case 2 had an abdominal operation 4 weeks ago and both cases were seronegative for HCV before the exposures. On admission to hospital, the alanine aminotransferase levels of cases were 2730 and 1410, and the aspartate aminotransferase level of cases were 1120 and 910, respectively. Case 1 and 2 were positive for antibodies to HCV, and HCV RNA was detected in serum of patients by polymerase chain reaction. Other hepatitis markers and auto antibodies were detected as negative. HCV was speciated as genotype 1b in both cases. In both of the patients, interferon à-2b was administered as induction treatment for the first 5 days with daily 5 MU and then 3 MU thrice-a-week for 24 weeks. Viral clearence occurred after 2 weeks for case 1 and 2. Viral clearence of cases sustained after 3 and 5 years of the treatment, respectively.
Conclusion: Early treatment of acute hepatitis C with interferon alone with induction in first 5 days, may be chance for preventing chronic infection whether the genotype of virus is 1b, which gives poorer response during treatment of chronic infection.

R2281 Seroprevalence of hepatitis E in Nahavand, Iran: a population-based study

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Background and objective: Hepatitis E virus (HEV) infection has recognised as the most common form of acute viral hepatitis among young adults in many developing countries. However, community-based surveys on this infection are scarce. This study looks at the seroepidemiology of HEV infection in the city of Nahavand (with 72 000 inhabitants), in the western part of Iran.
Subjects and methods: This cross-sectional study was conducted in February of 2003 among people of age 6 years and over in Nahavand. The six urban regions of Nahavand were considered as strata and 1824 subjects were recruited through a random stratified sampling (304 subjects from each stratum). Questionnaires were completed by face-to-face interview (including socio-economic and demographic variables) and blood samples were taken and tested for anti-HEV IgG using ELISA. STATA 8 was used to analyse data considering complex sampling design.
Results: A total of 799 males (43.8%) and 1025 (56.2%) females were included (34.7 ± 19.5 and 32 years as mean $\pm$ SD and median of age, respectively). The overall seroprevalence of hepatitis E was 9.6% (95% CI: 8.2–10.9). Seropositive subjects had mean and median age of 42.6 ± 15.6 and 40.5 years, respectively. Fifty-nine of seropositive subjects (34.7%) were male, 55 of them (32.4%) were illiterate and median of their family size was 4.0 persons. The prevalence of anti-HEV IgG antibody was not equally distributed among age groups with highest rate observable in the 30–39-year-old subjects (30%). Seropositivity for anti-HEV IgG was positively associated with age (OR = 1.02;%95CI: 1.01–1.03), sex (F/M) (OR = 1.52,%95 CI: 1.09–2.11) and educational level (illiterate/literate) (OR = 1.81,%95 CI: 1.28–2.55). No association observed between family size and seropositivity [OR = 0.82,%95 CI: 0.60–1.13]. Logistic multivariate regression indicated age and sex as the only significant risk factors of seropositivity. Adjusted ORs for age and sex were 1.03 (%95 CI: 1.01–1.04) and 1.61 (%95 CI: 1.13–2.28), respectively.
Conclusions: The high prevalence of positive serology observed in this study urges more researches in Nahavand and other regions of the country considering related risk factors. It is also important

to study the relative contribution of HEV infection to the burden of acute viral hepatitis.

R2282 Seroprevalence of hepatitis A, B and C in hospital personnel

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Objectives: Our study was conducted to estimate the seroprevalence of hepatitis A, B and C and to identify the risk factors determining occupational infection with the virus among the health personnel of a Greek major hospital.
Methods: Samples of serum from 225 persons both male and female, doctors and nurses who work in different wards of our hospital, were tested for the detection of HAV (total antiHAV and IgM antiHAV), HBV (HBsAg, HBeAg, antiHBs, antiHBc, antiHBe) and HCV (antiHCV)-markers. All tests were performed by immunoenzymatic methodology (MEIA, AXSYM-ABBOTT).
Results: Of the 225 persons who were controlled were found to be positive: for HBsAg 2 (0.89%), for total antiHBc alone 6 (2.6%), for simultaneous presence of antiHBc and antiHBs 26 (11.5%), for antiHBs due to vaccination 122 (54.2%). A total of 112 persons (49.7%) had developed title antiHBs > 100 mIU/mL.A total of 74 persons (32.9%) received complete three doses while in 48 persons (21.3%)the vaccination was incomplete. One person (0.44%) was positive for antiHCV, 106 persons (47.1%) were positive for total antiHAV and none was found to be positive for IgM anti-HAV.
Conclusions: The direct contact with patients and handling of blood and other body fluids and secretions are risk factors related to occupational infection with hepatitis virus. Therefore the effort for the prevention must be continual and the control measures with a HBV vaccination policy should be strictly enforced for the elimination of hepatitis transmission among health care workers.

R2283 The frequency of steatosis in chronic hepatitis B and C by ultrasonography

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Objectives: Steatosis is commonly present in chronic hepatitis C. Although liver biopsy is the gold standard in determining presence of steatosis, its presence can be determined by ultrasonographic examination with high sensitivity. Our aim in this study was to evaluate whether steatosis present in hepatitis B and hepatitis C, to determine their frequency in both hepatitis type, and to explore possible relationship between presence of steatosis and the liver function tests.
Methods: The patients with chronic hepatitis, B and C or both, were included in this study between January 2002 and September 2003. The patients having diabetes, cirrhosis or alcohol history were excluded from the study. Firstly, liver steatosis in patients with chronic hepatitis was investigated by ultrasonographic examination. The serum levels of alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase, cholesterol and triglyceride were obtained. Their serum levels were compared between groups in patients having steatosis or not.
Results: A total of 115 adults patients with chronic hepatitis including hepatitis B ($n = 90$), hepatitis C ($n = 22$) and hepatitis B + C ($n = 3$), were included study. Of these patients, 67% ($n = 77$) were male and 33% ($n = 38$) were female. Their mean age was 37 ± 9 years (range, 21–53 years). Steatosis presences were 33.3, 54.5 and 100% in hepatitis B, C and B + C, respectively. The serum levels of alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase, cholesterol and triglyceride were not statistically different between the groups with steatosis or not in all patients. There was no significant relationship between steatosis and gender or age also.
Conclusions: Steatosis was lower in patients with chronic hepatitis B than those with hepatitis C and hepatitis B + C. However, our results suggest that not only presence of steatosis was high in patients with hepatitis C, but also it was high in patients with hepatitis B.

R2284 Efficacy of lamivudine in treatment of HBeAg-positive chronic hepatitis B

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Objectives: Presence of HBeAg in serum of patients with chronic hepatitis B indicates active disease. This study was conducted to evaluate the efficacy of 1-year of lamivudine therapy in these patients.
Methods: A total of 25 cases of HbeAg-positive anti HBeAg negative chronic hepatitis B were treated with 100 mg lamivudine orally, daily for 1 year in Babol, Iran from September 2000 to March 2003. HBeAg, HBV-DNA, anti HBe and alanine aminotransferase(ALT) in all cases were assessed every 3-months interval during therapy. Proportions were compared with Fisher's exact test.
Results: A total of 15 men and 10 women with the mean age, 24.6 ± 6 years and mean-ALT levels of 59.8 ± 35 IU/L and mean score of histological activity index (HAI) 6.5 ± 3.5 were treated. After treatment, nine (60%) men and five (50%) women became HbeAg-negative ($P = 0.466$). Anti HBe was observed in 11 (44%) cases. HBV-DNA became negative in four (26.7%) men and one (10%) women ($P = 0.313$). Serum ALT levels returned to normal values in 22 (88%) cases.
Conclusion: One year of lamivudine therapy has benefical effect in these group of patients, however some patients need longer duration of therapy.

R2285 Tumour necrosis factor alpha, interleukins (IL)-1beta, IL-2, IL-4 serum levels in patients with chronic hepatitis C treated with interferon alpha

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Objectives: The aim of this study was to investigate the serum levels of cytokines tumour necrosis factor (TNF) -alpha, interleukin (IL)-1beta, IL-2, IL-4 in patients with chronic hepatitis C (CHC) treated with interferon alpha.
Methods: The serum cytokine levels (TNF-alpha, IL-1beta, IL-2, IL-4) in 10 healthy blood donors (I group), 35 patients with CHC without antiviral treatment (II group) and in 20 of patients treated with interferon alpha for 3–6 months (3 MU thrice a week, III group) by means of ELISA test was performed. Mann–Whitney test was used for statistical analysis.
Results: The serum level of TNF alpha was 24.0 pg/mL in I group, 128.3 pg/mL in II group and 190.9 pg/mL in III group (P I–II = 0.006, P I–III = 0.005). The serum level of IL-1beta was 4.4 pg/mL in I group, 376.1 pg/mL in II group and 553.0 pg/mL in III group (P I–II = 0.0001, P I–III = 0.0001). The serum levels of IL-2 was 20.9 pg/mL in I group, 49.2 pg/mL in II group, 77.3 pg/mL in III group (P I–III = 0.031). The serum levels of cytokine IL-4 was 21.9 pg/mL in I group, 79.8 pg/mL in II group and 147.0 pg/mL in III group (P I–III = 0.047).
Conclusion: During the course of treatment with interferon alpha the serum cytokines levels grow. The increasing amount of pro-inflammatory cytokines (TNF alpha and IL-1beta) can damage various organ including liver. The increase of cytokine IL-4 reflects activation of T-helper type 2, which promote sustained viral persistence. Cytokines dysbalance aggravation during the course of interferon alpha treatment probably may decrease therapy effect.

R2286 High prevalence of HIV, HBV, HCV infection in Gypsy population residing in Chahar, Mahal and Bakhtiari province

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Objective: Gypsies are groups of people who are not dependent on any special area. Because of permanent immigration and their life style that has been targeted to earn daily bread, they might be exposed to multiple partner sexual contacts, addiction and various kind of infections, like hepatitis B, C and AIDS. Purpose of this study is to estimate prevalence of these infections in gypsies of south west of IRAN (Shahr-e-kord).
Methods: This study was done in Sureshjan Pass near Shahr-e-kord. 226 persons of gypsies were underwent tests for hepatitis B, C and HIV. Collected serums were tested for serologic markers of HBsAg, anti-HBc, anti-HCV, anti-HIV. The kits used were based on third generation of enzyme immune assay (EIA) for HBV, HIV, and second generation of EIA for HCV. All positive sera were tested again. In fact positive sera in first step were selected and retested for HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, anti-HCV, anti-HIV antibodies. The kits used in this step had different mark of manufacturing.
Results: Serologic results indicated that 54 individuals (20.5%) were positive for HBV markers and 22 of them (9.8% of overall) had infectious hepatitis B, 13 of them (5.8% of overall) had only HBsAg positive, 11 individuals (4.9% of overall) were at an immune state for HBV and eight persons (3.5% of all) had isolated Anti-HBc. Also, seven persons (three women and four men) (3.09%) had positive anti-HCV and four persons (two women and two men) (1.7%) were HIV positive. The prevalence and relative risk of HBV, HCV and HIV in this group has been faced much higher rate than normal population. (relative risk is 2.6, 3.9, and 23, respectively)
Conclusion: The life style of gypsies as nomadic and margin dwelling, absence of any moral and hygienic disciplines, and multiple partner sexual contacts can transmit communicable diseases and cause increase prevalence of them in different societies. We suggest that, a good and effective health care system for gypsies should be considered so that saves the society from the risk of these diseases.

R2287 Detection of genotype and mutations of hepatitis B virus during lamivudine therapy

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Objective: The aim of this study was to compare two laboratory methods used to determine HBV genotypes and mutations of HBV during lamivudine therapy.
Material and Methods: A total of 14 serum samples from patients affected by HBV chronic hepatitis receiving lamivudine therapy, were included in the study. The serum samples tested contained virus level between 103 and 108 copies DNA/mL (PCR Amplicor Monitor Test, Roche). ALT and DNA-VHB levels (basal and several during the therapy) were determined. Genotype and mutations of HBV virus were studied by INNO-LiPA (LiPA HBV Genotyping kit and LiPA HBV-DR, Innogenetics) and sequence analysis (Trugene HBV Genotyping kit, Bayer).
Results: Among the 10 patients, eight were antiHBe+ and two HBeAg+. Medium basal values: ALT 120.5 UI/L, DNA-VHB 1.20×107 copies/mL. Seven patients had detectable DNA levels during the therapy. The three patients with negative DNA levels in a moment during the therapy were the only ones with basal DNA <106 copies/mL and were infected with genotype B or B/D. Six patients had mutant virus, in one of them the mutation appeared in the basal sample. Among the six patients, two were infected with genotype A and had the mutation pattern M204I + wt, two infected with genotype D had the L180M + M204V + wt and two with genotype D had the M204I or M204V $\pm$ wt. The virus mutant appeared after 9–26 months of therapy, the M204I appeared in the first 12 months, the L180M + M204V after 18 months and the M204V after 26 months. The results obtained by sequencing were in agreement with LiPA except in the detection of mixed populations (wild type and mutant). In an 83% of the cases, the viral load increased >1 log10 prior to detection of mutations by LiPA. The medium DNA level after the presence of mutations was lower for genotype B than for genotype A and D. The ALT level was lower, similar or higher than the basal value for genotype B, D and A, respectively.
Conclusions: (i) LiPA and sequence analysis demonstrated a good concordance in genotyping and in detecting lamivudine resistance mutation, but LiPA had superior sensitivity for identification of mixed populations (wild type and mutant). (ii). An increase of HBV DNA level was detected mainly before the detection of mutants by LiPA, and before the increase of ALT. (iii). Only patients infected with genotype B showed medium DNA level and ALT values lower than the basal value after the presence of mutations.

R2288 Genomic variability of pre-S/S region of hepatitis B virus from patients in the Czech Republic

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Objectives: For global epidemiology of hepatitis B virus both phenotype variability i.e. HBsAg subtypes and genotype variability are used. For determination of routes of transmission in local outbreaks, comparison and analysis of nucleotide sequences of HBV DNA from individual patients is needed. The knowledge of heterogenity of HBV genome in patients from given geographic region is useful. The aims of this study was to determine heterogenity of pre-S/S region of HBV in the Czech Republic and to use this knowledge for analysis of local outbreak of HBV infection among patients of a haemodialysis unit.
Methods: HBV pre-S/S region DNA amplified by PCR from sera of 182 hepatitis B patients was sequenced. Consensus nucleotide sequences were obtained using CLUSTAL W (European Bioinformatics Institute, GB) followed by manual editing using Bioedit 5.09. Phylogenetic trees were constructed with MEGA 2.1 using UPGMA Kimura two-parameter model.
Results: HBV-DNA was amplified by PCR from sera of 166 patients from different regions of the Czech Republic. Part of pre-S/S region from PCR products were sequenced. Nucleotide sequences were aligned and phylogenetic tree was constructed. In the tree were found 14 pairs of identical sequences, one cluster with three identical sequences, one cluster with four, one with five and one with 12 identical sequences. These 18 clusters were analysed from geographic point of view and retrospectively affordable data about epidemiological relatedness of cases were considered. Fourteen clusters were found in the group of patients with genotype A, three clusters in patients with genotype D and one cluster in the group with genotype B. The new phylogenetic tree was constructed when nucleotide sequences from 16 patients infected with HBV during the outbreak in a haemodialysis unit were added. The source of infection and route of transmission of HBV in this outbreak was identified with contribution of phylogenetic analysis.
Conclusion: This study provided data about heterogenity of the part of HBV genome-coding surface antigen among HBV infected patients in the Czech Republic. Study confirmed that sequence analysis of pre-S/S region of HBV DNA is useful epidemiological tool for identification of routes of transmission of HBV infection, mainly in medical facilities. Supported by grant IGA MZCR No. NI 6796-3.

Herpes virus

R2289 Cytomegalovirus infection in a Portuguese hospital: January 2000–September 2003

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Objectives: The purpose of this study was to determine the prevalence of cytomegalovirus (CMV) infection and evaluate the changing pattern of seroprevalence of CMV antibodies by age and sex, in patients followed at a central hospital in Lisbon, between January 2000 and September 2003.
Methods: Retrospective analysis of laboratory records. CMV antibodies (IgM and IgG) were analysed in serum samples, using the microparticle enzyme immunoassay (MEIA) technology (ABBOTT - AXSYM System).
Results: During the study period 1305 samples were analysed, 198 of children under 15 years (102 female and 96 male) and 1107 adults (784 female and 323 male). In the overall study population, 68.4% of patients have been exposed to the disease (IgM−/IgG+), 18.0% had active disease (IgM+/IgG− or IgM+/IgG+) and 13.6% were healthy (IgM−/IgG−) for the disease. The seropositivity for CMV IgG antibodies (with IgM−) was 66.2% among children under 15 years, over than a half having <1 year, and was 68.7% among adults with a predominancy of women. Women between 16 and 35 years are the most affected by the disease, corresponding to almost 60.0% of the women with active disease and with history of exposition to the virus. The number of cases with active disease had a slightly increase in 2001, but now it tends to decrease. However, the amount of patients that have been exposed to the disease is high and tends to increase, while the number of seronegative cases is stabilizing.
Conclusions: CMV is highly endemic in our population, with a prevalence of 86.4%. Women between 16 and 35 years are the most affected by the disease. It is important in this period, when women can become pregnant, to do the test. Despite the amount of CMV IgM−/IgG+ in children of <1 year, there was no significant difference among the different age groups. It means that the infection can occur at any time of our lives.

R2290 Cytomegalovirus (CMV) colitis in an immunocompetent patient

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Objectives: CMV infection in immunocompetent individuals is rarely a cause of clinical illness. Gastrointestinal manifestation of CMV infection occurring in such patients have been reported in only few cases.
Methods: We report a case of CMV colitis in an immunocompetent elderly patient.
Results: In August 2003, a 76-year-old woman was admitted to hospital for abdominal pain, diarrhoea and fever of 10 days duration. She was treated with levodopa for Parkinson's disease. Physical examination revealed an ill-looking, pale woman, with dehydrated skin and mucous membranes. Abdomen was mildly distended with tenderness in the lower quadrants, with no guarding or palpable masses. A stool specimen was positive for occult blood, but negative for ova and parasites, performance of a *C. difficile* stool toxin assay yielded normal results, and cultures were negative for pathogenic bacteria. An abdominal radiograph showed some dilated small bowel loops and few sings of air-fluid levels in the colon. Ciprofloxacin was administered for 7 days without improvement. A colonoscopy was performed and showed multiple, confluent, aphtoid lesions and friable mucosa in the descending and sigmoid colon. Microscopic examination showed chronic inflammatory infiltrates. Numerous intranuclear inclusions were detected in the endothelial cells. Immunohistochemical staining with anti-CMV monoclonal antibody was positive in the intranuclear inclusion bodies. CMV serology performed after the histopathological diagnosis of CMV colitis was positive for IgG antibodies (139 UI/mL) and negative for IgM. Tests for HIV were negative. A 2-week therapy with intravenous ganciclovir was completed. A second colonoscopy showed amelioration of the lesions in the descending and sigmoid colon. Biopsies were negative for intranuclear inclusion bodies. The patient was discharged and she is now healthy after a follow-up period of 3 months.
Conclusion: Clinically significant CMV infections usually occur in immunocompromised patients, such as patients with AIDS, transplantation, or cancer; critically ill patients are also at risk. Advanced age has been reported to play a role in the CMV reactivation because of the decline of immunity. Our case had no evident causes of immunodepression, but the advanced age might have played a role in the reactivation of CMV and in the severe clinical manifestation of the infection. We conclude that CMV colitis should be considered in differential diagnosis of diarrhoea in elderly.

Emerging infectious diseases

R2291 The first case of haemolytic-uraemic syndrome caused by STEC O26 in Slovakia

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Background: Shiga toxin-producing *Escherichia coli* (STEC) are associated with life-threatening sequelae such as haemolytic-uraemic syndrome (HUS). Particularly STEC O157 appears to be the most frequent. However, it has been recognised for a number of years that STEC belong to a broad range of O serogroups. We report a case of HUS caused by Stx2-positive STEC O26.
Methods: Girl (4 years) was admitted to Hospital for Sick Children with signs of HUS. Stool samples from patient and her family living in the same house were collected. There were used selective media (CT-SMAC, Enterohaemolysin agar, Oxoid, UK) for STEC detection, immunomagnetic separation with anti-O157 beads (Dynal, Norway), Vero cell assay and latex agglutination detecting shiga toxin 1 and 2 (Stx1,2) (Oxoid, UK), multiplex PCR using specific primers for genes encoding Stx1, Stx2, enterohaemolysin and intimin. Epidemiological information was obtained by semi-structured interviews undertaken by Epidemiology Unit staff with all family members and contacts.
Results: On the basis of collected samples we have isolated sorbitol-fermenting *Escherichia coli* O26 strain. Isolate originated from patient with HUS. Isolate harboured the stx2 gene, the eae gene encoding intimin and the ehxA gene encoding enterohaemolysin. Stx2 activity was also demonstrated. Stool samples taken from family members were negative for STEC O26.
Conclusions: Our data indicate the first documented case of HUS caused by shiga toxin 2-producing *Escherichia coli* O26 in our geographical area.

R2292 Electrochemical release of metal ions for water disinfection

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At present day the creation of effective and affordable water-disinfecting methods is one of important tasks in arid and semi-arid regions, where fresh water resources are limited and unevenly distributed, and drinking water often contains extraordinary large numbers of pathogenic bacteria. In remote regions of Uzbekistan portable water is the main reason of infectious diseases. The water disinfecting method presented in this work is based on the destructive impact of low concentrations of metal ions on bacteria in water. During the disinfection process, alloyed electrodes are placed into the water body and a current applied to the electrode causes the release of metal ions. The metal ions bind to the bacterial cell wall, causing its disruption and lyses. The efficacy of using different metal ions (Ag+, Cu2+, Au) combinations (within the limits of current drinking water regulations) for killing typhoid–paratyphoid, *Legionella pneumophila*, *Salmonella*, *V. cholera* etc. was examined. The cultivation, culture enrichment and the testing bacteria were performed following the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1995) for the evaluation of disinfection. Tests which were carried out by various independent labs and universities during the period of 1999 through 2003 have shown the dependence of bacteria killing time against metal ion concentration, different initial bacteria concentrations (from 10^3 to 10^{12} CFU/L), and the influence of different ion (Cl^-, SO_4^{2-}, S^{2-}, Fe^{2+}, Fe^{3+}) concentrations on the disinfection process. The best disinfection is obtained by using an alloy of silver/copper/gold composition with concentrations of metals in the ratio 70–90%/10–30%/0.1–0.2%, respectively. In the Aral Sea region (Uzbekistan) water disinfecting devices that were based on the developed method were installed on several hundreds manual water pumps, that allowed to decrease infectious diseases in this region.

R2293 Outbreak of cutaneous leishmaniasis in a nonendemic area of central Iran

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Objectives: To determine the epidemiological status of the cutaneous leishmaniasis outbreak, isolation and identification of the parasite in the study area.
Methods: This study was conducted from November 1999 to March 2002 in Ghanavat rural district of Qom province. Studies on human infection were carried out among students and the population of three villages once a season. A special form was completed to record necessary information for each households and students. Parasites were isolated from patients and identified by RAPD-PCR technique. Small mammals were caught in Sherman traps once a month and examined for the presence of amastigotes. Household dogs were also physically examined for the presence of any ulcer(s) or scar(s). Sandflies were collected and identified biweekly from indoor and outdoor fixed places in a village using 30 sticky traps from the beginning to the end of active season. Some female sandflies from rodents burrows were dissected and examined for the presence of promastigotes in September 2000 and 2001.
Results: A new outbreak of zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania* major was detected in the study area. Among 1069 inhabitants examined in December 1999, 5.1% had ulcers and 12.4% had scars. The most highly infected age group was 5–9 years for ulcers with a rate of 6.6%. The incidence of the disease was calculated 2.7 and 1.4% among the inhabitants and 2.8 and 2.3% among the students in 2000 and 2001, respectively. *Meriones libycus* (66.7%), *Nesokia indica* (27.3%) and *Hemiechinus aurithis* (6%) were present around the district. No leishmanial infection was seen in the slides. Sixteen domestic dogs examined and none of them appeared to be infected. A total of 7425 sandflies representing four species were collected and identified during April–October 2000. The common sandflies in indoors and outdoors were *Phlebotomus papatasi* and *Sergentomyia sintoni*. The active season of *P. papatasi* was from late April to early October in indoors. Natural leptomonad infection was found only in *S. sintoni* in rodent borrows.
Conclusion: We are dealing with ZCL in this new focus with an outbreak in 1999. *Leishmania* major is the agent, *M. libycus* and *P. papatasi* are probable reservoir and vector, respectively. The occurrence of this outbreak seems to be the result of construction of buildings near colonies of rodent burrows and also travelling of inhabitants to other ZCL foci of Iran.

R2294 Post-flood leptospirosis in the Czech Republic

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Objectives: Leptospirosis is a typical zoonosis with natural nidality and its occurrence in the climatic conditions of the Czech Republic is sporadic and the incidence is normally about 0.3 cases per 100 000 inhabitans.
Methods and results: In 1997 and 2002, however, the incidence of leptospirosis has been influenced by the actual natural phenomenon – catastrophic floods, which increased the numbers of serologically diagnosed and registered cases three times, i.c. to 0.9/100 000 in comparison with previous years. In 1997 there have been examined for leptospirosis a total of 7.156 subjects in the Czech Republic, and the disease was diagnosed and registered in 94 of them (and in 2002 in 92 patients respectively). Two-third of these cases came from inudate areas, half of them in direct relation with the floods. Four of registered cases (1997) of Weil disease have died in the Czech Republic. The difference between the actual and reported morbidity is critically discussed. The poster contains graphs, maps and tables, which described above mentioned.

R2295 *Mycobacterium kansasii* in Bilbao and Bizkaia

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Background: The aim of this study is to know clinical significance of isolates and the epidemiology of the infection.
Patients and methods: We reviewed 334 clinical records of patients with *M. kansasii* isolated from 1994 to 2002 in Bilbao area and from 2000 to 2002 in Bizkaia county. First, we used ATS diagnostics criteria for nontuberculous mycobacterial disease to the cases and then were further classified into according to modified criteria into probable, possible, probable-definitive, and colonisation. PCR-RFLP and AFLP analysis were also applied to clinical isolates.
Results: A total of 199 patients met ATS criteria (59.6%), probable disease 21 (6.3%), probable-definitive 37 (11.1%), possible 19 (5.7%) and colonisation 58 (17.4%). We considered ATS and probable disease as *M. kansasii* disease: 220 patients. These were 184 (83.6%) HIV- and 36 (16.4%) HIV+ with male predominance (5/1). 126 patients lived in Bilbao where the peripheral districts showed higher incidence 6.94‰. In Bizkaia county there were an incidence of 5.07‰ in the period studied. The predisposing factors were 25.9% COPD, 11.4% hepathopaty, 8.6% gastrectomy and 6.8% neoplasia. There was a higher frequency of COPD in HIV – ($P < 0.05$) and greater proportion of hepathopathy between HIV+. The respiratory symptoms were 70.9% cough, 24.1% dyspnoea, 18.2% haemoptysis and 20.5% chest pain. Fever, anorexia, weight reduction, cough and expectoration were more frequent in HIV+ patients ($P < 0.05$). X-rays showed infiltrates (106) or cavity lesions (106) and both (28). The predominant presentation was unilateral and in the right upper lobe. HIV− and + did not differ signifi-

cantly in X rays. Cavity lesions were associated with haemoptysis ($P < 0.05$). Isoniazid, rifampicin and ethambutol was initiated or previous theraphy was changed when *M. kansasii* was recovered in 201 patients (34 HIV+ and 167 HIV−). Subtype I was the only subtype isolated and seven clones were obtained, one of them with 42 strains of HIV− and other one with 10 of HIV+. Conclusions: ATS criteria, with their requirement for multiple positive specimens may be excessively strict for clinical purposes. Only subtype I was found although is heterogeneous.

R2296 Study of *Escherichia coli* DH5a lysogenisation with verotoxin1-producing bacteriophage

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Verotoxigenic *E. coli* (VTEC) are of the most important worldwide public health problems, causing outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome. This strains are genetically and phenotypically highly heterogenous, and produce verotoxin types 1 and 2, that are encoded within lambdoid prophages (temperate bacteriophages).Expression of these toxins is linked to bacteriophages induction and their genes are frequently lost in clinical isolates of *E. coli* upon subcultivation. So these genes are very instable, and act as a spreading factor of related disease.
Methods: The possibility and time-dependent lysogenisation of *E. coli* DH5á with the bacteriophage stock that prepared from verotoxin1 producing *E. coli* were studied. Lytic growth of the phage-particles carrying the verotoxin1 gene, of the PA101 strain was induced after treatment with mitomycin C. Production of verotoxin1 was measured by VTEC-RPLA kit in infected bacteria.
Results: Our results indicated that verotoxin1 bacteriophage is a mobile element, and could be easily transferred to other strains, with transduction. So VTEC strains act as a phage-producing centres in nature and could spread them to other bacteria.
Discussion and conclusion: Based on the results of this study, using phage-neutralising agents in health cares are necessary for preventing such infections.

R2297 Crimean Congo haemorrhagic fever infection simulating acute appendicitis

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Objective: An unusual cause of acute abdominal pain simulating acute appendicitis was presented.
Case: The patient was admitted with the complaints of fever, malaise, headache, nausea, vomitting, diarrhoea, severe bleeding. She was from Northeastern part of central Anatolia, where livestock husbandry was employed. On admission, she was severely ill. In her laboratory investigation, the minimum level of white blood cell count was 513/mm^3, haemoglobin level was 5.3 g/L and platelet count was 8910/mm^3. The maximum level of AST was 3195 IU, ALT was 1443 IU and LDH was 8190. Ribavirin therapy was started with the possible diagnosis of CCHF. While her clinical condition was improving, a sudden abdominal pain became her major problem. She had an acute pain with rebound at her right lower quadrant. She was consulted with general surgery department. Explorative laparotomy was performed with the possible diagnosis of acute appendicitis. The haemorrhage within the muscles was observed. It was concluded that, the haemorrhage was a complication of the infection with CCHF virus. Her CCHF serology was studied ELISA in Pasteur Institute in Lyon. Acute serum was found to be CCHF IgM-positive, and the convalescent serum was found to be CCHF IgG positive.
Conclusion: Acute abdomen because of severe bleeding could be a possible complication among the patients with CCHF infection. Therefore, these patients should be investigated, and haemorrhage should be ruled out before the decision of explorative laparotomy in CCHF suspected cases.

R2298 Identification of *Borrelia burgdorferi* from *Ixodes ricinus* ticks from Southern Moravia, Czech Republic

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Objectives: The prevalence of *Borrelia burgdorferi sensu lato* in Southern Moravia was investigated in suburban woods Pisárky (city of Brno), Czech Republic from 1996 to 2003.
Methods and results: Using the method of dark field microscopy and following PCR was mean ticks positivity in 1997–2000 7.3%, just examining with the PCR method in 2001–2002 positivity was 4.9% and using cultivation method and following DFM and PCR methods in 2002–2003, the reached positivity was 11.8% under the same condition of 14 days interval of collection. The last 2002–2003 collection resulted in 305 *Ixodes ricinus* ticks (21 larvae, 243 nymphs, 19 females and 22 males) collected from July to October 2002. Midgut tissue of each tick was dissected out and was transferred into BSK-H (Sigma) medium. After incubation each medium was examined by dark-field microscopy (DFM) for the presence of borreliae. The mean infection rate was 14.8%. Among 22 male adults 13.6% were positive, from 19 female adults 26.3% were positive. 243 nymphs showed 14.4% positivity and among 21 larvae 9.5% were positive. Isolation attempts resulted in 21 isolated strains. The positive samples were identified by PCR and following PCR-RFLP, confirmed by gradient SDS-PAGE. 35 samples were positive by PCR for the presence of *B. burgdorferi sensu lato*. Species identification by PCR-RFLP detection revealed strains of *B. afzelii*, *B. garinii* and *B. burgdorferi sensu stricto*. Some isolates showed a mixed population of these strains.
Conclusions: The results of all year collections refer to risk of human and animal infection by borreliae in the area of city of Brno.

R2299 The presence of antibodies against *Borrelia burgdorferi sensu lato* in rodents and occurence of this spirochaete in the mites parasitizing on them

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Borrelia burgdorferi sensu lato, causing Lyme disease, is transmitted by ticks (especially family Ixodidae), but is found also in some other blood-sucking arthropods. Rodents are considered to be the most frequent and important host group of these vectors. The prevalence of *Borrelia burgdorferi sensu lato* in wild rodents and in their small mites was examined. A total of 174 wild-living rodents belonging to two families (Muridae and Microtidae) was examined. Rodents were caught in a locality Bazantula in the north of Moravia in two consecutive years (2001–2002). Yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) predominated in obtained number of individuals. A presence of antibodies against *Borrelia afzelii* in the blood of rodents was found out by using indirect ELISA method. The mean positivity was 43.7% and the majority of positive samples were representatives of *Apodemus sylvaticus*. There were no significant difference in positivity between both rodent families as well as between genders. The wet ground character and often flooding of this locality results negligible number of ticks on vegetation and on single rodents, on the contrary for the occurrence of gamasid mites this area affords very favourable environment. 72 ectoparasites of order Acarina were collected here from the hair of rodents in 1999. DFM method confirmed the presence of spirochaetes in 14 specimens (19.4%). DNA of *Borrelia burgdorferi sensu lato* was found out in six cases of these 14. Spirochetes from one sample were successfully isolated and determined as *Borrelia afzelii* by using gradient SDS-PAGE.

R2300 Association of *Chlamydia pneumoniae* seropositivity and lung cancer

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Objectives: We aimed to evaluate the relationship between *Chlamydia pneumoniae* infection and lung cancer.
Methods: Seventy-nine, histopathologically diagnosed primary lung cancer patients were evaluated; of 79, six were female, 73 were male. The age range was between 28 and 77 years. Of the patients 13 were small cell carcinoma and 66 were nonsmall cell carcinoma (42 epidermoid carcinoma, 17 adeno carcinoma and seven large cell carcinoma). Also, 50 healthy people were evaluated as the control group. The indirect immunofluorescence test (Euroimmun, Germany) was used to detect *Chlamydia pneumoniae* specific IgG antibodies. The seropositivity was defined as the titre being greater than 1/100.
Results: Seropositivity was detected 69.6% of cancer patients and 43.2% of control group ($P < 0.01$). In the comparison of histological types, seropositivity was mostly detected in epidermoid carcinoma (30/42) and small cell carcinoma (11/12).
Conclusion: Our results revealed that there is a high seropositivity in *Chlamydia pneumoniae* in lung cancer patients. But it is clearly not sufficient to claim that *Chlamydia pneumoniae* infection is a cause of lung cancer. Much more prospective studies are needed in the future.

R2301 Clinico-epidemiologic feature and outcome analysis of haemorraghic form of Crimean Congo haemorraghic fever in Iran

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Crimean-Congo haemorrhagic fever is a zoonotic disease, which has been reported from the most parts of Europe, Asia and Africa. In this research the epidemiological features and the clinical and laboratory manifestations of the disease in Iran has been studied. From 51 probable cases from 1999 to 2002, in 13 cases the diagnosis was confirmed by positive IgM ELISA against the Crimean-Congo haemorrhagic fever virus. The mean incubation period was 4.2 days and the disease was more prevalent in men and middle age and the maximum incidence was in August and September. The clinical manifestations included extremely sudden, severe headache, myalgia, loss of appetite, nausea and fever. Patients rapidly exhibited a haemorrhagic tendency. Epistaxis, bleeding from the gums, petechia, purpura, melena, haematochesis, haematemesis, bleeding of venopuncture sites was common. Large echimotic areas appeared on the trunck, arms and legs and menmetrorrhagia was seen in female patients. Less prevalent signs were relative bradycardia, hypotension, tachypnea, abdominal pain, watery diarrhoea, icterus and lethargy. The most prevalent laboratory manifestations was haematuria, proteinuria, prolonged partial thromboplastin time, AST value >100 IU/dL, and less prevalent signs included anaemia, leucopenia, ALT value >100 IU/dL and prolonged prothrombin time. In this study for eight patients oral Ribavirin (in very ill patients by nasogasteric tube) was utilised in addition to supportive measures including infusion of cristaloids, packed cells, fresh frozen plasma and platelets and management of shock and renal failure and intensive care. Among these patients one patient died. In contrast in five patients who did not resieve Ribavirin only two survived.the cause of death in all of the patients were interactable haemorrhage and multiple organ failure. n the convalescent period except for the lassitude and asthenia (and development of erythema nodosum in one patient), no significant sequelae were seen in survivor. In the view of the relatively high incidence of CCHF in Iran and neighbouring countries, the education of the epidemiological features and the clinical and laboratory manifestations and providing laboratory facilities for rapid and accurate diagnosis of the disease has a significant priority.

R2302 Epidemiological monitoring of Astrakhan spotted fever

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ASF is emerging disease, appeared in the result of technogenic transformation of environment. About 2000 cases of ASF have been registered during 1983–2000 in Astrakhan region. The incidence increased from six cases in 1983 to 216 cases in 2000. From 2000 to 2003 incidence increased in 37.3 times when compared with 1983. The most of cases have been registered in the regions closely located to gas-condensate complex – 114.5–100 000 of population. 80% of cases have been confirmed serologically in IFT. The incidence coincides with seasonal dynamics of the vector-ixodid tick *Rhipicephalus pumilio* mostly in July–August. Index of abundance ticks on dog is 1.8–3.4.
Conclusion: ASF continues to be public health problem in endemic region.

R2303 Evaluation of ocular lesions in hospitalised patients with significant bacteraemia

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Objectives: In this study, we investigated frequency, aetiology and risk factors of retinal lesions in bacteraemic and septic patients.
Methods: A total of 150 adult bacteraemic, septic and control hospitalised patients were included in the study. Demographic data, area of admission, underlying diseases, Wilson's severity index, APACHE II scoring, Charlson's comorbidity index, community or nosocomial acquisition of bacteraemia, and responsible microorganism were recorded on the previously prepared forms. Blood cultures were obtained from all patients at least two times. All patients were examined for ocular lesions with the same ophthalmologist in 48–72 h after the first visit.
Results: Thirty-one (20.7%) patients were septic-bacteraemic, 43 (28.7%) were septic nonbacteraemic, 19 (12.7%) were severe inflammatory response syndrome SIRS; 16 (10.7%) were nonseptic nonbacteraemic infectious; and 23 (15.3%) were control. We found bacteraemia related ocular lesions (BROLs) in 22 (14.7%) patients. Winston scores were lower in the patients those have BROLs than others. According to the underlying diseases; there were more BROLs at the septic or bacteraemic patients with cancer (27.3%) and central nervous system (CNS) diseases (31.8%) than the others. The most detected agent at bacteraemia that has BROLs was *Pseudomonas* spp (27.3%), the second agent was *Staphylococcus* spp (22.7%).
Conclusion: As a result; BROLs were more frequent at bacteraemic-septic patients (12.7%) than the other groups. The most predisposing underlying diseases for BROLS were cancer and CNS diseases. Ocular examinations can give a clue for sepsis for this type of patients.

Table. Ocular lesions according to the patient groups

	Bacteremic non-septic	Septic non-bacteremic	Septic bacteremic	SIRS	Non septic. non-bacteremic	Control
No lesions	12 (8.0%)	30 (20.0%)	7 (4.7%)	14 (9.3%)	13 (8.7%)	15 (10.0%)
Non-related lesions	6 (4.0%)	10 (6.7%)	5 (3.3%)	5 (3.3%)	3 (2.0%)	8 (5.3%)
Lesions related with bacteremia	0	3 (2.0%)	19 (12.7%)	0	0	0

R2304 West Nile virus meningoencephalitis in central Tunisia: report of 13 cases

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Background: West Nile Virus (WNV) was first isolated in 1937 from the blood of an infected women in the West Nile province of what is known as Uganda. WNV infection is endemic in the middle east, sub-saharian Africa and Asia. In Tunisia a first outbreak was observed in 1997. Transmitted from birds to human through the bite of culicine mousquitoes, most WNV infections are mild or clinically unapparent. However, people older than 50 years are at highest risk of severe neurological disease.
Aim: This report describes clinical presentation, epidemiologic data and outcome of patients with WNV meningoencephalitis.
Methods: Retrospective study of 18 cases of WNV meningoencephalitis, recorded in a recent outbreak occurred in Central Tunisia from July to October 2003. Diagnosis was confirmed in 15 cases by detection of WNV antibodies with IgM capture and indirect IgG ELISAs from sera or cerebro spinal fluid (CSF).
Results: Data were obtained from 13 patients: 10 males and three females, with mean age = 58 years (22–80 years). All patients were from rural origin. Nine patients had meningitis (isolated in seven cases, associated to acute flaccid paralysis in two cases) and four patients had meningoencephalitis. CSF showed pleocytosis in all cases with lymphocytic predominance in six cases and moderate protein elevation in six cases. Hyponatraemia, lymphopenia and cytolysis were noted in respectively four, two and three cases. Only fatal case occurred in elderly patient, two had flaccid paralysis sequelae.
Conclusion: Since it's the second outbreak of WNV in our country and substantial morbidity in elderly persons, WNV surveillance among patients, in vectors and birds is necessary.

R2305 Diabetes insipidus due to *Streptococcus pneumoniae* meningitis

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Diabetes insipidus (DI), which is characterised by polyuria and polydipsia due to excessive urinary loss of solute-free water, can be either central (CDI) or nephrogenic. The syndrome of inappropriate hormone secretion is a well-known complication of bacterial meningitis, but, CDI is extremely rare in these patients. A 54-year-old man with a central diabetes insipidus as a complication to pneumococcal meningitis is presented. The patient was admitted to our hospital with a 4-day history of severe headache and vomiting. Two days before admission, he developed fever (39°C) and malaise. The initial examination showed slight confusion and nuchal rigidity. Lumbar puncture revealed increased cerebrospinal fluid (CSF) pressure, purulent appearance and leukocytes 1200/mm3 (85% PNL and 15% lymphocytes), protein 450 mg/dL, glucose 5 mg/dL. The Gram stain of CSF was positive for *Streptococcus pneumoniae*. Culture of blood and CSF yielded *S. pneumonia*e. According to the antimicrobial susceptibility test, ceftriaxone 4 days and dexamethasone 32 mg/day were initiated. Dexamethasone was stopped at fourth day of treatment diabetes insipidus developed on day 6 after the admission. The diagnosis of CDI was established by the response of the urine osmolarity and serum sodium levels by water deprivation test and the administration of 5 μg of arginine vasopressin. Desmopressin acetate was added to treatment and fluid adjustment was performed in the patient. Ceftriaxone was stopped fourteenth day of treatment. Urine density, serum osmolarity and urine output in 24 h of the patient has become normal levels by treatment of desmopressin. Despmopressin was stopped at tenth day of the treatment since the urine output levels has become less than normal. Although infection-precipitated CDI is a rare occurrence, its recognition is important. Therefore, CDI should be kept in mind in patients with bacterial meningitis.

R2306 *Salmonella* findings out and on a poultry farm

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We formulated a hypothesis, that *Salmonella* is more frequent isolated from poultry farm eggs than out of farm eggs.
Aim: (i) To research frequency of *Salmonella* findings in poultry farm and out of farm eggs; (ii) To research *Salmonella* present in food samples for chicken on and out of poultry farm; (iii) To research frequency of *Salmonella* findings in faeces samples and cloacal smears of chicken on and out of poultry farm.
Material and methods: We were taking samples from 880 chicken eggs. From 10 farms we were taking samples from 597 eggs (86%) 22 times. From small left farms we were taking samples from 96 eggs. During the same period we were taking samples from 187 (21%) out poultry farm eggs. We were taking 88 samples of chicken excrement. We homogenised each egg, and contents planted on liquid nourishing bases which favoured *Salmonella* multiplying.
Results: We isolated *Salmonella* from five (0.57%) of 880 inspected chicken eggs. There is a significant difference in *Salmonella* findings out and on poultry farm ($P = 0.008$; Fisher's correct test). *Salmonella* findings in chicken excrement: No. of samples – 79, and no. of positive samples – 13 (14.8%). 11 (12.5%) SE, and two (2.3%) *Salmonella typhimurium* (STM) *Salmonella* findings in concentrate. We isolated STM in two (4.3%) of 47 inspected samples. Both of samples are from Salas farm. We registered characteristic plasmid profile, presented with constitutive plasmid by 38 MDa, in SE isolated from chicken excrement on farm.

Origin of eggs	Isolated *Salmonella*	Not isolated *Salmonella*	Total
Eggs from farm	1 (0.14%)	692	693
Eggs out of farm	4 (2.14%)	183	187
Total	5 (0.57%)	875	880

Conclusion: (i) We reject active hypothesis, because we often find *Salmonella* in out of farm eggs then poultry farm eggs which in opposite to our hypothesis. (ii) The results of this study shows from 50 to 100 times often presence of *Salmonella* in eggs in regard to existing estimates. Therefore, they are significant ATI way of transmission, provoked by this carrier (cause).

Vaccines

R2307 Evaluation of hepatitis B vaccine protection in Iran's routine immunisation programme

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Objectives: More than 5% of the people are chronic carrier of hepatitis B virus. Acute and chronic hepatitis and their complications are health problem around the world. Immunisation is the best-known method of prophylaxis. Condition of vaccination has direct impact on amount of antibody formation and prophylaxis. To study the prophylactic effects of vaccination according to Iran mass immunisation protocol, a prospective research was carried out in Uromiyeh since 2002.
Materials and methods: Sixty neonates of HbsAg negative mothers were admitted to this study. One month after the last vaccine,

according to Iran general program, all infants' sera were tested for anti-HbsAg.
Results: The final results of 39 patients who completed the study include: (i) 21 males (53.85%). 18 females (46.15%) (ii) average anti-HbsAg production in males and females were 546.86 and 639.781 IU/L. Mean of all samples was 589.74 IU/L. Lower limit was 18 U and upper one was 1500.
Conclusion: The study shows 100% successes for routine programme because for prophylaxis only 10 U is enough. Comparing with other studies, the best results are extracted from this programme so the study recommends the persuasion of the programme.

R2308 Immunogenicity of an anti-Hav vaccine in infants

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Background and objectives: Hepatitis A is a common viral infection causing substantial morbidity and mortality. The anti-HAV vaccination in infants would guarantee the control of infection. However the immunogenicity of the HAV vaccine in infants could be impaired by the presence of passively acquired maternal HAV antibodies. Aim of this work was to evaluate safety and immunogenicity of anti-HAV inactivated vaccine administered during the first 4 months of life to anti-HAV seronegative babies.
Methods: Ninety-two babies born at IRCCS Policlinico San Matteo, in northern Italy, during 8-months period (September 2002–April 2003) were vaccinated with a two dose schedule (4 and 10 months of age), following obtained informed written consent from one parent. All the babies were characterised seronegative at birth and did not presented HAV-RNA positivity in a series of three stool samples taken at 1, 2 and 3 months of age. The detection of anti-HAV IgG was performed by MEIA method (AxSYM HAVAB 2.0, Abbot Italy) quantitave dosage using a calibration curve with five points: 0, 5, 10, 20, 50, 100 mIU/mL. The study was approved by the Ethical Committee of IRCCS Policlinico San Matteo.
Results: Neither local side-effects (redness/swelling) nor general (fever >38°C) were observed. After the first dose of vaccine 70/82 (85.4%) babies developed a protective titre (>10 mIU/mL): geometric mean titre (GMT) was 17.5, median 18.65; 25° centile 12.4; 75° centile 26.4 mIU/mL. After the second dose of vaccine all babies developed a protective titre: GMT 926.8; median 849.7; interquartile range (that include 50% of the sample): 565.2–1829 mIU/mL.
Conclusion: Anti-HAV vaccine resulted safe and highly immunogenic in seronegative babies, even if administered in first year of life. Our findings suggest that children born by anti-HAV negative mothers could be vaccinated early during the first year of life together with other routine vaccinations.

R2309 Rapid colorimetric assay for determination of viability of BCG vaccine

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Objective: The potency of BCG vaccine is evaluated as a content of colony forming units (CFU). The current method recommended by the EP and WHO for determination of potency of BCG vaccine depends on counting the number of CFU produced after culture on solid medium. Estimation of CFU count takes 3–4 weeks. Tendency to clumping of mycobacteria and the type of medium used may cause variable results. The aim of this study was developing of a colorimetric assay of tetrazolium salts reduction as a rapid method of BCG vaccine viability control.
Methods: Tetrazolium salts in presence of BCG were metabolically reduced to highly coloured end products called formazans. The quantity of formazan produced was directly proportional to CFU count. Tested BCG vaccine prepared from Moreau substrain is commercial preparation used for vaccination in Poland. Twenty lots of BCG vaccine were controlled by tetrazolium salts test alternatively to culture method. For the preparing the calibration curve the working reference BCG lot s. 4012002 was used. The different variants of BCG and tetrazolium salts concentrations were tested. BCG dilutions were transferred to microtitre plate and 25 µl of XTT [2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide] (Sigma) were then added with intermediate electron carrier - menandione sodium bisulphate (MSB, Sigma) to each well. Control wells had saline instead of either XTT or the vaccine. The optical density of each well was measured at 450 nm using a Labsystems Multiskan MS plate reader.
Results: The best calibration curve was obtained for range of BCG concentration 5×10^4–20×10^6 CFU/mL and twofold dilutions. The optimal concentration of XTT was 3 mg/mL. The viability of tested BCG were read from optical calibration curve. The assay using XTT showed great sensitivity in detection limits 4×10^5–16×10^6 CFU/mL. In parallel, all lots of BCG vaccines were tested in cultures on Ogawa solid medium. The number of CFU was counted after 3 weeks. In mentioned viability limits the correlation of viable count of all test samples from one test method to the second was never <0.82 using Pearson's correlation.
Conclusions: Tetrazolium salt (XTT) assay is rapid method of evaluation of BCG vaccine potency that produces a result in 48 h. It is significant correlation ($r \geq 0.82$) between results of conventional viable count method and tetrazolium salts reduction method used for control of potency of BCG vaccine.

R2310 Protection against leptospirosis with the peptide PP(R) of 25 amino acid from Hap1 (haemolysin-associated protein) in gerbils (*Meriones unguiculatus*)

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The leptospirosis is a widespread human and animal disease caused by pathogenic leptospires. The medical and economic losses caused by such forms of the zoonotic disease justify the use of *Leptospira* vaccines in human or animal populations at risk. However, available vaccines enhance a lipopolysaccharide-directed immune response, which is serogroup-specific.
Objectives: Our previous work determined that immunisation with the haemolysis-associated protein Hap1 (as known LipL32) expressed by adenovirus or plasmid induced in gerbils a significant protection against a virulent *Leptospira* challenge, while the recombinant protein Hap1 did not provide any significant protection. To avoid use of vector, we identified a peptide of 25 amino-acid from the Hap1 protein and used it in experimental challenge assay.
Methods: Gerbils were immunised three times with either PP (30 μg) either PP coupled to the protein carrier KLH (30 μg), controls received PBS at the same times. Two weeks after the last immunisation, gerbils were challenged by intra-peritoneal route. Each animal received 0.5 ml of fresh culture of the virulent *L. interrogans ss* serovar *canicola* (10^7 leptospires). Gerbils were daily observed, and mortality rate was recorded for 28 days after challenge. Serological response was followed by ELISA.
Results: ELISA against PP was realized on sera from gerbils protected against a leptospirosis challenge after immunization either by adenovirus either by plasmid encoding for Hap1. The IgG immuno response of gerbils immunised with PP-KLH or PP was analysed by ELISA against the peptide PP. Statistical analyses of mortality incidence and survival curves of gerbils showed that the both groups of animals (vaccinated with the peptide PP-KLH or PP alone) were significantly protected against lethal onset of leptospirosis.
Conclusion: Our results showed that the protective effect within pathogenic strains of *Leptospira sl* is shared by the peptide PP from the Hap1 protein. Peptide vaccination would efficiently prevent leptospirosis. This finding should facilitate the design and development of new generations of vaccines against pathogenic leptospires.

R2311 Seroprevalence of rubella antibodies among university female students

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Objectives: Rubella is a relatively mild viral infection. It's complications are arthritis, thrombopenic purpura, and encephalitis, but its prognosis is generally favourable. The rubella virus can induce problems in the next generation due to fetal infection. Seroprevalence of rubella in Turkey is still insufficient and national immunisation schedules do not include rubella vaccination. In this study we aimed to investigate the seroprevalence of rubella at child bearing age in an unvaccinated population in Erzurum, Turkey, to discuss whether routine rubella vaccination is necessary, and if so when it should be administered.
Methods: Serum specimens of 176 volunteers of healthy university female students aged 18–22 years were randomly sampled for screening rubella-specific IgG/IgM antibodies by an automated enzyme immunoassay system (Clark Laboratories, Inc. Rubella IgG ELISA).
Results: A total of 174 subjects were positive for rubella-specific IgG antibody, giving a prevalence of 97.7%. Rubella-specific IgM antibody was not detected in none of the students.
Conclusion: The present study indicate that majority of the females in child-bearing age in our region have an immunity to rubella due to natural infection. This may be interpreted that routine rubella vaccination is not necessary. However, the incidence of autism in the UK has increased markedly over the past decate. Some have proposed that this may be related to introduction of the MMR (measles–mumps–rubella) vaccine in 1988. But in some recent studies it has been reported that there is not any relation between MMR vaccination and the risk of autism. Nonetheless, we think that with such a relative low proportion of susceptibility (2.3%) among university students, a rubella vaccination programme may be enforced to prevent possible outbreaks of congenital rubella infection in the future.

R2312 Significance and interpretation of serological investigation of antibodies after vaccination against tick-borne encephalitis

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Objectives: Czech Republic is considered as an important endemic tick-borne encephalitis (TBE) region in Europe. The morbidity moves about 4–7/100 000 inhabitants per year for last decade. It represents 415–750 TBE illnesses per year. The active vaccination is very often practised in children and young people in our conditions, rather less in adults and elderly persons. Protection after basic immunisation is guaranteed for 3 years at least (vaccine information sheet) or according to the specific antibody status maybe longer.The aim of our study was to assess contribution of detection postvaccination antiTBE IgG for rational decision in the immunisation practice. Interpretation problems may occur from cross-reactions with other flaviviruses and neutralising capacity of ELISA detected IgG antibodies is a question too. Testing of anti-TBE antibodies avidity was done.
Methods: A total of 120 persons with basic vaccination schedule were enrolled and antibodies persisting after basic vaccination in time aplication of the first, second and third booster were investigated. Antibodies were assayed in ELISA IMMUNOZYM FSME-IgG and were compared with neutralisation test (NT). Avidity assay of anti-TBE antibodies: in-house system of protein denaturating immunoassay (avidity-EIA).
Results: The investigation of antiTBE IgG antibodies after 3 years since the last revaccination demonstrated their persistence longer than the guaranteed 3 years. Geometric mean titres (GMT) ranged 514–690 VIEU/ml in ELISA and 18–32 (titres) in NT depending on the number of applicated doses. The avidity index was higher in group of vaccinated persons compared with patients in acute disease state.
Conclusions: (i) The IgG ELISA results correlate well with the viruses-neutralising antibody titres in check successful vaccination. It was not found false positivity IgG in ELISA in investigated group of vaccinated persons; (ii) the IgG antibodies elicited after vaccination are of high avidity; this characteristic correlate with greater serum antiviral activity; (iii) we observed persistence of postvaccinal antibodies for period longer than 3 years in children and young person particularly. In the immunisation practice there are frequent cases, when is detection of antibodies advisable to use (neurological illnes, allergic patient, pregnancy, etc). ELISA is available method for this situation. Results interpretation is necessary to do in connection with age, history of vaccination and health status.

Infection in transplant recipients

R2313 Four cases of *Mycobacterium tuberculosis* infections in liver transplant recipients in Korea

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Objectives: Korea has been one of endemic areas of *M. tuberculosis*, but there has been no data in liver transplant recipients.
Methods: Four cases of *M. tuberculosis* infection were reviewed among 98 liver transplant recipients between 1996 and 2002.
Results: Two patients were diagnosed tuberculosis before transplantation, one probable case and one possible case. After transplantation, one proven case and one probable case were diagnosed. In 52-year-old male, screening test before transplantation revealed multiple right upper lung nodule. Biopsy findings were compatible with *M. tuberculosis* and anti-tuberculosis medications (isoniazid, rifampicin, ethambutol and pyrazinamide) were started 38 days before transplantation. No tuberculosis associated illness was found during 12 months after transplantation. In 51-year-old male, pleural effusion was found during preoperation routine check. Anti-tuberculosis medication was started because of high (123 IU/L) ADA level in pleural fluid. The regimens were isoniazid, ethambutol, rifampin and pyrazinamide that continued for 12 months with no specific complication. Other 50-year-old woman complained night sweating and back pain at 10 months after transplant. ADA levels of pleural fluid was elevated to 142 IU/L and acid-fast bacilli stain of spine was positive. *M. tuberculosis* was cultured from the spine. During 12 months' anti-tuberculosis therapy no recurrence was found. Thirty-eight year old male complained haemoptysis 13 months after transplant. He was diagnosed with sputum AFB smear test. Isoniazid, rifampin and ethambutol were started but 1 month later rifampin was held due to rash. Five months later he stopped medication by himself. But no active lesion was found at follow up chest X-ray. Forty-five months later, he expired because of liver failure.
Conclusion: In Korea, incidence of liver transplantation related *M. tuberculosis* infection is low (1.0%) and INH prophylaxis seems not needed. Most patients could be treated with the first line anti-tuberculosis medication and no relapse was observed during the follow-up periods.

Paediatric infections

R2314 Adenoviruses as causative agents of infections in children

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Objectives: To define the frequency of positive anti-adenoviral IgG and IgA antibodies in children hospitalised during the current year (2003) in our children's hospital.
Methods: A total of 355 children younger than 14 years of age were examined, all of whom had been treated as inpatients for symptoms of viral, febrile illness. Anti-adenoviral IgG and IgA antibodies were assessed by conventional enzyme-linked immunosorbent assay (ELISA). The major antigenic determinant that was used, is common for all adenoviral subtypes that produce illness in humans. IgG quantification was performed using the single-cut-off method, which provides high accuracy and reproducibility of results. High IgG and positive IgA antibody titres were considered to be indicative of acute infection (early phase). The finding of a low IgG antibody titre alone was interpreted as a normal immune response to recurrent, symptomatic or clinically silent infection by various adenoviral subtypes.
Results: Of a total of 355 sera examined, 62 (17.4%) were found to be positive for anti-adenoviral antibodies. The 62 patients with positive antibody tests against adenoviruses had been mainly treated for upper (pharyngitis, tonsillitis) or lower (bronchiolitis, pneumonia) respiratory-tract infections. 45% of all patients (355) were found to be positive for IgG antibodies only, a finding suggesting past infection, whether symptomatic or clinically silent.
Conclusion: Adenoviruses constitute a significant causative agent of respiratory infections in children, with a higher incidence during spring time (54% of all cases examined).

R2315 The importance of EBNA1-IgG (nuclear antibody) for the confirmation of recent/acute infection from Epstein-Barr virus in children's hospital

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Objectives: The early diagnosis of the recent/acute infection from EBV is of great importance for the diagnosis of the disease and the avoidance of the frequent, unnecessary use of antibiotics. The determination of serological pattern of EBV in patients who probably suffer from infectious mononucleosis after the addition of the EBNA1-IgG antibody in the serological screening by the microbiological laboratory.
Methods: Our material was 233 serum samples of children aged 6 months–14 years admitted at out children's hospital during the last 8 months or visited our outpatients' department with clinical suspicion of infectious mononucleosis. The sera were tested for VCA (viral capsid antibody) IgG and IgM of EBV using the method of indirect immunofluorescence (IFA) and for EBNA1-IgG using the immunoenzymatic method ELISA.
Results: From the 233 serums tested, 34 were found positive for recent/acute infection of EBV (14.6%). The profile of the antibodies of the positive samples is shown in the table below.
Conclusions: The measurement of VCA-IgM and VCA-IgG antibodies only in the EBV serology results in the loss of 29.3% of the cases of recent/acute infection, a problem which can be solved with the help of EBNA1-IgG [profile: EBNA1-IgG(−), VCA-IgG(++)]. Therefore the determination of EBNA1-IgG is considered necessary in order EBV infection to be diagnosed with high fidelity.

Table 1. Positive Samples for EBV recent/acute infection

N	%	VCA-IGM	VCA-IgG	EBNAI-IgG
4	11.8	+	−	−
20	58.9	+	+	−
10	29.3	−	+	−

R2316 Evaluation of prenatal screening and intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease

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Objectives: The evaluation of prenatal screening for vaginal and rectal colonisation and intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal (GBS) disease in the Hospital Universitario de Canarias (HUC) in the last 4 years.
Methods: The prenatal screening of the pregnant women was realised at 39–40 week gestation with the swab collection both the lower vagina and rectum and cultured 24–48 h in a tube of Granada Medium (Biomedics). The candidates to intrapartum antibiotic prophylaxis was the colonised pregnant women and the pregnant women with risk factor (according with the last CDCs recommendations). In 2003, we have changed the Granada Medium for the Instant Granada Medium and we have also incremented the nurses per delivery-rooms and a better implementation of the intrapartum antibiotic prophylaxis. From 2000 to November 2003, we have evaluated the screening pregnant women percentage, the colonised pregnant women percentage, the incidence of the early-onset and the late-onset GBS bacteraemia and Enterobacteriaceae bacteraemia.
Results: During 2000, 2001, 2002, until November 2003, the number of deliveries at HUC was 2418, 2543, 2448 and 2037, respectively. 37.3, 38, 40, 40%, of the pregnant women was screened and 10, 11, 10 and 15% were colonised per year. In 2000 there were 4/1000 early-onset GBS bacteraemia per live births; 4.4/1000 in 2001; 3.2/1000 in 2002 and 0.5/1000 in 2003. The late-onset GBS bacteraemia per live births were 0.4/1000, 0.4/1000, 1.6/1000 and 0/1000, respectively. The evolution of Enterobacteriaceae bacteraemia projected an incidence of 11.1/1000 live births in 2000, 7.07/1000 in 2001, 6.1/1000 in 2002 and 13.2/1000 in 2003. In 2003 we have incremented by 5% the detection colonised pregnant women and the incidence of early-onset bacteraemia has declined by 87.5% from 4/1000 live births in 2000 to 0.5/1000 live births in 2003.
Discussion: Declines in perinatal GBS diseases incidence in 2003 suggest that prevention strategies have been implemented succesfully, though it could be improved. Continued surveillance of neonatal bacteraemia other than GBS is needed.

R2317 Pertussis is the main cause of prolonged cough illness in children

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Background: The morbidity of pertussis in Lithuania in fact is higher than registered officially. According to existing practice, children in Lithuania are being vaccinated at 3, 4.5 and 6 months of age using cellular pertussis vaccine and booster dose at 18 months of age. The aim of our study was to establish *B. pertussis* role of prolonged cough illness in children, by evaluating pertussis epidemiological and clinical data.
Patients and methods: A total of 70 children with prolonged cough illness hospitalised in Vilnius University Children's Hospital, were observed during 8 months of 2001 year. Individual and

epidemiological data, clinical manifestation and laboratory results (*B. pertussis* IgM and IgA antibodies, ELISA, Labsystem, Finland) have been evaluated.
Results: Pertussis was diagnosed in 53 children. To 49 children the diagnosis of pertussis was confirmed by serology tests and for four children, only by clinical findings. Children age was 1 month–15 years (mean age 7.6 ± 5.4 years). Nine of 16 infants were not vaccinated at all, seven infants were not fully vaccinated against pertussis. Fifty-eight per cent before they fell ill with pertussis, got in to contact with persons with prolonged cough illness in the family, school or day-care centres. The duration of cough before pertussis diagnosis was confirmed was at average 47 days (mean 4–270 days). Most of children (88.7%) had paroxysmal cough, 41.2%, typical 'whoop' and 13.7%, apnea, 39.6% of the patients had a cough leading to vomiting. Four children were treated in ICU. All recovered.
Conclusions: Pertussis is the main cause of prolonged cough illness in children. Most of the children had typical clinical symptoms of pertussis.

R2318 Relationship between serum selenium levels, lipid peroxidation and acute bronchiolitis in infancy

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Objectives: The possible interaction between serum malondialdehyde (MDA) and selenium (Se) levels and the occurrence and severity of acute bronchiolitis in children was investigated in order to discuss the possible role of Se for the disease management.
Methods: Thirty-four infants with acute bronchiolitis were enrolled into the study group at the paediatric emergency room in Dicle University Hospital, Diyarbakir during September 2002 and May 2003, and were compared with 25 age-matched healthy controls. Serum samples were taken for serum Se and MDA measurements, and clinical score was assessed at admission. Blood was taken again from the children with bronchiolitis at 2 months after discharge from the hospital.
Results: MDA as a marker of free radical activity was significantly higher in patients with acute bronchiolitis than at postbronchiolitis stage and the controls 4.2 ± 2.5, 1.4 ± 0.8 and 0.7 ± 0.2 nmol/L, respectively ($P < 0.001$). Infants with bronchiolitis had lower mean serum Se levels at the acute stage than after 2 months (31.7 ± 28.9 μg/L vs. 68.4 ± 26.4 μg/L, respectively); both of which were significantly lower than the control group measurements (145.0 ± 21.9 μg/L) ($P < 0.001$). There was a negative correlation between serum MDA and Se levels in the patient group ($r = -0.85$, $P < 0.001$). There were no significant correlations between serum Se or MDA levels and patient's clinical score at admission, age of the patient, child's immunisation status, parental smoking habit and family crowding index.
Conclusions: Increased MDA levels and impaired Se status demonstrate the presence of possible relationship of these parameters with pathogenesis of acute bronchiolitis, and antioxidant supplementation with Se may be thought to supply therapeutic effect against bronchiolitis.

R2319 Is there any relationship between *Chlamydophilia pneumoniae* infection and juvenile idiopathic arthritis?

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Objectives: The aim of the study is to investigate the role of *Chlamydophilia pneumoniae* in the development and exacerbation of juvenile idiopathic arthritis (JIA).
Methods: Blood samples were taken from 60 JIA patients in active period and 4 weeks after this period. Synovial fluid samples were taken from 20 of the 60 patients. Besides that, 22 familial Mediterranean fever (FMF) patients in active period and also 35 healthy children were taken into the study as the control group. Synovial fluid samples were taken from only three children with FMF. IgG, IgM, IgA levels against *C. pneumoniae* were studied from the serum samples with immunofluorescence method and IgG antibody and PCR studies for *C. pneumoniae* DNA were studied from the synovial fluid samples.
Results: A total of 29 (48.3%), 18 (81.8%) and 11 (73.3%) of JIA, FMF and healthy children cases were found preinfected with *C. pneumoniae*, respectively. Preinfection with *C. pneumoniae* among FMF patients was found significantly higher than JIA cases. We did not find a significant difference by comparing JIA cases with healthy children. The markers of chronic *C. pneumoniae* infection are IgG >1/512 and IgA >1/40 and this situation have been observed only in six patients with JIA, one with FMF and in two healthy children. Synovial fluid antibodies were found higher than 1/512 and four times higher than normal serum in one JIA and three JIA patients, respectively. *C. pneumoniae* DNA was not detected in any synovial fluid sample from FMF or JIA patients by PCR.
Conclusions: *C. pneumoniae* infection does not have a triggering or a progressive effect on clinical situation in JIA aetiopathogenesis, resulting from multifactorial etiological factors. New, extensive and serial studies (especially PCR studies for synovial tissue) are needed in order to confirm our indirect results in case level.

R2320 Diagnostic value of virological, bacteriological and cytological screening in children with chronic tonsillitis

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Objectives: Chronic tonsillitis (CT) is a common disease in clinical practice. Specialised cell junctions (SCJ) of reticular epithelium of the tonsillar crypt regulate paracellular movements of antigens. The breakdown of SCJ during CT due to pathogens action may facilitate the exfoliation of epitheliocytes into lumen of tonsillar crypts. The aim of the study was to compare diagnostic value of cytological, bacteriological and virological tests among patients with chronic tonsillitis and determine the susceptibility of isolated microorganisms to antimicrobial drugs.
Methods: Sixty chronic tonsillitis patients in disease exacerbation stage (10–18 years old) were investigated for the presence of different bacteria, viruses and desquamated epithelial cells in the crypts of palatine tonsil. The rapid diagnosis of adenovirus, parainfluenza, influenza A and B, and respiratory-syncytial virus infection was made by viral antigen detection using immunofluorescence direct procedure from pharyngeal aspirates. The HHV1, 2 and CMV DNS was detected by polymerase chain reaction. Immunohistochemical staining was performed in Sweden Orebro University on 30 tonsil specimens obtained from tonsillectomy. Tissue sections were stained with CMV and EBV antibodies. A bacterial throat culture was obtained from each patient by swabbing both tonsils with a cotton-tipped swab. Microorganisms' biochemical indentification was performed using Crystal datorsystem tests.
Results: We have shown that in CT the tonsils are reservoir of streptococci, staphylococci, adenoviruses and Epstein-Barr virus. The isolated microorganisms are resistant as a minimum to two from eight used antimicrobial drugs. Obtained data showed an increased desquamation of epithelial cells that correlates with intense reproduction of viruses and microorganisms.
Conclusion: It is suggested that chronic inflammation is maintained by colonisation and multiplication of CT specific pathogens. In CT exacerbation pathogens proliferation lead to alteration in SCJ morphology with consequent shedding of the epitheliocytes. The present study suggests the synergy between crypt cytology, and bacteriological and virological findings.

R2321 *Haemophilus influenzae* serotype e cross-infection from a cystic fibrosis patient in a paediatric hospital in Portugal

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Background: *Haemophilus influenzae* (HI) has been responsible for a number of human diseases, including chronic respiratory infections as cystic fibrosis. Most of HI strains are noncapsulated, being the capsulated strains classified in six serotypes, a–f. The introduction of Hib vaccine in many countries contributed to a decline of this serotype. Many studies have been done to find out the possible changes that may occur in the epidemiology of the HI disease, namely changes related to capsule replacement and virulence of non-b strains.
Objectives: Phenotype and molecular characterisation of three HI strains isolated in a Children's Hospital in OPorto, Portugal, involved in a cross-infection. Two of the strains were isolated, within 1 month from a boy, 11 years old, with cystic fibrosis. The other strain was collected from a nurse that was in contact with this patient, 4 days after the first isolate of the child.
Methods: Biotype, serotype (agglutination procedure and PCR), β-lactamase production (nitrocefin) and antibiotic susceptibility (microdilution assay) to 14 antibiotics (ampicillin, amoxicillin-clavulanate, cefotaxime, cefaclor, cefuroxime, cefepime, meropenem, tetracycline, chloramphenicol, trimethoprim–sulphamethoxazole, rifampicin, ciprofloxacin, azithromycin and erythromycin) were performed to characterise the phenotype of isolates. Breakpoints used were according to NCCLS 2003. A molecular approach was used to evaluate the clonality of isolates (pulsed-field gel electrophoresis).
Results: All three strains were β-lactamase negative, belonged to biotype IV and had the same capsular serotype, e. They had the same antibiotic susceptibility pattern, being only resistant to trimethoprim–sulphamethoxazole DNA typing permitted the identification of the same pattern in the three strains showing that all had the same origin and were closely related.
Conclusion: These results are in agreement with a cross infection of HI serotype e from a cystic fibrosis child to the nurse that was in contact with him. One month later the child was still infected with the same strain. Serotype e strains are still rare in Portugal, although they are emerging after the introduction of the Hib vaccine. It is of most concern to continue monitoring serotypes of HI responsible for different infections, to detect possible changes that may occur after the introduction of the vaccine in our country.

R2322 Coexistence of pseudomembranous enterocolitis and Crohn's disease: a rare occurrence

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Introduction: The clinical picture of *Clostridium difficile* infection ranges from asymptomatic infection to severe diarrhoea, pseudomembranous enterocolitis, toxic megacolon and colonic perforation. Here we present a patient with pseudomembranous enterocolitis, evolved in the setting of Crohn's disease.
Case: A 9-year-old girl presented with a history of abdominal pain that had started 10 days before. She had reportedly been given an enema containing hydrogen peroxide in a medical facility; then she started to pass watery, bloody stools. On admission to our clinic, she had septic appearence and abdominal rigidity. Her clinical status deteriorated gradually afterwards. There were no pathological findings in laparoscopy except for some haemorrhagic fluid in the abdomen. She recovered with meropenem plus metronidazole and was discharged. Two weeks later, she presented with vomiting, diarrhoea, fever, dehydration with distention and tenderness of the abdomen. Plain abdominal films showed gas-fluid levels. Ultrasonography revealed ascites. 'Accordion sign', noted in abdominal computed tomography and endoscopically-detected yellow membranes on the rectosigmoid mucosa were both suggestive of pseudomembranous enterocolitis. The presence of *C. difficile* toxin A confirmed this diagnosis. Metronidazole in combination with oral vancomycine was started and the patient's symptoms gradually subsided. A few days later, she began to complain about abdominal pain again while still on antibiotics. Rifampin and intravenous immune globulin were added to her therapeutic regimen. Metronidazole was stopped and vancomycine dosage was reduced. Repeat endoscopy was completely normal. Days later, her clinical status began to deteriorate once again. She had diagnostic laparotomy and since the appearance of the colon was very suggestive of inflammatory bowel disease, partial colectomy was performed. The histopathological examination of the surgical specimen showed characteristics of Crohn's disease. She was given prednisolone and sulfasalazine and is doing quite well since then.
Discussion: This is the second case report of coexistence of pseudomembranous enterocolitis and Crohn's disease in electronically searchable medical literature. Crohn's disease may be an underlying pathology which facilitates pseudomembranous enterocolitis and we propose that these coexistence be kept in mind when dealing with patients showing the clinical manifestations of either disease.

R2323 The outcome in children with congenital cytomegalovirus infection

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Objectives: In this report, the authors have presented clinical picture, course and therapy used in 18 infants with congenital CMV infection hospitalised at the Department of Paediatric Infectious Diseases in Brno within January 1998–August 2003.
Methods: The diagnosis of congenital CMV infection was established partly clinically on the base of typical symptoms of congenitally acquired disease, in a part of infants even from personal anamnesis (mother's disease during pregnancy) and especially verified by means of laboratory isolation of CMV virus in urine and by means of serological examinations.
Results: In the set of 18 newborns with confirmed congenital CMV infection, 11 of them have permanent neurological consequences involving motor of psychomotor problems. Five of them suffer from sensoric affection (4× disturbance up to loss of hearing, 1× strabism).
Conclusions: (i) As most maternal infections are asypmtomatic, repeated serological screening of all susceptible seronegative women would be required throughout pregnancy. (ii) There is no effective treatment for either mother or infant, and the only intervention that could be offered to a woman with primary infection is termination of the pregnancy. (iii) There is no established treatment for congenital CMV infection, although ganciclovir or foscarnet have been suggested for use to inhibit disease progression. However, there are no approved antivirals for use in neonates to prevent progression of CMV disease. (iv) The results have confirmed the well-known fact that more frequent and more serious consequences are observed in neonates with symptomatic CMV infection after the delivery.

R2324 Streptococcal toxic shock syndrome together with multifocal osteomyelitis and myocarditis

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Introduction: In addition to multisystem involvement streptococcal toxic shock syndrome (STSS) results in soft tissue damage like necrotising fasciitis, necrotising myositis, pyomyositis and gangrene. The association of osteomyelitis and STSS has not been previously reported in children.

Case: A previously healthy 13-months-old male infant was brought in for fever, rash, seizures and swelling and decreased mobility of left forearm and right thigh. His initial complaints were fever and swelling of left forearm which had started 3 days before and other complaints had appeared during the last 24 h. Physical exam revealed impaired consciousness, hypotension, poor peripheral circulation, generalised erythematous rash, swelling of left forearm and right thigh together with erythema, increased warmth, decreased mobility, and necrosis over right hand and foot. His laboratory tests revealed elevated renal and liver function tests, elevated creatinine phosphokinase, thrombocytopenia and prolonged PT and aPTT, together with increased D-dimer levels. Intravenous ceftriaxone was started and clindamycin was added after the blood culture grew Group A – β-haemolytic *Streptococcus*. Shock and DIC were taken under control with dopamine infusion and fresh frozen plasma and thrombocyte transfusion. Liver and renal function tests returned to normal, however heart failure developed during follow-up. Myocarditis was diagnosed by low voltage EKG, and dilated heart and poor ventricular functions shown by echocardiogram, which was treated successfully by dobutamine. Cardiac functions returned to normal within 10 days. Computed tomography of the right femoral region revealed intramuscular abscesses, which were drained. Osteomyelitis of the left ulna and thrombosis of the left axillary vein was also diagnosed and the thrombus resolved with heparin treatment. During follow-up swelling of left foot developed and direct films and bone scan showed osteomyelitis of left and right foot first metatarsal bone. Osteomyelitis resolved without surgical drainage after 8 weeks of antibiotic treatment.
Discussion: Soft tissue involvement in STSS is due to focal invasion during bacteraemia. The pyomyositis in our case shows that in addition to the necrotic soft tissue involvement in STSS, multifocal osteomyelitis can also develop. The other interesting feature of this case is the development of myocarditis, which is observed very rarely in STSS and is attributed to circulating toxins.

R2325 The consequence of the aetiological agent in the clinical course of paretic involvement in children

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Objectives: To find out the clinical course of paretic involvement relevant to the aetiology in three major centres for infectious diseases in Czech and Slovak Republic.
Methods: Five-year retrospective study between 1999 and 2003 evaluates all cases with peripheral and central pareses. Detailed epidemiological, clinical and laboratory data were available from 273 children. Serological examinations for neurotropic viruses or bacterial agents were performed according to epidemiological circumstances. CSF was analysed for cytology, proteins, glucose, detection of bacterial antigens and antibodies against neurotropic viruses, respectively antiborrelial antibodies or PCR.
Results: Isolated facial palsy was diagnosed in 53% of children whereas facial palsy associated with aseptic meningitis was proven in 41% of children. Only 21% of them suffered from complete unilateral paralysis. Both sides were affected equally (48%) and bilateral facial palsy appeared in 4%. Other paretic involvements occurred rarely: cranial neuritis of sixth nerve (two), hemiparesis (one), monoparesis (two), quadruplegia (one) or paraplegia (one). Erythema migrans was confirmed in 11% of children. Febrile course was observed in 49% and the duration of signs of pareses was median 14 days during admission, altogether median 24 days. Abnormal CSF analysis appeared in 50%. Pleocytosis was detected in 47% and isolated abnormal values of total protein in 3% of children. Borrelial aetiology was proven in 43% of children, meningoneuritis (35%) was diagnosed by intrathecally produced antibodies in the first CSF sample. 5% of children suffered from facial palsy of herpetic aetiology. Enteroviruses caused only 1% of paretic involvement. The part of other viral agents in the aetiology was <1%. The aetiology remains unknown in 46% of children. Several statistically significant distinctions ($P < 0.05$) were found in the clinical course, and CSF findings between the group with bacterial and viral or unknown aetiology.
Conclusion: The clinical manifestation of neuroborreliosis is unilateral or bilateral facial palsy, respiratory meningoneuritis in children, mostly diagnosed from paretic involvements. Viral aetiology was proven in only 7% of cases. To reveal the full spectrum of causative agents requires routinely using molecular biological techniques. The most feared manifestations of CNS infections: encephalomyelitis, meningomyeloradiculitis, Guillain-Barré syndrome tend to occur rarely in children.

R2326 Lyme carditis

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Objectives: To report two severe cases of Lyme carditis in children and to point out the impact of early antibiotic therapy.
Patients: Two children were admitted to Children's University Hospital in Brno with complete heart block of unknown origin. Basic laboratory tests, electrocardiogram (ECG), serology for cardiotropic viruses and Lyme borreliosis were performed. Prior heart disease was excluded.
Results: Previous tick bite was noticed in one child, stay in endemic area in the second one. Onset of the first cardiac symptoms: weakness, palpitations, syncope and chest pain in July and September, suggested borrelial aetiology. Acute ECG changes showing complete heart block allowed to initiate the antibiotic therapy (ceftriaxone/cefotaxime). Four to seven days later, first-degree heart block was registered, I.PQ 0.36 s/II.0.39 s. In one case an escape rhythm persisted (PQ 0.38–0.50 s) for 8 days. One month later, no symptoms or signs of atrioventricular disturbance and normal PQ 0.16–0.17 s were present. High titres of IgM antibodies against *B. burgdorferi sensu stricto* were confirmed in the first samples, five specific antigens reacting in immunoblot. Seroconversion was detected in 18 days. In the second child, significant decline of specific IgM and IgG titres was found 2 months later, only OspC and p41 were detectable in immunoblot. Cephalosporins of the third generation were given for 28 days intravenously. Neither child did require temporary cardiac pacing.
Conclusion: Cardiac manifestation was seen for the first time in our children. Borrelial infection caused by *B. burgdorferi sensu stricto* is rarely seen in Europe. Early diagnosed severe involvement of the heart with appropriate antibiotic therapy had an excellent prognosis for both children even associated with critical atrioventricular conduction abnormality. Four-week course of antibiotic treatment was sufficient to prevent further sequelae and effectively eradicated borrelial infection. The prognosis depended on the management of the acute stage.

R2327 *Pneumocystis carinii* pneumonia in the neonatal intensive care unit

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Pneumocystis carinii (*P. carinii*) is a classical, opportunistic, pathogenic factor, belonging to mycetes (Ascomycetes). It mostly affects people with lowered immunity. It can be dangerous also in neonatal intensive care units (NICU) because of immature immune system of newborn infants especially premature ones. There are few other risk factors as parenteral nutrition, long antibiotics treatment, possible hospital's infection and adrenocortical hormonal treatment.
Objective: The aim of the work was clinical analysis *P. carinii* infection in the newborn infants.
Materials and methods: There have been analysed 57 newborns with *P. carinii* pneumonia, hospitalised in NICU of the Department of Obstetric and Perinatology Pomeranian Medical Univer-

sity. In examination group there were 55 premature (26.7 ± 1.9 weeks of gestational age and birth weight 990 ± 197 g) and two full-term newborns with congenital abnormalities. There was no patient infected HIV. All of them needed mechanical ventilation. Suspection of *P. carinii* pneumonia was made on the basis of characteristic clinical symptoms and also radiological changes (interstitial pneumonia). Clinical diagnosis was proven by microscope inspection of bronchoaspiration (BAL) with the usage of Giemsa's stain method. Control microscope inspection were taken after 7–10 days of treatment. Biochemical tests were taken to localise infection: C-reactive protein concentration, white blood cell count, blood platelets count, the immature/total neutrophil ratio was inspected.

Results: *P. carinii* pneumonia was diagnosed from sixth to 52nd day of living (20 ± 12 days). In the moment of diagnosis 48 infants were mechanically ventilated, none of them were breathing without ventilation but after earlier extubation. First symptoms of *P. carinii* pneumonia were apnoeas and saturation decreases without self-existing bradycardia. The most frequent signs were: apnoea, grey or pale skin, excessive secretion in respiratory tract, convulsions and abdominal distension. Chest X-ray showed interstitial pneumonia. Laboratory tests were nonspecific. Diagnosis was confirmed by presence of *P. carinii* cysts in microscopic test of BAL. Associated cotrimoksazol and penthamidyne treatment was performed for 14 days. In four newborns treatment does not any clinical effect, three of them died.

Conclusion: Pneumocystis interstitial pneumonia is opportunistic infection causing serious complications in severely ill, HIV-negative, mechanically ventilated newborns.

R2328 Community-aquired pneumonia in children 1997–2003

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Objectives: Community-acquired pneumonia (CAP) has usually in immunocompetent children a benign course. The aim of the study was evaluation of primary diagnoses in children sent to hospital, determination of aetiological agents and resistance and elaboration of guidelines for primary-care settings.

Patients and methods: Children with suspicion of pneumonia between 0 and 18 years of age admitted to the clinic of infectious diseases were included in the study. The diagnosis of pneumonia was established during the first 48 h of hospitalisation and verified by X-ray examination. Clinical symptoms, laboratory parameters, complications, mortality and therapeutic approach were monitored. Blood cultures were taken before antibiotic treatment was started. In children with atypical symptoms were done also serological examinations.

Results: During the studied period was CAP diagnosed in 192 children but only 39 (20.4%) children were sent to hospital with this diagnosis. Other diagnoses were: bronchitis, suspicion of neuroinfection, suspicion of GIT infection. In 55 children was treatment with antibiotics started before admission. Blood cultures were taken in 46 patients: 8× *Streptococcus pneumoniae*, 1× *Neisseria meningitidis* type C, 1× *Streptococcus mitis*, 5× *Staphylococcus epidermidis* (presumably contamination). In one case was *S. pneumoniae* found in aspirate and in one patient was positive PCR in the serum. All strains were sensitive to penicillin and erythromycin. Serological examinations were done in 47 children: in 15 cases were detected respiratory viruses, in *Mycoplasma pneumoniae*, in two cases was found mixed infection – viruses and *M. pneumoniae*, 3× *Chlamydia* spp., 6× *Varicella pneumonia*. Typical X-ray picture of lobar pneumonia was found in five children; in three of them was confirmed *S. pneumoniae*. In 15 children was diagnosed pleuropneumonia, and in three of them was detected *S. pneumoniae*. 36 children were admitted to ICU.

Conclusions: (i) Identical diagnosis CAP before admission was in 20% only. (ii) Blood culture is a gold standard for diagnosis; the other possibilities are detection of antigens or PCR. (iii) *S. pneumoniae* has good sensitivity to penicillins and macrolides. (iv) Viral aetiology is more frequent than *M. pneumoniae*, and *Chlamydia* spp. is rare in children. (v) Penicillins represent first choice therapy for lobar pneumonia or pleuropneumonia in children, macrolides for atypical pneumonia.

R2329 Varicella and its complications: a 5-year-long retrospective analysis of hospitalised patients

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Objectives: The aim of the study was to analyse complications of varicella both in healthy and immunocompromised individuals and to elaborate guidelines for vaccination.

Methods: Retrospective analysis of patients hospitalised in selected departments of infectious diseases in 1997–2001 years in the Czech Republic (CR) and comparison of obtained figures with total number of cases reported in EPIDAT.

Results: During study period 1977 cases of varicella were hospitalised in CR. In 10 selected depts.infect.dis. were hospitalised 1317 (66.6%) patients. 79% of patients were children below 18 years of age, 21% were adults. The average duration of hospitalisation was 8.1 day, median duration 7 days. In 147 (11.2%) children were registered risk factors in anamnesis, 78 patients were on immunosuppressive treatment. Complications were observed in 851 (64.6%) patients: in 282 (21.4%) patients local and in 569 systemic. Systemic complications were observed in 42.4% of children and in 46.4% of adults ($RR = 1.18$; $P = 0.23$). There was no significant difference in occurrence of complications between groups with or without risk factors: the occurrence of local complications in the group with risk factors was 29.3% compared with 29.6% in nonrisk group ($RR = 0.99$; $P = 0.98$). Incidence of systemic complications in risk group was 33.3%. Significantly higher incidence of systemic complications – 44.4% was among adult patients ($RR = 0.75$; $P = 0.01$). Altogether 569 primary complications were observed – 104 cases of encephalitis, 156 cases of liver involvement and in 21 patients was diagnosed atypical pneumonia (average age 26.9 years). 141 patients were treated in intensive care units and three patients required reanimation care. The average duration of stay in intensive care unit was 6.0 days (median 5 days). Only 17 patients had a history with risk factors. 527 patients were treated with aciclovir.

Conclusions: (i) There was no significant difference in occurrence of complications between immunocompromised and immunocompetent patients, higher incidence of complications was observed in adults. (ii) Most frequent complications were liver involvement and encephalitis and in adults atypical pneumonia. (iii) The course of the disease was significantly influenced by administration of aciclovir. (iv) Vaccination against varicella is recommendable for immunosuppressed children and adults and immunocompetent nonimmune adults.

R2330 Break points in gut microflora formation

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Formation of gut microflora is a gradual process in which several stages can be distinguished. During the first few days of life, the intestinal tract is rapidly contaminated with different aerobic and microaerophilic microorganisms. After 1 month the intestinal microflora anaerobic microorganisms gradually exceed the colonic aerobic flora. However, little information is available about break points of different microorganisms in gut microflora during the first 5 years of life. The aim of this study was to investigate the formation of children gut microflora starting from birth up to the 5 years age.

Material and methods: We investigated 107 faecal samples from 36 children during 5 years of life. Weighed samples were serially

diluted, seeded on nine different media and incubated in certain environment. After the identification of bacteria the quantitative composition was calculated: counts in log CFU/g and relative share in total counts of microorganisms (%).
Results: During the first 5 years of life the counts ($r = -0.565$; $P < 0.0001$) and the relative share ($r = -0.586$; $P < 0.0001$) of aerobic bacteria showed negative correlation with age. The counts of coagulase-negative staphylococci ($r = -0.484$; $P < 0.0001$), *Staphylococcus aureus* ($r = -0.542$; $P < 0.0001$), enterococci ($r = -0.452$; $P < 0.0001$) and enterobacteria ($r = -0.470$; $P < 0.0001$) decreased with the increase of age. At the same time the total counts of anaerobes did not change during 5 years, yet their relative share ($r = 0.586$; $P < 0.0001$) correlated positively with age. Differently, the relative share of anaerobic bifidobacteria ($r = 0.586$; $P \leq 0.05$) decreased from birth to 5 years, due to the decrease of their counts after 1–2 years of life. During 5 years of life the break points of bacterial counts were different high numbers of staphylococci starting from first month decreased to 3–6 month ($P < 0.01$), enterococci and enterobacteria decreased from 1 to 2 years to 5 years of life ($P < 0.0001$), bacteroides ($P < 0.05$) and clostridia ($P < 0.0001$) significantly increased starting from 3 to 6 months to 1–2 years and decreased ($P < 0.05$; $P < 0.0001$) from 1–2 to 5 years of life.
Conclusion: The break points of different aerobic and anaerobic bacteria counts in the gut microflora can be applied for estimation of the age-balanced state during 5 years of life.

R2331 Quantitative culture of bronchoalveolar lavage fluid (BALF) in Greek children

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Introduction: Culture of the secretion of the lower parts of lungs in paediatric patients is difficult and samples from certain pathological parts of the lungs are impossible to obtain with conventional methods. The flexible fibreoptic bronchoscopy (FFB) is a well accepted technique to obtain bronchoalveolar secretions.
Objective: We registered the microbial growth in cultures and the resistance to antibiotics after a FFB in certain paediatric respiratory patients.
Materials and methods: A total of 198 BALF samples were obtained and studied from children aged from 18 months to 12 years old. Indications for the bronchoscopy were: persistent or recurrent pneumonia or atelectasis, chronic cough and recurrent wheezing. From the investigation excluded children with immunodeficiency and cystic fibrosis. BALF was obtained either from the abnormal area of the lung, or from the right middle lobe in cases of nonfocal pathology in a standardised manner. Quantitative cultures were performed for common and anaerobic bacteria, fungi and mycobacteria. Positive was considered the sample with growth of $\geq 10^4$ and $\geq 10^3$ CFU/mL of an isolated pathogen together with presence of leucocytes.
Results: A total of 59 of 198 BALF (29.7%) cultures were positive for common bacteria, whereas in two (simblings) grew *M. tuberculosis*. The frequency of the isolated bacteria was as follows: *H. influenzae* 33/59 samples (55.9%), *M. catarrhalis* 19/59 (32.2%), *S. pneumoniae* 13/59 (22.0%), *S. aureus* five of 59 (8.4%), *Ps. aeruginosa* six of 59 (10.1%), *Candida albicans* three of 59 (5.0%) and other bacteria three of 59 (5.0%). In 12/59 (20.3%) of the samples two or more pathogens isolated in a growth of $\geq 10^4$ CFU/mL. A total of 42 of the bacteria positive samples obtained from children with focal lesions. The rest had no evidence of alveolar involvement ($P < 0.01$). Increasing resistance to antibiotics was found in *S. pneumoniae* to penicillin (46.1%), *H. influenzae* to trim/sulpha (24.5%), *S. aureus* to oxacillin (31.8%) and *M. catarrhalis* to ampicillin (70.0%).
Conclusion: *Haemophilus influenzae* is the most common pathogen isolated. BALF culture in children seems to give useful information, which contributes to the clinical consideration of the problem and the selection of the proper antibiotic.

R2332 Viral aetiology of acute lower respiratory infections among hospitalised children in Greece

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Objectives: The viral aetiology of acute lower respiratory infections (ALRIs) in hospitalised children under 3 years of age was studied.
Methods: During the winter seasons from 1998 to 2003, 1847 children with ALRIs were tested for the determination of the responsible aetiologic viral agent. Nasopharyngeal aspirates (NPAs) were analysed for the common respiratory viruses antigen detection (RSV, influenza A (IA) and B (IB), parainfluenza (PIV) 1 + 2 and 3 and adenoviruses), using indirect immunofluorescence assay (IFA).
Results: Viral agents were associated in 1032 of 1847 ALRIs cases (56%). The annual occurrence of viral ALRIs with the respiratory viruses detected is showed in Table 1: RSV was identified in 933 NPA samples (90,4% of all viral diagnoses). Influenza A virus (5%), adenovirus (1.6%), influenza B (1.5%), PIV (1 + 2) (0.9%) and PIV3 (0.7%) accounted for 9.6% of the viral diagnoses. RSV was prevalent in children under 1 year of age (99%).

	1998		1999		2000		2001		2002		2003		Total
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	(*n*)
RSV	88	46.3	93	46.5	164	49.5	214	56.5	220	62.3	154	39	933
Adeno	1	0.53	0	0	1	0.3	1	0.3	1	0.3	12	3	16
IA	7	3.7	2	1	12	3.6	3	0.8	7	2	2.1	5.3	52
IB	0	0	0	0	0	0	0	0	14	4	1	0.25	15
PIV (t+2)	0	0	0	0	0	0	0	1	0.3	8	2	9	
PIV 3	0	0	1	0.5	1	0.3	1	0.3	2	0.6	2	0.5	7
Total positive	96		96		178		219		245		198		1032
Total samples	190		200		331		379		353		394		1847
Positive samples (%)	50.5		48		53.8		57.8		69.4		50.25		56

Conclusions: RSV is the main aetiologic agent of viral ALRIs in young hospitalised children.

R2333 Prevalence and antimicrobial resistance of bacterial isolates causing paediatric blood-stream infections in a tertiary hospital in Tanzania

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Objectives: Blood-stream infections (BSI) are commonly associated with case-fatality rates in excess of 25% and infections caused by drug-resistant organisms represent an additional therapeutic challenge. The objective of this study was to assess the prevalence and resistance patterns of bacterial isolates causing BSI in children at Muhimbili National Hospital, Dar es Salaam, Tanzania.
Methods: Blood cultures were obtained from 1789 children (age 0–7 years) with fever or signs of serious infection admitted to the hospital during the period August 2001 to August 2002. Isolates were identified by standard methods and susceptibility tested by disc diffusion method. Since only one blood culture was obtained from each patient, we did not consider coagulase-negative staphylococci and other organisms of uncertain pathogenicity in this study.

Results: Among the 1789 children included in the study, 149 had Gram-negative rods, 61 had Gram-positive cocci, 19 had *Candida* spp. and one had *Mycobaterium tuberculosis* recovered from blood cultures. The most common Gram-negative isolates were *K. pneumoniae* ($n = 48$), *E. coli* ($n = 36$) and various *Salmonella enterica* serotypes ($n = 37$, two *Salmonella* Typhii, 15 *S.* Typhimurium, 19 *S.* Enteritidis and one *S.* Newport). The most common Gram-positive isolates were *Staphylococcus aureus* ($n = 20$), *E. faecium* ($n = 17$) and *E. fecalis* ($n = 15$). The Gram-negative isolates displayed high rates of resistance to most commonly used antimicrobial drugs, and even extended-spectrum β-lactamase phenotype was present in high proportions of the isolates.
Conclusion: Gram-negative bacteria such as *K. pneumoniae*, *E. coli* and *Salmonella* serotypes are the most prevalent organisms causing BSI in children at this Tanzanian hospital. High rates of resistance against commonly used antimicrobials raise concerns as to the efficiency of current treatment regimens, and there are no obvious affordable and appropriate alternative drugs available.

R2334 Neonatal sepsis due to *Klebsiella* species: prevalence, outcome and antibiotic sensitivity

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Introduction: Sepsis is a significant cause of morbidity and mortality in neonates. The most common pathogens of bacterial sepsis and antibiotic sensitivity patterns vary in different parts of the world. The aim of this study was to determine the most common pathogens of neonatal sepsis and antibiotic sensitivity and outcome of neonatal sepsis due to *Klebsiella* species.
Materials and methods: A retrospective descriptive study was carried out. The study was performed in a neonatal care unit in Kashan between October 2000 and October 2003.Only those neonates with positive blood culture were included. Patients with *Klebsiella* septicemia were categorised into two groups of early-onset and late-onset. Frequencies and Fisher's exact test (to compare early-onset outcome vs. late-onset) were calculated by SPSS. *P*-value <0.05 was considered significant.
Results: A total of 136 neonates had positive blood cultures out of 453 cases. The most common pathogens were *Pseudomonas*, *Klebsiella* and coagulase-negative staphylococci, respectively. In 43 cases (32%) *Klebsiella* species were isolated. These isolates were all resistant to ampicillin. Seventy-three per cent were resistant to gentamicin. Twenty-three per cent of *Klebsiella* isolates were multi-resistant (resistant to ampicillin, amikacin, gentamicin and all third generation cephalosporins). In patients with *Klebsiella* sepsis mortality was higher in early-onset sepsis than late-onset sepsis (Fisher's exact test, $P = 0.043$).
Conclusion: Considering our most common aetiologic pathogens of neonatal sepsis and the significant number of resistant *Klebsiella*, to ampicillin and gentamicin it seems prudent to consider revising the present choice of empirical antibiotic treatment.

R2335 The effect of bacterial septicaemia on the plasma motilin concentration in neonates

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Background: Plasma motilin concentration increase in first 2 weeks of life both in full-term and preterm healthy neonates. In severe infected neonates gastrointestinal symptoms such delayed gastric emptying, vomiting and paralytic ileus are frequently observed and their association with plasma gastrointestinal hormones activity remains unclear.
Objectives: To determine if bacterial septicaemia may influence plasma motilin concentration in sick neonates.
Materials and method: A total of 45 septic and 24 severe infected neonates mainly with pneumonia, without sepsis (32 full-term, 37 preterm) were included to the study. Control group consisted of healthy, born with Apgar score >9, breast-fed neonates. Birth asphyxia in 41% and clinical symptoms of gastrointestinal tract disorders in 50% of all severe infected neonates were noted. Plasma motilin concentration (pmol/L) in peripheral vein blood detected by radioimmunoassay method (Euro-diagnostic AB Sweden Set) were measured twice: between the second and fourth and between the 12th and 14th day of life in sick neonates. In control group the test was performed only once between the second and fourth day of life (ethical reasons).
Results: Plasma motilin concentration (first test) in septic full-term neonates ranged from 30.38 to 257.38, mean 90.6 ± 54.31 pmol/L was significantly higher ($P < 0.05$) than the mean value of plasma motilin in septic prematures (range from 30.35 to 133.54, mean 60.01 ± 26.59 pmol/L). There was no deference in the first test between mean motilin concentration in healthy full-term (range 37.51–131.24, mean 76.6 ± 27.4 pmol/L) and preterm neonates (range 38.85–99.91, mean 64.51 ± 14.43 pmol/L). Moreover no difference was noted between mean values of motilin concentration in neonates with other kind of infection both in first and second determinations. Significant increase ($P < 0.01$) of the mean motilin value was found during the first 2 weeks of life as well in full-term septic neonates and septic prematures.
Conclusions: Bacterial septicaemia in preterm neonates may cause the decrease of plasma motilin concentration.

R2336 ESBL producing Enterobacteriaceae among paediatric patients

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Objective: The aim of this study was to analyse occurrence of ESBL-producing Enterobacteriaceae in patients without haematological malignancies who were hospitalised in the institute of Pediatrics, Medical University of Gdansk.
Methods and materials: We analysed microbiologic records obtained in 2003. Strains were identified by classical method and VITEK cards (BioMerieux). Production of ESBL was detected by double disc method. ESBL colonisation was determined by a positive stool/rectal/throat culture without any sign of infection.
Results: In the studied period 249 patients were hospitalised. ESBL + isolates were recovered from 35 patients. 11 patients were infected and 16 colonised, eight patients were both colonised and infected. *Klebsiella pneumoniae* was isolated from 10 patients (20 isolates) then *E. coli* (20 isolates from nine patients) and *K. oxytoca* (six isolates from four patients), *E. aerogenes* (10 isolates from six patients). Isolates were recovered from stools and rectal swabs (56.6%), urine (28.3%), respiratory tract (9.4%) and intravenous catheter tips (5.7%). A total of 24 patients were admitted to the units with established ESBL colonisation. Eleven patients acquired ESBL during hospitalisation. 36.9% patients received cephalosporins, 18.4% aminoglucosides, 15.8% amoxyxylin/clavulanic acid and 28.9% were not given any antibiotic. 25% infecting ESBL strains were detected in patients with urinary-tract infections, 12.5% with epilepsy, 12.5% with suspected pneumonia and 10.4% with cholestatic jaundice. All strains were susceptible to carbapenems, about 90% to fluoroquinolones and about 80% urine isolates to nitrofurantoin, but about 80% isolate were resistant to aminoglucosides and cotrimoxazole. 70% patients who were ESBL negative on admission, were bottle-fed and 30% were breast-fed. 70% of those breast-fed were earlier admitted to different hospitals in our region.
Conclusions: High percentage of ESBL carriage among children complicates infection control and treatment of common urinary tract infection. Patients on admission and before discharged should be screened for ESBL carriage.

R2337 Occurrence of Gram-negative nonfermenters in neonatal intensive care units

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Objectives: To determine the prevalence, the susceptibility to antimicrobials and genotypic relationship of clinical isolates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* in neonatal intensive care units.
Methods: Gram-negative nonfermenter strains were isolated from different samples (blood culture, respiratory tract and urine) of neonates treated in the neonatal intensive care unit between January 2001 and November 2003. The strains were identified using the ATB and Vitek systems (bioMérieux) and genotyped with AP-PCR (ERIC-2). Susceptibility to antibiotics was evaluated by disc diffusion method and by E-test strip.
Results: Of 6544 blood cultures from three NICUs of the university hospitals 910 (14%) gave positive results. Among these cultures, 27 *P. aeruginosa* (3%), two (0.2%) *A. baumannii* and four (0.4%) *S. maltophilia* were isolated from colonised or infected neonates. 212 *P. aeruginosa* strains (14%), 13 *A. baumannii* (0.9%) and 56 *S. maltophilia* (3.8%) were isolated. Amikacin, imipenem and meropenem were the most effective antimicrobial agents against *P. aeruginosa* and *Acinetobacter baumannii* strains with sensitivity rates of 91/100%, 76.8/100% and 88.4/100%. The incidence of resistance to aminoglycosides, ceftazidime, ciprofloxacin and moxifloxacin among strains of *S. maltophilia* was 100, 34, 76 and 0%. No cotrimoxasole resistant strain was found. Based on the results of AP-PCR the strains could be divided many different clones.
Conclusion: Nowadays Gram-negative nonfermenter strains seem to be one of the leading causes of nosocomial infections, especially in intensive care units. These strains could have been isolated from respiratory tract samples more frequently, than other type of samples. Management of these infections may be difficult due to the inherent multidrug resistance of strains. The high genetic diversity among the isolates probably means that the multiple independent environmental sources are more relevant than the cross-transmission in nosocomial infections, therefore the independent infections are more probable.

R2338 Neonatal brucellosis: probable transmission from breast milk

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Brucellosis is primarily a zoonotic infection which can be transmitted to humans through direct contact with infected animals, their carcasses or ingestion of unpasteurised milk or milk products. Human-to-human transmission is rare. Neonatal infection can be acquired transplacentally or during delivery. However, some cases of breast-feeding transmission have been described. The diagnosis of brucellosis is difficult in infancy because the symptoms are nonspecific and the disease may be mild and self-limited.
Case: A 3-month-old child with acute brucellosis is reported. Fifteen days after delivery, the mother became acutely ill with fever and progressive malaise. Based on a positive seroaglutinattion test. The patient was treated with doxycycline and gentamicin. *Brucella melitensis* grew in blood cultures and agglutination titre in serum was significant. At the same time, the child was submitted to the Pediatrics department. He was healthy, without fever and, except for splenomegaly, the physical examination was normal. *B. melitensis* was also isolated in blood cultures. The infant had been exclusively breast-fed when his mother developed brucellosis. In order to avoid complications due to the use of tetracyclines, the patient was treated with rifampicin and cotrimoxazole with good evolution and tolerance.
Conclusions: *B. melitensis* transmission can occur through breast-milk and should be considered in children who were breast-feeding in mother recently infected

R2339 Fatal acute infectious purpura fulminans in a child caused by *Haemophilus influenzae* – a case report

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Objective: The aim of the study was to present a case report of child who suffered from acute infectious *purpura fulminans*. The progression of the disease was so rapid that we could not take specimens for culture. Postmortem cultures were obtained.
Case report: A mother of an 8-month-old child noticed high temperature and haemorrhagic rash on skin. Additionaly, bronchial infection was suspected. The child died within 10 h from the onset of symptoms. In the postmortem examination, the following day, no signs of respiratory tract infection nor infectious organ infiltration were found. There was only a haemorrhagic effusion of suprarenal glands. In postmortem cultures we isolated *Haemophilus influenzae* (HI) from cerebrospinal fluid and bronchial aspirate and *Streptococcus pneumoniae* (SP) from bronchial aspirate. Blood cultures were negative.
Conclusions: Acute HI infection could be very rapid and fatal. The recognised symptoms, which were nonspecific, included fever and haemorrhagic rush all over the body.

R2340 Study of the epidemiology of *Shigella* spp. in Tehran, Iran during 2002–2003

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Objective: Shigellosis is an infectious disease caused by a group of bacteria called *Shigella*. The illness is also known as 'bacillary dysentery' and is more severe than other forms of gastroenteritis. Shigellosis in children remains an important problem in developing countries. The purpose of this study was to investigate epidemiology of shigellosis in Tehran, Iran during a 1-year period.
Methods: Between January 2002 and December 2003, faecal samples of individual patients referring to the Children's Hospital Medial Center, Mofid Children's Hospital, Baqiatallah (a.s), Millad, and Firozabadi Hospitals were cultured for *Shigella* spp. using standard microbiological techniques. Isolates were identified by biochemical and serotyping methods.
Results: From a total 302 *Shigella* isolates, 178 were identified as *S. sonnei* (58.940%), 110 as *S. flexneri* (36.424%), 10 as *S. boydii* (3.311%), and four as *S. dysenteriae* (1.324%). In this study *Shigella sonnei* was the predominant species, followed by *S. flexnery*. Overall, 167 patients (55.3%) were males and 135 (44.7%) were females. *Shigella* was isolated frequently from children under 5 years of age, who accounted for 66% of all isolates. About 29.2% of all isolates came from persons aged 5–10 years, and 4.8% from persons aged over 10 years of age. The peak of infection occurred during summer. In Children's Hospital Medial Center Mortality rates of shigellosis was 2.14%.
Conclusion: Bacillary dysentery still constitutes a significant proportion of acute intestinal disease in the children of Iran. Unlike previous years, in this study *S. sonnei* was reported as the predominant serotype.

R2341 Serotypes and subtypes of *N. meningitidis* serogroup B from Argentinian children with invasive disease

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Introduction: *N. meningitidis* (Nm) is the leading causative agent of bacterial meningitis in children in Argentina.
Objective: To study the frequency of isolation for NmB serotypes and subtypes through a 3-year period, 2000–2002.
Material and method: Blood an CSF cultures and typification were made by standard methods, and identification with DIFCO serum at Microbiology Laboratory, Sor María Ludovica Children Hospital, La Plata, Argentina. Sero and subtyping with specific monoclonal antibodies by ELISA were made at National Institute for Infectious Diseases Dr Carlos G. Malbrán (Buenos Aires).
Results: A total of 102 strains were isolated. The number of isolated strains were 40, 29, and 33 for the year 2000, 2001 and 2002, respectively. The most frequent sero and subtypes were: (i) for the year 2000, B15.P1.7.16 (27.5%), B4.P1.NT (23.2%), B4.P1.15 (10%), B15.P1.NT (7.5%) and B1.P1.NT (7.5%); (ii) for the year 2001, B4.P1.NT (24.1%), B15.P1.NT (13.8%), B15.P1.7 (13.8%), B1.P1.NT (6.9%) and B4.P1.14 (6.9%); (iii) for the year 2002, B4.P1.NT (18.2%), B4 P1.15, B15.P1.NT, B15.P1.7 and B4.P1.14 (6.7% for each one).
Comment: Clinical laboratory data is useful for surveillance and to identify common antigens in order to obtain an eficacious vaccine.

R2342 Study of mortality rates of shigellosis and a report of three shigellosis cases resulted in death in the Children's Hospital Medical Center, Tehran, Iran during 2002–2003

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Objectives: More than 1 million deaths occur yearly due to infections with Shigella and the victims are mostly children of the developing world. Small children and the elderly are at greatest risk to experience mortality from a *Shigella* infection. In this research, we studied mortality rate of shigellosis during 1-year period in the Children's Hospital Medical Center, Tehran, Iran. Moreover clinical and microbiological features of three cases of shigellosis that resulted in death was studied.
Methods: Between January 2002 and December 2003, all of patients with gastrointestinal disease referring to the Children's Hospital Medical Center were studied for shigellosis. Of all patients that were identified as shigellosis cases, three patients died. Before death, these three patients were admitted with a history of watery diarrhoea, vomiting, and generalised tonic clonic seizures. They were transferred to intensive care unit (ICU) and were intubated. Intravenous antibiotic therapy with ceftriaxone was administrated immediately after admission. Faecal specimens were cultured for *Shigella* spp.using standard microbiological methods. Antibiotic susceptibility of bacterial isolates to the 20 antibiotic was performed.
Results: Of 140 causative bacterial isolates, 68 were identified as *S. sonnei* (48.6%), 65 as *S. flexnery* (46.4%), five as *S. boydii* (3.6%), and two as *S. dysenteriae* (1.4%). From of total cases, three patients died (mortality rates 2.1%). These three patients have been admitted with a history of watery diarrhoea, vomiting, and generalised tonic clonic seizures. No signs of severe dehydration were observed. The symptoms of patients were not improved with treatment, and all three patients were expired within 24 h of hospitalisation. Two patients were female and one other was male. The ages of these three patients ranged from 13 months to 8 years. In two patients, stool cultures were positive for *S. sonnei* and in one other patients, were positive for *S. flexnery*. Of three isolates, two had similar antimicrobial resistance patterns.
Discussion: Dysentery accounts for 20% of the 4.6 million diarrhoea-associated deaths among children in developing countries and Shigellosis accounts for the majority of dysenteric episodes. Many studies have shown that fatality may be as high as 10–15% with some strains. In this study mortality rates was 2.1%. Whereas mortality caused by other species than *S. dysenteriae* is rare but in this study *S. sonnei* and *S. flexnery* were causative agents of three deaths.

Mycobacterial infections (incl. diagnosis)

R2343 Comparison of the MB/BacT ALERT 3D system, the Lowenstein-Jensen medium and the Middlebrook 7H10/7H11 plate for recovery of mycobacteria

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Basle, CH

Objective: A retrospective study was carried out in order to compare the MB/BacT ALERT 3D system, the Lowenstein–Jensen (LJ) medium and the Middlebrook 7H10/7H11 plate for the recovery of mycobacteria from clinical specimens.
Material and methods: During a period of 25 months 6250 clinical samples were tested for mycobacteria. All isolates where data of all three media were available were included in the evaluation. Isolates from urine and blood were excluded because a different panel of media had been used.
Results: A total of 343 isolates of mycobacteria, namely 285 isolates of *M. tuberculosis* (MTBC) and 58 isolates of nontuberculous mycobacteria (NTM), were included in the evaluation. BacT/ALERT detected 308 isolates (90%), LJ medium 277 isolates (81%) and 7H10/7H11 detected 273 isolates (80%). The combination of BacT/ALERT and LJ medium detected 334 isolates (97%), the combination BacT/ALERT and 7H10/7H11 detected 327 isolates (95%) and the combination of LJ medium and 7H10/7H11 detected 315 isolates (92%).
Conclusions: The combination of BacT/ALERT and LJ medium performed only marginally worse than the combination of all three media. Although it missed four isolates of MTBC, it detected MTBC in other specimens of the same patients, thus not missing any case of tuberculosis. Accordingly, although it missed five isolates of NTM, it missed only one case (*M. marinum*) that was considered clinically relevant. The combination of Bact/ALERT and LJ medium is sufficient and costeffective for the isolation of mycobacteria in the clinical laboratory. It poses less problems with contamination than a panel of media that contains Middlebrook agar.

R2344 Comparison of two different therapeutic regimens of DOT strategy in patients with pulmonary tuberculosis

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Objectives: To compare the effect, complication, and treatment cost of two different therapeutic regimens of DOT's strategy in treating patients with pulmonary tuberculosis (TB) and positive sputum smear test.
Methods: A clinical trial was done on 200 patients aged 15–50 years with pulmonary tuberculosis and no history of previous treatment for TB, Immunocompromise, liver, and kidney diseases. (pregnant women excluded from this study) Selected patients divided into two groups randomly and one group was treated by using the Denver method (62 doses), and the other group treated

with routine standard method (180 doses), Sputum smear test was performed for each patient at the end of 2, 4, and 6 months of treatment, and finally collected data were analysed by SPSS software and Fisher method ($P < 0.05$).
Results: Cure occurred in 94% of patients treated by Denver method and in 93% with routine standard method, this result was not significant, also we could not find significant difference in complications occurred during using these two methods. (9% in Denver method vs. 6% in standard method)
Conclusion: With regard to using drugs just twice a week in Denver method and its lower cost treatment, Denver method will be better than standard method and patients compliance will be more in this method.

R2345 Comparison of the VersaTREK to the ESP Culture System II for mycobacterial detection and susceptibility testing

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Objective: Studies were conducted at two sites, Trek Diagnostic Systems Laboratory (TS) Sun Prairie WI, USA, and at Vincent's Hospital (SVH) New York, New York, USA, to validate the performance of the VersaTREK (VT) instrument for the detection and susceptibility testing of mycobacteria.
Methods: In a study conducted at TS, standardised suspensions of nine isolates of mycobacteria were tested in the VT and the ESP Culture System II (ESP). The time to detection (TTD) of a positive culture and typical curve response was evaluated. Reproducibility studies using five isolates of *M. tuberculosis* (*M. tb*) tested on three different days were conducted at TS. A seeded study comparing TTD in the VT and in the ESP using seven strains of *M. tb* was conducted at SVH. Susceptibility studies using strains of *M. tb* with known resistance profiles were conducted at TS, while a comparative study using clinical isolates was conducted at SVH. A clinical trial using split patient specimens for the ability to detect growth of mycobacteria is ongoing at SVH.
Results: The nine isolates representing seven species of mycobacteria were shown to exhibit comparable TTD and typical curve response when tested in both systems. The reproducibility studies with *M. tb* demonstrated that the average TTD for the VT was 5.53 days compared with 5.37 days for the ESP. Studies on seeded clinical isolates showed that the isolates incubated in the VT had comparable TTD to those incubated in the ESP. Susceptibility studies using isolates of *M. tb* correctly identified three isolates that were susceptible to the four first line agents, one that was resistant to INH and one resistant to ethambutol. Comparative susceptibility studies between the VT and ESP were in complete agreement with the four first line agents and PZA. An ongoing split sample clinical trial at SVH has not demonstrated any significant difference between the two systems.
Conclusions: The VT was demonstrated to be comparable to the ESP for the detection of mycobacteria and for susceptibility testing of *M. tb*.

R2346 Application of nested PCR and reverse hybridisation for diagnosis of central nervous system tuberculosis

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Tuberculosis of central nervous system (CNS) is regarded to be the less common form of extrapulmonary tuberculosis, but at the same time, the most severe with the highest mortality rate among all forms of the disease. Low rate of *Mycobacterium tuberculosis* detection in cerebrospinal fluid (CSF) when culture methods are used is the main diagnostic problem. In consequence, it results in low gauges of CNS tuberculosis in epidemiological data. Increasing frequency of the drug-resistant strains and their long-lasting identification with classical methods are other major contributing factors to the difficulties in rapid selection of the most appropriate treatment course.
Objectives: Evaluation with nested PCR of *M. tuberculosis* complex presence in CSF of patients with suspicion of CNS tuberculosis and identification of rifampin-resistant strains with application of reverse hybridisation. Improvement of the diagnosis by detecting the bacteria in blood samples, which is to our knowledge the first to be used.
Methods: A total of 52 CSF and 11 blood samples derived from 39 patients with suspicion of CNS tuberculosis (mean age: 40.7 years) and hospitalised at neurology departments of Lublin clinics between June 2000 and December 2003 was studied. The commercial kit of A&A Biotechnology (Poland) was used for DNA extraction from the samples. Protocols and reagents of Inno-Lipa Rif.TB (Innogenetics, Belgium) for nested PCR were used to detect *M. tuberculosis*. The target gene was RNA-polymerase β-subunit gene (rpoB) for *M. tuberculosis* complex. Reverse hybridisation was applied after amplification reaction to confirm positive result of nested PCR and to identify rifampin-resistant strains.
Results: During the study period 15 of 37 patients (40.5%) were detected *Mycobacterium tuberculosis* complex in the CSF and four of 11 patients (26.7%) in blood samples. Application of the hybridisation allowed to exclude three false-positive results of the nested PCR reaction and to identify four strains of 16 evaluated as rifampin-resistant.
Conclusions: We conclude that application of genetic tools increases the identification of CNS tuberculosis and a detection of rifampin-resistance strains allows quick selection of the accurate treatment. Blood samples may be useful in the disease diagnostics. Diagnosis with use of PCR indicates on more frequent incidence of CNS tuberculosis in epidemiological data then it has been previously regarded.

R2347 Evaluation of the MB/BacT system for antimicrobial drug susceptibility testing of *Mycobacterium tuberculosis*

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Objective: Evaluate the ability of the MB/BacT culture system to perform antimicrobial susceptibility testing of *Mycobacterium tuberculosis* to isoniazid, rifampin, streptomycin and ethambutol by comparing the results to those obtained by the agar proportion method on Lowenstein-Jensen (LJ) medium.
Methods: We performed paired antimicrobial susceptibility test (AST) on 34 *Mycobacterium tuberculosis* complex (Mt) clinical strains with the MB/BacT system (Biomérieux) using the BacT/ALERT SIRE kit and the agar proportion method on LJ using the Mycobacterial susceptibility testing (Biomérieux). The control strain H37Rv (ATCC 27294) was tested by both methods as a quality control. All strains were identified by the Accuprobe culture identification test (Gen Probe). Four antimicrobial drugs were evaluated: streptomycin (1.0 μg/mL in MB/BacT and 4.0 and 10.0 μg/mL in LJ), isoniazid (0.1 μg/mL in MB/BacT and 0.2 and 1.0 μg/mL in LJ), ethambutol (5.0 μg/mL in MB/BacT and 2.0 and 3.0 μg/mL in LJ) and rifampin (1.0 μg/mL in MB/BacT and 20 and 40 μg/mL in LJ). The tests were conducted according to the instructions given by the manufacturer.
Results: The control strain H37Rv performed as expected: susceptible to four drugs by the two methods. Of the 34 Mt strains tested with the MB/BacT system, we detected seven strains resistant to one or more drugs (three were multiresistants) and 27 strains susceptible to all four drugs. Full agreement of rifampin and isoniazid results was found for all isolates. The two methods showed concordance for the four drugs tested in 30 strains (88.2%). There was disagreement in one case (2.9%) for Ethambutol (resistant by LJ and susceptible by MB/BacT) and three cases (8.8%) for streptomycin (one susceptible by LJ and resistant by MB/BacT and two resistants by LJ and susceptibles by MB/BacT). Turnaround times for AST ranged from 5.5 to 13.0 days for MB/BacT.
Conclusions: These results indicate that the MB/BacT system is shown to be a reliable, rapid, fully automated, nonradiometric

system for the susceptibility test of *Mycobacterium tuberculosis* complex.

R2348 Second-line antituberculosis drug susceptibility in multiresistant *Mycobacterium tuberculosis* strains

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Objectives: MDR-TB is a worldwide problem and second-line drugs should be added to the therapy. In Turkey in spite of some regional studies, there is no national data about second-line drugs. In this study, the objective was to evaluate the drug resistance pattern of the second-line drugs in MDR-TB strains in Turkey.
Methods: This study was performed in Tuberculosis Referance Laboratory, RSNHC. The tests were done in 51 MDR-TB strains, which were from nine provinces among five of the seven regions in Turkey between August 2002 and August 2003. The number and the incidence (per 100 000) of the strains in the provinces Trabzon, Ankara, Van, Elazig, Kayseri, Duzce, Samsun, Izmir, Antalya were 17/18.05, 10/14.22, 8/14.36, 5/27.04, 4/13.96, 4/44.87, 1/34.07, 1/33.52 and 1/17.33, respectively. Indirect proportion method with Loewenstein-Jensen medium was applied for the susceptibility tests of the second-line antituberculosis drugs. The antibiotics used, and the concentration critics in microgram per millilitre were: ethionamide (ETH 20–30), thioacetazone (TIA 2), cycloserin (CYC 30–40), kanamycin (K 20–30), *p*-aminosalicylic acid (PAS 0.25–0.5), ofloxacine (OFL 2) and capreomycin (CAP 20–40). *M. tuberculosis* H37Rv strain was used for quality control.
Results: The number and the ratio of the resistant strains for ETH, CYC, K, PAS, OFL, TIA and CAP were 24 (47%), four (8%), three (6%), three (6%), two (4%), one (2%), 0. (0%) respectively. The 41% of the strains showed resistance to one drug, 8% to two drugs, 2% to three drugs, and 2% to four drugs.
Conclusion: In this study, second-line drugs which are important in MDR-TB therapy seem to be effective with >8% resistance ratio other than ETH. It is also realised that polydrug resistance is not high so that these drugs are promising for treatment.

R2349 *Mycobacterium tuberculosis* sigma factor genes expression after exposure to rifampicin

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Objectives: The aim of this work was to determine *Mycobacterium tuberculosis* sigA, sigE, sigF, sigH and sigI genes, and the reference 85B gene expression under stress conditions due to rifampicin exposure.
Methods: The sputum sample was processed, seeded on L-J medium, and incubated for 4 weeks in standard conditions. Than, *M. tuberculosis* culture was transferred to the Middlebrook 7H 9 medium, standardised to McFarland 0.5 and grown for further 48 h. The cells were exposed to rifampicin concentrations of 10, 20, 25, 35 and 40 μg/mL. Cell suspensions were collected after 1, 12 and 48 h of exposure. The obtained mycobacterial strain was susceptible to rifampicin. Total RNA was extracted from 100 μL of each broth culture by a modified guanidine–phenol–chloroform method. A one-step RT-QPCR reaction was done with ABI-PRISM 7700 sequence detection system (TaqMan). Sequence specific PCR primers and oligonucleotide detector probes labelled with FAM and TAMRA were used.
Results: Changes in 85B, sigA, sigE, sigH and sigI mRNA levels were noted in comparison with the control *M. tuberculosis* cultures. In the exposed cultures a decrease of 85B, sigE, sigH mRNA levels was seen. SigA and sigI mRNA were not detected in the control cultures. Exposure to rifampicin induced expression of these genes. SigF transcript was not detected neither in the exposed cultures, nor in the controls.
Conclusion: The results of our studies suggests that *M. tuberculosis* genes encoding sigma factors A, E, H and I, and 85B mRNA participate in cellular response/reaction to rifampicin exposure.

R2350 Beijing genotype in Bilbao

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Introduction: Due to the notifications of an increment in the number of multirresistant *Mycobacterium tuberculosis* around the world, the objective of our work was to know the situation of this trouble in our hospital. This centre is Basurto's Hospital, a tertiary Hospital with 800 beds that give cover to the area of Bilbao.
Material and methods: We have realised a retrospective study of the sensibility of *M. tuberculosis* in our hospital since 1998–2002. The used method to know the sensibility was Susceptibility Test Kit of BIO RAD® based in Cannetti's proportions method, and since 1999 we started use the liquid system BD BACTEC®MGIT 960, both of them following the maker instructions. MGIT 960 utilises these concentrations of antibiotics: isoniazid (I) (0.1 μg/mL), rifampin (R) (1.00 μg/mL), ethambutol (E) (5.00 μg/mL), streptomycin (S) (1.00 μg/mL) and pyrazinamide (P) (100 μg/mL). We have considered multirresistance if appear resistance for two first line tuberculostatics.
Results: For the 365 studied isolates, we only have found one multirresistant isolate. This resistant was for all first line tuberculostatics (I, R, E, S and P). The case was a male of 55 years old, VIH-, smoker and moderate drinker, worked as host. His antecedents were: Pleuritis 35 years ago and some residual pulmonary images in chest radiography. Before the patients got admitted in the hospital suffered a subacute syndrome based in: chest pain, cough with mucous expectoration ocassionally green and without fever. In the day of his ingress had sputums with blood. In the chest radiography were observed calcified nodular images in left lung and cavitated infiltrate in upper right lobule. They instaurated treatment with I, R and P but was changed due to Microbiology Service informs and reiterated positive baciloscopes. The resistant was primary for all of them and the genotypic study confirmed that was Beijing genotype.
Conclusions: In our medium is still very small the number of multi-resistant stems (1/365) being more frequent the resistant for one tuberculostatic as I (7%) or R (1.1%). Our case was a 55 years smoker male, host and without interest antecedents who presented a subacute pulmonary syndrome. We do not know any transmission from the patient to his contact group. Presented primary resistant for all first line tuberculostatics and the genotypic study showed that it was Beijing type.

R2351 *Mycobacterium tuberculosis* drug resistance in Adana, Turkey

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Objective: To evaluate the drug resistance pattern to first-line antituberculosis drugs for *Mycobacterium tuberculosis* strains isolated in our University Hospital.
Methods: In this retrospective study, isolation and susceptibility testing of *Mycobacterium tuberculosis* was carried out using the BACTEC 460TB system and/or the mycobacterial growth indicator tube (MGIT). From January 1, 1998 to December 31, 2002, a total of 774 strains of *M. tuberculosis* were analysed. The annual number of isolates was 107, 162, 172, 164 and 169, in the 5-years period between 1998 and 2002, respectively.
Results: Total resistance to any drug was 24.3% (26 strains) in 1998, 27.8% (45 strains) in 1999, 20.9% (36 strains) in 2000, 34.8% (57 strains) in 2001 and 29.6% (50 strains) in 2002. Of 774 isolates, 214 (27.6%) had resistance to at least one first-line antituberculosis drug, of which 115 (14.9%) had mono-resistance, 69 (8.9%) had multidrug-resistance (MDR-TB; resistance to at least isoniazid and

rifampicin). The overall proportion of isolates resistant to each drug were as follows: isoniazid, 23.8%; rifampicin, 10.2%; ethambutol, 6.3%; and streptomycin, 5.4%. Monodrug resistance was observed most often to isoniazid (11.4%, 88 strains). Resistance to rifampicin, ethambutol and streptomycin alone was rarely seen (0.9%, seven strains; 1.2%, nine strains and 1.4%, 11 strains, respectively).
Conclusion: These data have demonstrated that resistance of *M. tuberculosis* to first-line antituberculosis (TB) drugs, particularly to isoniazid and rifampicin, is a growing problem in our region. For this reason, the application of the effective treatment and TB control programmes are of a great importance.

R2352 Pneumoniae caused by *Mycoplasma pneumoniae*

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In the previous period of 6 years we analysed patients with improved clinical, radiographs and laboratories, pneumonias. The leading symptom and indication for hospitalisations in 29 (5.3%) patients were haemoptysis. Patients were in ages from 15 to 45 years, and mainly males: 18 (62.1%) and 11 (37.9%) females. Besides haemoptysis in 14 (48.2%) patients dominated high temperatures, cough, headache, chest pain and myalgia. 20 (41.3%) patients had subfebrile states. In three (10.3%) patients haemoptysis were the only symptom of illness. Radiographic manifestations on admission to the hospital, showed hilar lymphadenopathies accompanied with nonhomogenous shadows in the upper right lung in five (17.2%) patients and almost the same lesions on the right were find in four (13.5%) on the left side. 10 (34.4%) patients had in homogenous shadows in lower lobe. Prominent reticular findings were localised in the middle lobe in 2 (6.8%) patients. Basal spot shadows on the left with ipsilateral pleural effusion were found out in three (10.3%) patients. Round shaped shadowings in the left upper lobe were reported in five (17.2%) patients. In all 29 patients serologic reactions of complement binding confirmed *Mycoplasma pneumoniae* with antibody IgG and IgM titres. The effects of macrolides therapies were good in all patients after average period of 19.4 days.

R2353 Tuberculous otitis media in a renal transplant recipient

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Introduction: Tuberculosis was a serious public health problem. Tuberculous (TB) otitis media can be difficult to diagnose because it can easily be confused with other acute or chronic middle ear conditions. As TB oitis media in a rare disease, the diagnosis is usually delayed.
Case report: We present the case of a 25-year-old female, developed persistent oititis media at 5 years, which failed to respond to multiple courses of antibiotics and surgery. A patient underwent renal transplantasion in 1997 and she is still on corticosteroid and cyclosporine-A therapy. She had a chronic tympanic membrane perforation, abundant pale granulation tissue in the middle ear and ear drainage associated with progressive and profound hearing loss. Postmastoidectomy recurrence of granulation tissue, slow wound healing, persistent otorrhoea. ARB was detected by EZN that confirmed the diagnosis of *Mycobacterium* infection. She was given ethambutol 1500 mg/day, isoniazid 300 mg/day, rifampin 600 mg/day and morphozinamide 2500 mg/day.
Conclusion: Finally, the final diagnosis can be difficult because it requires special culture and pathologic studies. Early treatment is essential in order to avoid propagation of the disease and lasting loss of function.

R2354 Pott's disease: a case report

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Pott's disease, the most dangerous form of tuberculosis, still constitutes a public health problem in developing and underdeveloped countries. We reported a patient with loss of weight, a 7-month history of severe back pain, weakness, lack of appetite, progressive disability to walk, kyphotic deformity. Definitive diagnosis was performed by microbiological diagnostic procedures (acid-fast stain, conventional and radiometric cultures, polymerase chain reaction) and the patient was treated with the combination of surgery and antituberculosis chemotherapy. The combined application of culture methods with molecular identification of mycobacteria is an effective strategy to prevent a delay in definitive diagnosis and completely treatment of Pott's disease. This combination of surgery and antituberculosis chemotherapy produced good results in our patient. Physicians must not omit tuberculosis in the differential diagnosis of any spine inflammatory process so that specific treatment may be initiated as soon as possible.

R2355 Antimycobacterial activity of ofloxacin, ciprofloxacin and amikacin against *Mycobacterium tuberculosis*

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Objectives: Despite recent advances in chemotherapy and a steady improvement in health conditions, tuberculosis still represents a common disease in the world. The incidence of resistant *Mycobacterium tuberculosis* to single or multiple drug therapies employing standard drugs is increasing in almost all countries. The aim of this study was to investigate the *in vitro* susceptibility of resistant *Mycobacterium tuberculosis* isolates to some anti-mycobacterial agents.
Methods: In the present study, *in vitro* activities of ofloxacin, ciprofloxacin and amikacin against 45 drug resistant and 15 susceptible *Mycobacterium tuberculosis* isolates were studied by proportion method. Susceptible strains were used as a control group.
Results: All of the 60 isolates were found to be susceptible to ofloxacin and ciprofloxacin. From 24 isolates that were resistant to streptomycin, only six were resistant to amikacin. Fifteen susceptible strains as a control group were sensitive to alternative drugs.
Conclusions: The findings of this study suggest the usefulness of ofloxacin, ciprofloxacin and amikacin as alternative drugs in the treatment of resistant tuberculosis.