Health care issues, public health, pharmaco-economics

R1949

Malaria problem in Afghanistan: malaria scanning results of the Turkish medical aid group after the war

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Objectives: Malaria is a parasitic infection caused by *Plasmodium* species and it is especially seen in tropical and subtropical areas. We aimed to evaluate the effects of the infection in Afghanistan, which is an endemic place for malaria and had severe socio-economical lost after the war. We also compared these data with the ones that were recorded before the war.
Methods: Blood samples were taken from 376 malaria suspected patients who come to the health centre, established by the medical group of Istanbul Medical Faculty in 2002, Afghanistan. Blood samples were screened using the OPTIMAL Rapid Malaria Test and Giemsa staining method.
Results: In 95 (25.3%) patients diagnosis was malaria. In 65 patients (17.3%) the agent of the infection was *P. falciparum* and in 30 patients (8%) agents were other *Plasmodium* species.
Conclusions: Reported data of malaria in Afghanistan are limited by the only studies carried on Afghan refugees in bordering countries. In this study given data were collected from the present Afghan people living in their home country even after the war. We emphasized the increasing importance of the infection in Afghanistan where malaria is endemic and there is severe socio-economic damage after the war.

R1950

Prevalence of *Salmonella* carriers among food handlers in Hamadan, western Iran

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Objectives: *Salmonella* is one of the major causes of human diseases related to food consumption. Some infected individuals recovering from this infection become temporary or permanent carriers, harbouring the organisms in the gallbladder, biliary tract, or rarely in their intestine. The objective of this study was prevalence of *S. typhi* and *S. para-typhi* carriers among food handlers.
Method: In this study, 210 persons who were working in supplying and distribution of the food-stuffs were investigated. The fecal samples from food handlers were collected and cultured on a suitable media. The organisms were identified by relevant antisera. The data was gathered through questionnaire and analysed using EPI6 statistical software.
Results: Of 210 stool cultures, 5 cases (2.38%) were positive for *Salmonella* species. Two (0.94%) were *S. typhi*, two (0.94%) *S. para-typhi* B, and one (0.47%) *Salmonella non-typhi*. Positive cases were tested again after one month and one of them (non-typhi) was negative in culture. So, the rate of infection was 0.19%.
Conclusion: Our results showed that prevalence of *S. typhi* and *S. para-typhi* carriers among food handlers in Hamadan was approximately similar to those in the third world countries. Since food handler carriers are at high risk for transition of organisms, which involved in foodborne diseases, they must be checked regularly for health.

R1951

Seroprevalence of *Haemophilus influenzae* Type B in children aged 1–5 years old in the region of a primary health care unit in Antalya, Turkey

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Objectives: Aim of this study was to determine the natural immunity to *Haemophilus influenzae* type b (Hib) and contributed factors in children because there is limited data about the seroprevalence of Hib in Turkey.
Methods: Presented study was performed in healthy children aged 13–60 months living in the region of Primary Health Care Unit Number 17, Antalya. A total of 378 children were selected for sampling. No child had been vaccinated before against Hib. Probable factors were asked to parents of the children affecting Hib natural immunity. Questionnaire forms were filled by us. Blood samples were taken from those children. Capsular polysaccharide specific IgG antibody (anti-PRP) levels were determined in the sera by EIA methods. 0.15 microgram/ml is accepted as positive limit level of anti-PRP IgG antibody. Dependent and independent variables were evaluated by statistical methods.
Results: Antibody levels were below 0.15 μ/ml in 10 (2.6%) and antibody levels were above 0.15 μ/ml in 368 (97.4%) of children in the study group. No significant relationships were found geometric mean titres of antibodies with year's intervals ($p > 0.05$). Relationships were established between the Hib natural immunity and frequency of upper respiratory tract infection and bronchopneumonia ($p < 0.05$). No significant relationships were found between the natural immunity and age, gender, breastfeeding, socioeconomic level, attendance day care center, number of siblings, exposure to otitis media and sinusitis ($p > 0.05$).
Conclusion: In our study we determined the natural immunity rate was high even in very early ages in Antalya. This fact should be evaluated by nation wide surveillance programme of invasive Hib diseases and nasopharyngeal colonisation rate before making a decision for Hib routine vaccination in Turkey.

R1952

Determination of the quantity of Aflatoxin M1 contamination in traditional and UF cheese in Shiraz, Iran

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Objectives: Aflatoxins are toxic mold metabolites produced by toxigenic strains of *Aspergillus* species. They have an important role in the occurrence of some human diseases such as liver cancer, chronic hepatitis and cirrhosis. When animals eat food-stuffs containing aflatoxin B1, these toxins will be metabolized and excreted as aflatoxin M1 in their milk. A aflatoxin M1 is resistant to thermal inactivation and is not destroyed completely by pasteurization, autoclaving or the other food processing procedures. The aim of this study was to evaluate the quantity of aflatoxin M1 contamination in UF and traditional cheeses in comparison with the primary milk contamination in Shiraz city (Iran).
Methods: Forty-eight UF cheese, 48 traditional cheese and 48 milk samples which were used to produce those cheeses were collected from one of the cheese factories in Shiraz. The amount of aflatoxin M1 in these samples was determined by ELISA test Using Ridascreen Aflatoxin M1 kit (r-biopharm, Germany). Aflatoxins are Soluble in polar compounds so water phase of centrifuged milk samples and methanol phase of cheese samples, which were prepared with solvents such as methanol, PBS buffer and heptan were used to determine the quantity of aflatoxin M1.
Results: Thirty per cent of milk samples had contamination more than maximum tolerated level (50 ng/kg). The range of aflatoxin M1 contamination in UF cheese samples was 27–137 ng/kg and that of traditional cheese samples was 25–88 ng/kg. So none of the UF and traditional cheese samples have contamination more than maximum tolerated level, which has been accepted by European countries (250 ng/kg). This study showed that in transforming the milk to UF and traditional Cheeses 61.9% and 77.1% of aflotoxins were eradicated in whey, respectively while 38.1% and 22.9% of aflatoxins remained respectively, which shows no statistical differences between UF and traditional cheeses in contamination with aflatoxins.
Conclusion: Although 30% of pasteurized milk had contamination more than maximum tolerated level with aflatoxin M1, none of the cheeses, which produced from those pasteurized milk have contamination more than maximum tolerated level. We concluded that as aflatoxins are soluble in polar compounds, most of them will be gone out with whey and little of them will remained in cheeses.

R1953

Evaluation of awareness of healthcare workers about standard health precaution and isolation

M. Jamshidi, A. Jamshidi *(Bandar Abbas, IR)*

Objective: To evaluate knowledge of health care workers about health precaution and its association.
Methods: This descriptive study is done on 191 health care workers, working in teaching hospitals of Hormozgan university of medical sciences in southern Iran. Data is collected by giving a question are including questions about knowledge of healthcare workers (HCW) about standard preventing health precaution and demographic data. The results from questionnaires is analysed by EPI INFO2000 program. For finding association we used Fisher test and chi-square. We classified HCW according to scores that they take into four groups; a (good), b (moderate), c (weak), d (very weak).
Results: One hundred and ninety-one health care workers participate in this program. 85.5% was female with average age of 31.8 ± 5.6 .average work experience was 8.4 ± 6 years. 4.2% of health care workers was in group a, 57.6% in group b, 35.1%in group c and 3.1% in group d. There was no statistically significant correlation between scores and age, sex or work experience but there was significant correlation between different hospitals and different wards. High scores was seen in paediatric hospital and in ICU, CCU and emergency ward.
Conclusion: Results of this study showed that awareness of healthcare workers about preventing precaution is not enough in this university and educational program in this field is necessary.

R1954

Seroepidemiology of Crimean-Congo haemorrhagic fever in local and imported sheep in Isfahan province, Iran in 2002

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Crimean- Congo Haemorrhagic Fever is an Arboviral Zoonotic infection which several cases of that have been reported in Iran. Measurement of antibody level of Crimean-Congo Haemorrhagic Fever among local and imported sheep's in Isfahan province and presence of tick on their body are the main purposes of this study. This cross sectional study has been performed among 372 local and the same amount of imported sheep's, regarding to the presence of IgG antibody of Crimean-Congo Haemorrhagic Fever and tick on their body. The study was made with the special helps of Arbovirus laboratory of Iranian Pasteur Institute on 2002. 286 (76.9%) of local and 223 (57.8%) of imported sheep's have had seropositive results but these data were statistically meaningless (P = 0.094). Ticks have been found on the body surface of 115 (31%) of local sheep's but among imported one's were totally absent. The results of this study revealed the endemic spreading of CCHF in the sheep's in Isfahan province and it needs special attention to prevent the infection in the communities and occupational exposure.

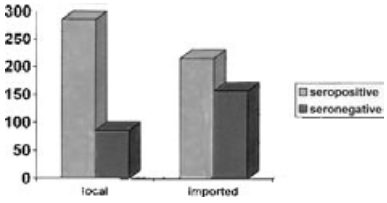

Fig. 1. Relative prevalence of CCHF serology in local and imported sheep is in Isfahan province.

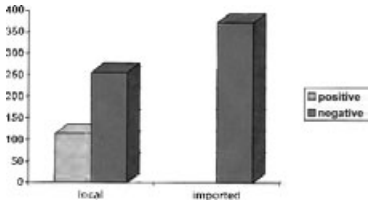

Fig. 2. Relative prevalence of the presence of tick on imported and local sheep in Isfahan province.

R1955

Epidemiology of *Helicobacter pylori* infection in Kashan

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Objectives: 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.
Methods: Two hundred and thirty-two patients with gastrointestinal signs were studied. Endoscopy was done by internist and 2–3 biopsies from antrum was taken. Specimens were processed by Shandon tissue Processor (Citadel). Prepared slides were stained by Hematoxycillin and Eosin & 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%. 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.
Conclusions: 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.

R1956

The study of prevalence of aetiology and outcome of septic shock patients admitted at obstetric and gynecologic department

Z. Shahshahan, S. Boroomand *(Isfahan, IR)*

Objectives: The incidence of sepsis, the precursor of septic shock, has continued to increase over past decades. The aim of this study was to evaluate the outcome and aetiology of septic shock in Iranian patients.
Methods: During the years 1992–2002 , we examined the blood, urine, uterus discharge culture of patients who were known cases of septic shock and the outcome and aetiology of the patients were recorded.
Results: Four hundred patients with a mean age of 30 and a range of 25–35 were included. The prevalence of aetiology was 299 (74%) septic abortion, 55 (14%) endometritis, 27 (7%) pyelonephritis, and 10 (2.5%) chorioamnionitis, and 172 (43%) died. The results of culture were: *E. coli* (50%) *klebsiella* (20%), *enterobacter* SP (15%) *streptococcus* SP (10.5%) and others (4.5%).
Conclusion: According to this study illegal abortion was the most common cause of septic shock, therefore education effective methods of contraception are recommended to women.

R1957

Co-infection of *Salmonella typhi*, *Salmonella paratyphi*, and *Giardia lamblis* after travelling abroad

J.-S. Eom, H.-S. Kim, J.-S. Kim *(Seoul, KOR)*

Introduction: We experienced a case of co-infection of *Salmonella typhi*, *Salmonella paratyphi*, and *Giardia lamblis* after traveling abroad. Case: A Korean male who was 23 year old had traveled India and Thailand for two weeks on January, 2004. He had a first febrile sense on 10 days later after traveling and started watery diarrhoea over 10 times in a day for a week. Just after he returned to Republic of Korea, he visited outpatient clinic of Division of Infectious Diseases in our hospital. He had been drinking water in bottles which were not completely corked and foods which were sold on the side of the streets in India and Thailand. He was acute ill looking and patient's body temperature was 40°C, pulse rate 78/min, respiration rate 24/min, and blood pressure 120/70 mmHg. Physical examinations were shown that very dehydrated tongue and decreased skin turgor but no other remarkable findings. Laboratory findings were; in CBC, WBC 3,450/μL (neutrophil 67.8%, lymphocyte 18.2%, monocyte 13.8%), hemoglobin 10.9 g/dl, platelet 143,400/μL; in chemistry, AST 106 IU/L, ALT 71 IU/L, albumin 3.0 g/dl, total cholesterol 66 mg/dl; peripheral blood smear for malaria was negative; stool occult blood positive. We examined stool wet smear by microcopy and discover the trophozoites of *Giardia lamblis*. We prescribed metronidazole 500 mg tid po but patient's fever was not decreased after three days later. At forth hospital day, *Salmonella typhi* was isolated from blood culture. So, we added ciprofloxacin 500 mg bid po and then patient's body temperature normalized after four days later. Each antibiotic administered for 10 days and then he discharged hospital after results of follow-up stool culture were negative. But, three days later, he had high fever and was hospitalized again. *Salmonella paratyphi* were isolated from his blood cultures. It was a question why *Salmonella paratyphi* had not been isolated from previous blood and stool cultures and had not eradicated despite administer of ciprofloxacin. There were no outbreaks of *Salmonella paratyphi* in our hospital and his community. He had not eaten out during admission and after discharge. We prescribed ciprofloxacin 500 mg bid po again. After 10 days later, he was cured and had no recurred.
Conclusion: Although we do not know the correct cause for late onset of *Salmonella paratyphi* bacteraemia, we suggest strongly that the co-infection of three pathogens was caused by food-borne infection during his traveling.

New drugs

R1958

In vitro activity of ceftobiprole against recent clinical isolates of *Pseudomonas aeruginosa* from hospitalised patients in Europe and USA

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Objectives: Ceftobiprole (BPR), formerly BAL9141, is the first of a new class of cephalosporins and has broad-spectrum activity towards a wide range of Gram-negative and Gram-positive hospital-acquired pathogens including MRSA. *Pseudomonas aeruginosa* (PA) is a common nosocomial pathogen, which has developed high resistance rates to various antibiotics. In this study we investigated the in vitro activity of BPR and other commonly used anti-pseudomonal antibiotics against a collection of clinical *P. aeruginosa* isolates.
Methods: A total of 407 organisms originating from hospitals in Europe (Belgium, France, Germany, Italy, and Spain) (n = 204) and throughout the USA (n = 203), were studied. All isolates were non-repeat isolates from hospitalized patients during 2001–4. MICs of BPR, ceftazidime, ciprofloxacin, imipenem, piperacillin/tazobactam, and tobramycin were determined by broth microdilution according to NCCLS guidelines. BPR breakpoints used were 4 mg/L susceptible, 8 mg/L intermediate, and 16 mg/L resistant.
Results: Similar other recent reports, 14.2 and 9.4% of isolates were imipenem resistant in EU and USA, respectively. At 8 mg/L, 71.6/78.3% EU and US, respectively PA isolates were inhibited by BPR, compared to 76.5/79.8% for ceftazidime. The two drugs exhibited a high degree of cross-resistance.

		MIC (mg/L)					
Region	Agent	Range	MIC_{50}	MIC_{90}	%S	%I	%R
Europe n = 204	Ceftobiprole	1–>32	4	32	50.0	21.6	28.4
	Ceftazidime	0.5–>32	2	>32	76.5	2.5	21.1
	Ciprofloxacin	≤0.06–>4	0.25	>4	62.7	6.9	30.4
	Imipenem	≤0.25–>16	1	16	78.4	7.4	14.2
	Piperacillin/Tazob.	0.5–>128	8	>128	82.4	–	17.6
	Tobramycin	≤4–>32	≤4	>32	75.0	0.5	24.5
US n = 203	Ceftobiprole	0.25–>32	4	16	64.5	13.8	21.7
	Ceftazidime	≤0.25–>32	2	32	79.8	5.4	14.8
	Ciprofloxacin	≤0.06–>4	0.25	>4	66.0	6.4	27.6
	Imipenem	≤0.25–>16	1	8	86.2	4.4	9.4
	Piperacillin/Tazob.	≤0.25–>128	8	>128	80.8	–	19.2
	Tobramycin	≤4–>32	≤4	8	89.2	2.0	8.9

Conclusion: In addition to its anti-MRSA activity, the spectrum of activity of BPR towards *P. aeruginosa* resembles that of ceftazidime.

Pharmacokinetics, pharmacodynamics, drug interactions, tolerability

R1959

Importance of the infecting organism in antibiotic penetration into the middle ear in experimental otitis media

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Objective: To determine the rate of antibiotic penetration into the ME in experimental acute otitis media caused by *Streptococcus pneumoniae* (Sp), *Haemophilus influenzae* (Hi), or a combination of both (Sp + Hi).
Methods: A gerbil model of otitis media caused by either Sp, Hi, and Sp plus Hi was induced. Animals were sbc treated with amoxicillin (AMX), cefuroxime (CXM) or erythromycin (ERY) 2, 10, and 18 h after bacterial challenge and antibiotic efficacy was evaluated clinically and bacteriologically. Antibiotic concentrations were determined in the serum of healthy animals as well as in the middle ear 90 min after drug administration in infected animals.
Results: The percentage of drug penetration into the middle ear in animals with otitis media in relation to the inoculated organism was as follows:
Table 1

Drug (mg/kg)	Sp	Hi	Sp + Hi
AMX (2.5)	29.8	–	–
AMX (5)	19.7	9.4	23.8
AMX (10)	–	7.8	–
AMX (20)	–	9.8	–
CXM (5)	–	7.7	21.8
CXM (20)	–	9.4	–
ERY (2.5)	54.3	–	–
ERY (50)	–	43.6	40.6

Conclusions: 1) The rate of penetration into the middle ear for betalactam antibiotics ranged approximately from 20 to 30% when animals were infected with Sp (alone or combined with Hi) but such a rate was approximately 8 to 10% when the organism involved was Hi. 2) The erythromycin rate penetration ranged approximately from 40 to 50% whatever it was the microorganism involved.

R1960

Penetration of telithromycin into nasal mucosa and ethmoid bone of patients

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Objectives: The ketolide telithromycin has a focused spectrum of activity against respiratory tract pathogens, including penicillin- and erythromycin-resistant pneumococci, as well as intracellular and atypical bacteria. The aim of the present study was to investigate the penetration of telithromycin into nasal mucosa and ethmoid bone of patients to provide kinetic data.
Methods: The study was designed as an exploratory population kinetics trial. A total of 29 patients undergoing rhinosurgery for chronic sinusitis were evaluated. The patients received a single oral dose of telithromycin 800 mg. Samples of blood, nasal mucus, nasal mucosa and ethmoid bone were collected during surgery 3, 6, 9, 15 and 24 h post-drug administration. Drug concentrations were determined by HPLC with fluorimetric detection.
Results: The telithromycin mean (SD) concentrations 3–24 h after administration are listed in the Table. The achieved concentrations were generally above the MIC90 of common pathogens in upper respiratory tract infections (*S. pneumoniae* 0.12 mg/L, S. aureus [MSSA] 0.06 mg/L, *M. catarrhalis* 0.12 mg/L; data from the PROTEKT surveillance study). The relative

systemic exposure, expressed by the ratio of the area under the concentration-time curve in tissue vs plasma, was 1.0 for nasal mucus, 5.9 for nasal mucosa and 1.6 for ethmoid bone, respectively.

time h	n	plasma mg/L	n. mucous mg/L	n. mucosa mg/kg	ethm. bone mg/kg
3	6	0.73 (0.32)	0.66 (0.82)	3.77 (2.4)	1.12 (0.31)
6	6	0.53 (0.27)	0.21 (0.24)	2.46 (0.81)	0.79 (0.54)
9	6	0.22 (0.05)	0.22 (0.06)	1.53 (0.81)	0.33 (0.16)
15	6	0.06 (0.02)	0.17 (0.14)	0.52 (0.20)	0.14 (0.09)
24	5	0.02 (0.01)	0.13 (0.10)	0.32 (0.27)	0.06 (0.02)

R1961

The chinolones significantly increased the release of verotoxin2 from clinical VTEC O26 in vitro

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Objectives: Verotoxin-producing *Escherichia coli* (VTEC) cause hemolytic-uremic syndrome (HUS) mostly in children. Children hospitalised at Intensive Care Units are given antibiotics prophylaxis. However, it has been recognized that certain antimicrobial drugs have capacity to enhance the release of verotoxin. We have investigated the influence of antimicrobial drugs used against gram-negative bacteria to verotoxin2-positive clinical VTEC O26 in vitro.
Methods: The clinical VTEC strain isolated from 4-year-old child suffering from HUS was incubated with ampicillin/sulbactam, meropenem, cefepime, piperaciline, piperacilline/tazobactam, cefotaxime, ceftazidime, gentamicine, amikacin, ciprofloxacine and trimethoprime/sulfamethoxazole using dilution method. After 2 h and 4 h incubation, the verotoxin was separated and the presence of verotoxin was confirmed by ELISA with monoclonal antibodies against verotoxin2. We used *t*-test for statistical analysis.
Results: Based on ELISA tests, we have not found any significant increase or decrease of verotoxin release after 2 h incubation. However, after 4 h incubation we have found that ciprofloxacin ($p < 0.05$) significantly induced the production of verotoxin 2 and verotoxin release from clinical VTEC strain was significantly decreased by meropenem and amikacine ($p < 0.05$) compared with clinical VTEC strain incubated without antibiotics.
Conclusions: Antimicrobial drugs play role not only as inhibitors of the bacterial growth but some of them can activate the production of important virulence factors. Therefore, it is important not only isolation of VTEC from HUS patients but also detection of the influence of antibiotics used for prophylaxis in hospitals to verotoxin production of isolated strain.

R1962

Continuous versus intermittent intravenous administration of antibiotics: a meta-analysis of randomised controlled trials

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Background: Intermittent intravenous administration of antibiotics is the first line approach in the management of severe infections worldwide. However, the potential benefits of alternative modes of administration of antibiotics, including the continuous intravenous infusion, deserve further evaluation. We performed a meta-analysis of randomized controlled trials (RCTs) to compare the effectiveness and toxicity of the continuous versus the intermittent mode of intravenous administration of various antibiotics.
Methods: Data for this meta-analysis were identified from PubMed (01/1950 to 09/2004), Current Contents, Cochrane central register of controlled trials, and references from relevant articles. RCTs comparing the continuous with the intermittent intravenous administration of the same antibiotic regimen, and examining effectiveness, mortality and/or toxicity were included in the analysis. Experimental trials, studies focusing on pharmacokinetic and/or pharmacodynamic parameters, trials concerning antifungal, antiviral, or antiparasitic agents, and surgical prophylaxis trials were excluded. We used data conforming to standard outcome definitions of clinical failure and nephrotoxicity.
Results: A total of 20 comparisons from 9 randomized controlled trials (RCTs) studying α-lactams (6), aminoglycosides (2), and vancomycin (1) were included in the meta-analysis. Publication bias and statistically significant heterogeneity was not observed among the analysed studies. A better outcome was observed with the continuous intravenous administration of antibiotics; the pooled odds ratio for clinical failure (10 comparisons from 7 RCTs) was 0.73 (95% CI, 0.53–1.01) for the continuous compared to the intermittent intravenous administration. Regarding mortality (5 comparisons from 5 RCTs) and nephrotoxicity (5 comparisons from 4 RCTs), no differences were found; the pooled odds ratio and 95% CI for mortality and nephrotoxicity was 0.89 (95% CI, 0.48–1.64) and 0.91 (95% CI, 0.56–1.47), respectively, comparing the continuous with the intermittent intravenous administration of antibiotics.
Conclusion: Compared with the intermittent, the continuous intravenous administration of antibiotics is more efficient regarding clinical effectiveness. In an era of gradually increasing resistance rates among most pathogens, the potential advantages of the continuous intravenous administration of antibiotics on several clinical outcomes should be further investigated.

R1963

Penetration of moxifloxacin into rat mandibular bone and soft tissue

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Objectives: Odontogenic infections result in submucosal infiltrates and abscesses. They are able to penetrate into facial softtissues and bone. These mixed infections are mainly caused by both facultative and obligate anaerobes. Based on in vitro activity, the new 8-methoxyquinolone antibiotic moxifloxacin (MXF) seems to be suited for the antibiotic therapy of these odontogenic infections. The aim of this experimental study was to determine the levels of MXF in plasma, soft tissue (M. masseter) and the mandible.
Methods: Forty-nine male fasted albino Wistar rats (aged 6 weeks) weighing 190–300 g were used throughout the study. A dose of 10 mg/kg MFX dissolved in water was administered orally per gavage. Blood samples of 7 animals (6 MFX, 1 control) were obtained 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 8 h and 24 h, respectively, after administration. The rats were killed with Ketalar (10 mg/kg i.m.) and tissue samples were collected. After homogenization (tissue samples) and protein precipitation all

samples were analysed by HPLC/fluorescence detection assay. The pharmacokinetic evaluation was conducted based on non-compartmental analysis.
Results: MFX concentrations above lower limit of quantitation were measured from 0.5 h up to 8 h after administration. The exposure in terms of AUC (0 – ∞) amounted to 1821 μg h/L (plasma), 2463 μg h/kg (mandibles) and 5192 μg h/kg (M. masseter). The concentration–time curves of tissues were more plateau-shaped compared to plasma. Calculated AUC ratios tissue:plasma were M. masseter:plasma = 2.85 and mandibles:plasma = 1.35.
Conclusion: Administration of antibiotics is considered as an important part of therapy during and/or after surgical procedures in the maxillofacial area. Because of its good penetration into bone and muscle tissues demonstrated in Wistar rats, MXF might be an option for clinical application. Based on the experimental data, controlled studies have to evaluate the clinical efficacy of MXF, also compared to other antibiotics.

R1964

Pharmacokinetic and pharmacodynamic aspects of high dose oral levofloxacin therapy for bone infections

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Objectives: To assess pharmacokinetic and pharmacodynamic aspects of high dose oral levofloxacin in the therapy of bone infection due to fully susceptible organisms.
Methods: All patients with microbiologically documented bone infection, treated at our Institution from 01/04 to 10/04 with oral levofloxacin, were included in the present analysis. Two regimens were routinely used according to age, body weight and renal function: 500 mg bid or 750 mg qd. According to different isolates other active antibiotics were administered. Patients underwent weekly clinical, biochemical and plasma therapeutic drug monitoring (TDM). Dosage adjustments were made according to plasma levels to maintain optimal exposure. Outcome was assessed at 8 weeks of treatment (16 weeks for discitis) and at least 1 month after the end of treatment.
Results: Fifteen patients were included. Mean age was 60 years (SD 17). 10 were males. Mean weight was 77 kg (SD 13). Median creatinine clearance was 91 ml/min (range 41–155). 7 pts had a prosthetic joint infection, 5 a post-traumatic ostheomyelitis (PTOM), 2 spondilodisicitis and 1 septic arthritis. Infections were monomicrobial in 13 cases (4 MSSA, 6 CoNS, 1 each *E. faecalis*, *E. coli*, *K. ozaenae*); polymicrobial in 2 (CoNS and *P.aeruginosa*, MSSA and MRSA). All but one isolates (MRSA) were levofloxacin-susceptible (available MIC for 9 isolates ranged 0.04–0.5 mcg/ml). Mean administered dosage was 8.9 mg/kg/daily (SD 2.2). Average C_{min} and C_{max} during the overall treatment were 1.4 and 7.7 mcg/ml, respectively. Average C_{max}/MIC ratio in the five patients with a punctual MIC was 133.21. Median treatment duration was 62 days, (range 17–135). At 1 month follow up after the end of treatment, 12 pts were deemed cured (clinically and by C Reactive Protein normalization). The 3 patients in whom levofloxacin failed 2 were prosthetic joint infections in which infected prosthesis was not removed, and 1 PTOM. No drug withdrawal was necessary due to adverse events, but 3/15 pts experienced mild arthralgies.
Conclusion: In our experience,TDM tailored high daily dose oral levofloxacin enabled adequate plasma levels and high cure rates, with no significant adverse events throughout long-term treatment. Plasma drug level monitoring is an exceedingly useful tool in the management of bone infections, suggesting dose reduction in pts at risk of adverse events and dose increase in pts suboptimally exposed.

R1965

Penetration of levofloxacin in sinusal mucosa of patients with chronic sinusitis following oral administration of a single 500 mg dose

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Objectives: To assess levofloxacin penetration in sinusal mucosa of patients with chronic sinusitis undergoing functional endoscopic sinusal surgery (FESS).
Methods: Seven patients (mean ± SD; age, 45 ± 18 yrs; weight, 77 ± 13 kg; sex, 5/2 M/F; CLCr, 1.28 ± 0.32 mL/min/kg) were administered a standard 500 mg single oral dose of levofloxacin at 1 h (n = 4), 2 h (n = 1) or 3 h (n = 2) before surgical intervention. Intraoperative mucosal tissue and blood samples were collected simultaneously in each patient during FESS with the intent of assessing and comparing plasma and tissular concentration of levofloxacin. Levofloxacin concentrations in plasma and tissue homogenate were analysed by means of a validated HPLC method.
Results: Levofloxacin concentrations in plasma (mcg/mL) and tissue homogenate(mcg/g), respectively, after 1 h ranged 0.43–8.52 mcg/mL, and 0.78–9.87 mcg/g; after 2 h were 7.27 mcg/mL and 8.91 mcg/g; and after 3 h ranged between 3.59 to 5.14 mcg/mL, and 6.46 to 9.34 mcg/g. Tissue-to-plasma ratio of levofloxacin at different sampling times ranged between 1.16 and 1.82.
Conclusion: These preliminary findings suggest that single 500 mg oral dose of levofloxacin may ensure therapeutically effective concentrations in the sinusal mucosa of patients with chronic sinusitis. Of note, considering that fluoroquinolones exhibit concentration-dependent bactericidal activity and that optimal exposure may be achieved when peak-to-MIC ratio > 8–10 has been achieved, these data enable to conclude that a single 500 mg oral dose of levofloxacin may ensure effective exposure in sinusal mucosa against bacterial pathogens with an MIC < 0.8–1 mcg/ml, that is a value lower than the MIC90 of most pathogens usually responsible for bacterial sinusitis. Accordingly, these pharmacokinetic data are consistent with levofloxacin 500 mg once daily to be an effective antibiotic in the treatment of bacterial sinusitis.

R1966

A situation that mimics viral hepatitis: statin induced rhabdomyolysis

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A 44-year-old woman was admitted to the emergency room with the complaints of abdominal pain, generalized muscle pain, cramps, and dark urine lasting for the last five days. She was well orientated with a body temperature of 37.3°C, blood pressure of 110/80 mmHg, and pulse rate of 84/min. Systemic examination did not reveal any pathological finding. The biochemical tests showed elevated levels of ALT (1196 U/L), AST (1200 U/L), LDH (1768 U/L), creatinine (2.1 mg/dL), and BUN (62 mg/dL). Viral hepatitis, leptospirosis and hepatorenal syndrome were considered in the differential diagnosis and the patient was transferred to the Infectious Diseases Department. She was diabetic and hypertensive for a year and was taking gliclazide 60 mg/day, lisinopril 20 mg/day. She was also taking

fluvastatin 80 mg/day for hypercholesterolemia for a year which was changed to simvastatin 40 mg/day for the last two months by her doctor. The biochemical tests revealed that serum CPK was 119.300 U/L in 1/40 diluted serum sample, myoglobin was 4.000 ng/ml, viral hepatitis panel and Leptospira microagglutination test was negative. Her renal functions rapidly deteriorated. She was diagnosed as statin rhabdomyolysis and underwent to hemodialysis. The patient slowly improved and recovered with hemodialysis and discontinuation of statin drug. The incidence of rhabdomyolysis with statin monotherapy was reported as 0.04–0.2%. Our patient was taking high dose statin and was unaware of side effects. All patients taking statins should be informed about the side effects of these drugs.

R1967

The rationale for higher dose levofloxacin for the treatment of serious infections

J. Kahn, N. Davis *(Raritan, USA)*

Background/Objective: Advances in medical care continue to be associated with improved survival rates and better patient quality of life. A consequence of these developments is an increased vulnerability to serious infection due to traditional pathogens (or commensals and relatively avirulent organisms) that may be resistant to multiple antimicrobial agents. There are few new antibacterial agents on the horizon. One strategy for dealing with this threat is to maximize the utility of each of our antibacterial drugs by dosing them more rationally. The purpose of this paper is to summarize the rationale for utilizing higher doses of levofloxacin in the management of serious infections.
Methods: We have reviewed and will report on all available data from subjects exposed to daily levofloxacin doses of 750 mg or more.
Results: After more than seven years of use in the US, levofloxacin has maintained its excellent susceptibility profile. Drug levels seen in infected patients are significantly higher than those achieved in healthy volunteers and enable coverage of organisms with higher MICs. Because of its concentration-dependent bactericidal activity, higher doses of levofloxacin increase the speed and thoroughness of bacterial eradication in vitro and can inhibit the emergence of fluoroquinolone resistance in studied organisms. Clinical trials with the 750 mg dose have been undertaken in complicated skin and skin structure infections, nosocomial pneumonia, and febrile neutropenia; results were at least equivalent to comparator agents. Use of this dose in community-acquired pneumonia and acute bacterial exacerbations of chronic bronchitis has allowed for more rapid resolution of symptoms and a shortening of the course of therapy. Dosing in healthy volunteers at does up to 1500 mg per day resulted in no significant alteration of the adverse event profile. Clinical trial data show that the safety of 750 mg daily is similar to the 250 and 500 mg forms.
Conclusion: Levofloxacin has documented efficacy and safety credentials at clinical doses of at least 750 mg once daily. At these higher doses, it is an attractive candidate for monotherapy of some serious infections or for combination therapy with such other agents as the carbapenems or 4th generation cephalosporins. Additional clinical trials using these approaches seem warranted.

In vitro activity of antimicrobial agents

R1968

Sensitivity to meropenem and imipenem pseudomonas strains isolated from various patients' material

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Introduction: *Pseudomonas* stem consists of resistible, aerobian, Gram-negative, non fermentation bacilli. They might cause various human infections and they are most frequent agents of intrahospital infections.
Purpose: Investigation of *Pseudomonas* strains sensitivity to meropenem and imipenem.
Material and methods: At the IGO CCS in Belgrade, from January 1st, 2004, till September 16th, 2004, a total of 131 strains of *Pseudomonas* from various patients' material were isolated. In 98 isolated strains, sensitivity to meropenem and imipenem was investigated by Gel Diffusion Technique, using commercial antimicrobial susceptibility test discs – Oxoid.
Results: Sensitivity to meropenem and imipenem was registered in 52/98 (53.06%), while 32/98 (32.65%) showed sensitivity to meropenem and resistance to imipenem. Another 10/98 (10.2%) of isolated strains were resistant to both antibiotics subject of this study. In 4 cases (4.08%), sensitivity to meropenem (S) was investigated, while sensitivity to imipenem was not tested. Out of 131 isolated strains, 79 were *Pseudomonas aeroginosa*, and in 61 strains, sensitivity to both meropenem and imipenem was tested. 36/61 (59.01%) showed sensitivity to both antibiotics. 16/61 (26.22%) were sensitive to meropenem and resistant either intermediary resistant to imipenem. 7/71 (11.47%) were resistant to both investigated antibiotics. 52/131 were *Pseudomonas* species, and in 37, sensitivity to meropenem and imipenem was investigated. 16/37 (43.24%) were sensitive to both antibiotics. 16/37 (43.24%) showed sensitivity to meropenem and resistance to imipenem. 3/37 (8.1%) isolated strains showed resistance to both investigated antibiotics.
Conclusion: Isolated *Pseudomonas* strains demonstrate a high resistance to imipenem. In the investigation period, *Pseudomonas* strains resistant to meropenem were isolated for the first time ever. Considering that karbapenems are drugs of choice for the treatment of multiresistant strains, appearance of high resistance points to the necessity of their rational use.

R1969

The bacterial causes of nosocomial infections associated with short and long term-catheter

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The urinary tract infection is the most frequent site of nosocomial infections, accounting for approximately 40% of all NCIs. Catheterization is the most risk factor that predispose for such UTI. Two hundred sixty-six strains (76%) out of 348 catheterized patients were isolated. Out of these 266 strains, 212 cases were identified as NCIs, 54 cases (20%) were identified as community acquired infection. The isolated strains were found to be 202 (76%) Gram-negative bacilli, 32 (12%) *S. aureus* and 32 (12%) *Candida* isolates. The organisms were more common in NCIs than in community-acquired infections. Nosocomial infections were higher in the urology ward than in other ward involved in

the study. The most effective antibiotic against *S.aureus* was vancomycin (100%). For Gram-negative bacilli, the most effective antibiotics were amikacin (for *E. coli*), tobramycin (for *P. mirabilis*), cefotaxime (for *K. pneumoniae*) and ceftazidime (for *P. vulgaris* and *E. cloacae*). High level of antimicrobial resistance was noticed among *P. aeruginosa*, the most active antibiotic was amikacin. ESBL production was nearly the same in both NCL and community acquired infection, and it was highly prevalent among isolates of *P. mirabilis* (26%), followed by *E. coli* (8%). Other isolates showed no ESBL activity. Rough comparison of susceptibility results of E- test (ceftazidime strip) with that of disc diffusion and agar dilution methods in detection of the susceptibility test of the organisms to the antibiotic used. As regard the plasmid profile, it seemed that there is no particular association between the plasmid content and ESBL activity of the isolated nosocomial strains of both *E. coli* and *P. mirabilis* to explain interspecies relationship.

NCI = nosocomial infection
UTI = urinary tract infection
ESBL = extended spectrum B-lactamase

R1970

Bacteraemia – prevalence and antibiotic resistance among clinical isolates from Slovakia

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Objectives: Bacteraemia represents a serious complication in hospitalized patients. Problems of therapy of bacteraemia increase with the emergence of antibiotic resistance. The aim of this study was to monitor the prevalence of pathogens and trends of resistance in bacteria isolated from blood.
Methods: Six University Clinics and/or Regional Hospitals have participated in the study and a total of 1795 isolates were collected during 2002–2004. Microbiological diagnosis was performed according to standardized methods in participating laboratory. Antimicrobial resistance was estimated by the disc diffusion method by NCCLS.
Results: The most prevalent organisms were coagulase negative staphylococci (CONS) (28.7%), *Staphylococcus aureus* (14%), among Gram-negative bacteria *Escherichia coli* (13.4%), *Klebsiella pneumoniae* (9.3%) and *Pseudomonas aeruginosa* (6.4%) followed by enterococci, *Enterobacter* spp. and *Acinetobacter* spp. All CONS and *S. aureus* were susceptible to vancomycin, resistance to oxacillin was observed for 55–74% of CONS and only for 2–19% of *S. aureus* isolates. *Enterococcus* spp. isolates were fully susceptible to vancomycin and teicoplanin. Resistance to amoxicillin-clavulante increased from 9 to 35% among *E. coli*. Ciprofloxacin-resistance of *K. pneumoniae* isolates increased from 19 to 41%, despite this, ciprofloxacin together with carbapenems (100% susceptibility) was the most effective drug against *K. pneumoniae*. The most effective antibiotics against *P. aeruginosa* were meropenem, amikacin, piperacillin/tazobactam. Resistance to ceftazidime increased from 13 to 40% among *P. aeruginosa*. Imipenem retained 100% activity against *Enterobacter* spp. isolates, considerable was increasing of resistance to ciprofloxacin (from 15 to 58%). Resistance to meropenem, amikacin, ampicillin/sulbactam and cefoperazon/sulbactam in *Acinetobacter* spp. was relatively low.
Conclusions: Staphylococci, i.e. CONS and *S. aureus* have been identified as the most frequent causal agents of bacteraemia during all study period. The most significant rise in resistance was observed in ciprofloxacin against *Enterobacter* spp., *E. coli* and *K. pneumoniae.* Considerable is still good activity of carbapenems in Gram-negative and 100% efficiency of vancomycin and teicoplanin in Gram-positive bacteria. Surveillance of antibiotic resistance provides data about efficiency of antimicrobials in given locality and is important part of measures maintaining good antibiotic activity.

R1971

Comparative in vitro activity of ertapenem against ESBL-producing *E. coli* and *K. pneumoniae* isolated in Spain

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Objectives: To study the in vitro activity of ertapenem against ESBL-producing *E. coli* and *K. pneumoniae* isolated in Spain.
Methods: *E. coli* (289) and *K. pneumoniae* (174) isolates from a Spanish multicentre study performed in 2000 (40 hospitals) and from the University Hospital Virgen Macarena in Seville (1995–2002) were included. ESBL production and antimicrobial susceptibility (betalactams, betalactams + betalactamase inhibitors, ciprofloxacin, aminoglycosides and co-trimoxazole) was determined by microdilution (NCCLS guidelines). Ertapenem activity was analysed according to clonal relationship (REP-PCR), ESBL-type (PCR and ESBL-gene sequencing) and acquisition of infection (community or nosocomial).
Results: The in vitro activity of ertapenem was as follows: (Graphic 1)

E. coli (n = 289)	Ertapenem	Imipenem	Meropenem
RANGE	<0.03–4	<0.03–1	<0.03–0.5
MIC_{50}	<0.03	0.125	<0.03
MIC_{90}	0.125	0.25	<0.03
K. pneumoniae (n = 174)			
RANGE	<0.03–4	<0.03–1	<0.03–0.25
MIC_{50}	<0.03	0.125	<0.03
MIC_{90}	0.25	0.25	0.06

All the isolates were susceptible to carbapenems. Resistance (resistant + intermediate isolates) of ESBL-producing *E. coli* and *K. pneumoniae* were: piperacillin-tazobactam 22% and 53%, ciprofloxacin 71% and 34%, co-trimoxazole 65% and 60%, amikacin 8% and 7%, gentamicin 36% and 48%, and tobramycin 44% and 63%, respectively. No significant differences were detected among the clones found. Ertapenem was equally active against all ESBL families (SHV, TEM and CTX-M) evaluated. Only four *E. coli* and one *K. pneumoniae* isolates had a MIC of ertapenem >0.5 μg/ml but still in the susceptibility range. Excluding these isolates, all coming from hospitalised patients, no difference in activity was observed according to the origin of infection.
Conclusions: Ertapenem showed an excellent in-vitro activity against ESBL-producing *E. coli* and *K. pneumoniae*. Ertapenem activity was similar to that of imipenem and meropenem but higher than that of other antimicrobials tested. No differences in activity were observed according to geographical origin, clonal relationship and type of ESBL.

R1972

Effect of beta-lactamase-inhibitor concentrations on in vitro test results with piperacillin/tazobactam and piperacillin/sulbactam

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Objectives: Recently, DIN 58940 was altered, now recommending to test the beta-lactamase inhibiting effect of Sulbactam in presence of a constant concentration of 4 mg/L instead of

previously 8 mg/L. The present study was performed to assess the effect of this change on the MIC distributions of *E. coli* for Piperacillin/Sulbactam (P/S) as compared to Piperacillin/Tazobactam (P/T) with Tazobactam tested at a concentration of 4 mg/L.

Methods: The in-vitro activity of Piperacillin without and with Tazobactam or Sulbactam, respectively, against *E. coli* (n = 2856 in Leipzig, n = 420 in Berlin) was tested by means of microdilution MIC determinations as recommended by DIN 58940 using a fixed concentration of 4 mg/L of Tazobactam and 4 as well as 8 mg/L of Sulbactam.

Results: On the basis of MIC breakpoints according to DIN, the superiority of P/T over P/S is maximal in Piperacillin-resistant *E. coli* and minimal in Piperacillin-intermediate *E. coli.* The recently recommended reduction of the fixed Sulbactam concentration (from 8 to 4 mg/L) in susceptibility tests leads to reduced susceptibility in Piperacillin-intermediate *E. coli*, too. The results are as follows: (table)

Cumulative % of strains inhibited at MIC (The intermediate range is in bold)

				Cumulative % of strains inhibited at MIC (The intermediate range is in bold)							
Piperacillin	Origin	n	Antibiotic	0.5	1	2	4	**8**	**16**	**32**	64
resistant	Leipzig	650	PIT (4)	1.1	4.5	42.0	64.6	**72.5**	**78.0**	**81.2**	86.2
	Berlin	74	PIT (4)	0.0	6.8	48.6	67.6	**70.3**	**77.0**	**82.4**	83.8
	Leipzig	650	PIS (8)	0.8	1.5	10.8	22.0	**31.5**	**45.2**	**56.9**	70.2
	Berlin	74	PIS (8)	0.0	1.4	17.6	28.4	**39.2**	**55.4**	**70.3**	82.4
	Berlin	74	PIS (4)	0.0	0.0	0.0	10.8	**17.6**	**39.2**	**55.4**	74.3
intermediate	Leipzig	631	PIT (4)	3.3	15.4	72.1	90.8	**96.5**	**99.5**	**100**	100
	Berlin	107	PIT (4)	0.0	21.5	77.6	90.7	**99.1**	**100**	**100**	100
	Leipzig	631	PIS (8)	10.1	24.7	63.5	84.0	**93.3**	**97.8**	**99.4**	100
	Berlin	107	PIS (8)	7.5	31.8	78.5	91.6	**99.1**	**100**	**100**	100
	Berlin	107	PIS (4)	0.0	1.9	23.4	78.5	**90.7**	**100**	**100**	100
susceptible	Leipzig	1575	PIT (4)	5.5	23.0	87.0	99.6	**99.9**	**100**	**100**	100
	Berlin	239	PIT (4)	2.9	36.0	90.4	100	**100**	**100**	**100**	100
	Leipzig	1575	PIS (8)	57.4	87.7	98.0	99.8	**99.9**	**99.9**	**99.9**	99.9
	Berlin	239	PIS (8)	64.4	94.1	100	100	**100**	**100**	**100**	100
	Berlin	239	PIS (4)	15.5	67.4	98.3	100	**100**	**100**	**100**	100

Conclusion: The recently recommended 4 mg/L fixed concentration of Sulbactam in testing procedures shows that the in-vitro activity of P/T exceed those of P/S not only in Piperacillin-resistant but also in Piperacillin-intermediate *E. coli*. These results are credible because of the stronger inhibition of TEM-1 lactamases by Tazobactam in comparison to Sulbactam.

R1973

The inhibition of microbial adhesion in the presence of immune compounds on the human cell culture model

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Objectives: To comparatively evaluate the influence of the commercial preparations of normal human immunoglobulin (Russia), Intraglobin and Pentaglobin (Biotest, Germany) on the clinical strain *S. aureus* adhesion to the human embryonic skin fibroblasts in culture.

Methods: The clinical isolate of *S. aureus* with the highest adhesive capacity was selected in the model of it's co-incubation with the target cells in culture. The inoculums was prepared at a baseline concentration of 108 cfu/ml after 24 h grown on meat-peptone agar. The antiadhesive activity of the normal human immunoglobulin (NHI), Intraglobin and Pentaglobin at a baseline concentration of 20 μg/ml was assessed. The estimation of the influence of the agents under investigation on the bacterial adhesion process was performed on the above mentioned model of co-incubation of microorganism and the target-cells in culture as well as using 1 h pre-incubation with agents to be tested. Also the experiments were held in the presence Protein A from the staphylococcus cell wall as well as clostridial collagenasum and animal hyaluronidasum. The intensity of bacterial adhesion and antiadhesive properties of used compounds were expressed as following indices: Index of adhesion (IA) calculated as mean number of attached microbes per one eukaryotic cell; Percent of affected cells of the monolayer (AC%); Microbial contamination of the monolayer (MC) obtained as a product of IA•AC%; Percent of inhibition of bacterial adhesion (II%) calculated from the figures of IA with respect to the control.

Results: All preparations revealed more or less significant inherent antiadhesive activity in vitro, which became apparent in the reduction of all indices characterizing colonization of target-cells. Most effective proved to be the preparations with 1 hour pre-incubation with clinical isolate of *S. aureus* which protected fibroblasts against the bacterial adhesion in more than 92% in this case.

Conclusion: The protective effect of NHI and Intraglobin disappeared with addition of Protein A, collagenasum or hyaluronidasum to nutrient medium but remained the same for Pentaglobin. According to its specific polymeric structure and prospective form as well as content of IgM Pentaglobin possesses much more antiadhesive activity in the presence of Protein A, collagenasum or hyaluronidasum. In the presence of Protein A and enzymes IgM makes the antiadhesive activity of Pentaglobin irreversible.

R1974

EDTA (ethylenediamine tetraacetic acid) enhances the activity of tetracycline in *P. aeruginosa* by increasing drug accumulation

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Objectives: In this work we investigated the potentiating effect of EDTA on tetracycline, versus resistant *P. aeruginosa* strains.

Methods: We first assessed, through MIC determination, the effect of EDTA/tetracycline association on two tetracycline resistant *P. aeruginosa* strains. Next, through fluorescence microscopy, we evaluate the accumulation of tetracycline inside bacterial cells in presence or not of EDTA; moreover, pre-exposing cells to EDTA and successively (after washing them) to tetracycline, we evaluate if differences in tetracycline accumulation in presence of EDTA could be due only to its effect on the bacterial cell wall.

Results: Data show as EDTA, at sub-MIC concentrations is able to reduce the MIC for tetracycline. Fluorescence microscopy shows as cells, in presence of EDTA, accumulate much more tetracycline inside them respect cell directly exposed to tetracycline alone and also respect cells pre treated with EDTA and successively exposed to the drug.

Conclusion: Thus we argue that EDTA is able to improve tetracycline MICs of resistant *P. aeruginosa* strains, not only permeabilizing the external cell wall, but increasing in other ways the bacterial tetracycline accumulation, perhaps interfering with the drug extrusion machinery.

R1975

Repeated in vitro exposure of *Clostridium difficile* to metronidazole, vancomycin and linezolid does not result in resistance

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Objective: *Clostridium difficile* remains the major cause of nosocomial diarrhoea. First line therapeutics for the treatment of *C. difficile* associated diarrhoea are metronidazole and vancomycin. Meanwhile impaired susceptibility was observed for both substances, however not directly linked to treatment failures. On the other hand, as many as 20% of patients had at least one recurrence of CDAD after the initial therapy was

discontinued. Routine susceptibility testing is performed in few and specialized laboratories only. Therefore data on activity of both substances are limited. The potency of resistance development against vancomycin and metronidazole is not known. Linezolid is a new antimicrobal agent active against gram-positive bacteria.
Methods: Selection of resistant mutants of the ATCC strain 43255 (MIC in mg/l for metronidazole, vancomycin, linezolid: 0.064, 0.5, 0.75) was performed on supplemented agar plates containing increasing amounts of the antimicrobials. Bacteria collected from five agar plates (after 48 h growth) were collected and centrifuged. The pellet was re-suspended and exposed to the next higher antimicrobial concentration on agar plates.
Results: Repeating this procedure several times 7, 2 and 3 generations of bacteria for metronidazole, vancomycin and linezolid were generated, respectively. The bacteria were finally exposed to 5 mg/l metronidazole 2.0 mg/l vancomycin and 5 mg/l linezolid. Using Etest MHC values of 1.5 (23 × MHC), 2 (4 × MHC) and 2 (2.7 × MHC) mg/l could be confirmed.
Conclusion: Higher MHC values but not resistance could be selected in vitro in *C. difficile* against metronidazole, vancomycin and linezolid. This confirms the clinical observation that *C. difficile* bacteria resistant to metronidazole or vancomycin do not attribute to therapeutic failures in the treatment of CDAD.

R1976

Susceptibility rates of pathogens isolated from ICU and haematology patients against different antibiotics recommended for empiric treatment

B. Grabein *(Munich, D)*

Objectives: Infections in the ICU and haematology unit are a main risk factor for mortality due to inadequate antibiotic treatment. In this study we evaluated the in vitro activity of different broad spectrum antibiotics. Cefepime, imipenem, meropenem and piperacillin/tazobactam are recommended treatment options in international and German guidelines for treatment of severe infections in ICU or haematology patients. Local data regarding epidemiology and resistance rates should be considered in treatment decisions.
Methods: Over a period of three month in 2000, 2001, 2002 and 2003 pathogens isolated from patients in the ICU or haematology unit have been collected. In vitro activity was investigated by broth microdilution according to NCCLS standards. Reference test strains were *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212.
Results: The strains were mainly isolated from lower respiratory tract (tracheal aspirate, sputum) followed by blood and urine. In total 160 isolates of enterobacteriaceae, nonfermentive bacteria (*P. aeruginosa* and *Acinetobacter* spp.) and staphylococci (MSSA, MSSE) have been isolated in the year 2003 and in vitro activity of piperacillin/tazobactam (P/T), ceftazidime (CAZ), cefepime (CEP), imipenem (IMI) and meropenem (MERO) has been determined by MIC-testing. Differences in susceptibility rates regarding enterobacteriaceae (n = 71) were seen in *Enterobacter* spp. (CEP 100%, IMI 100%, MERO 100%, P/T 72% and CAZ 67%) and *Klebsiella* spp. (CEP/IMI/MERO 100%, CAZ 95%, P/T 86%). In nonfermentive bacteria (n = 38) *P. aeruginosa* and *Acinetobacter* spp. susceptibility rates were highest for MERO (86%, 100%) followed by CEP (86%, 94%) IMI (76,%, 100%), CAZ (81%, 94%) and P/T (71%, 94%). Susceptibility of *P. aeruginosa* against IMI decreased from 100% in the year 2000 to 76% in the year 2003. Susceptibility rates in other bacteria remained mainly stable over the years regarding all antibiotics. In methicillin susceptible staphylococci (n = 51) IMI, MERO, CEP and P/T showed excellent activity (MIC90?1 mg/l), whereas CAZ activity was significantly lower (MIC90 8 mg/l).
Conclusion: In our investigation regarding enterobacteriaceae, nonfermentive bacteria (*P. aeruginosa*, *Acinetobacter* spp.) and staphylococci (MSSA, MSSE) isolated from ICU and haematology patients susceptibility rates where highest for MERO and CEP followed by IMI, CAZ and P/T.

R1977

Carbapenem comparisons in *P. aeruginosa* isolated from Brazilian hospitals: MYSTIC Program Brazil 2004

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Objective: To compare the susceptibility patterns of *P. aeruginosa* clinical isolates for imipenem and meropenem in the MYSTIC Program Brazil 2004.
Methods: Four hundred and thirty-three *P. aeruginosa* clinical isolates were collected by 18 centres participating in the 2004 MYSTIC Program edition in Brazil. Minimum inhibitory concentrations (MICs) were determined to imipenem, meropenem and another seven antimicrobials at a central laboratory by E-test methodology according to manufacturer's instructions. Interpretations followed the NCCLS document M100-S14. Susceptibility and cross-resistance rates to both carbapenems were calculated.
Results: *P. aeruginosa* clinical isolates (n = 433) showed 45% susceptibility (S), 3.2% intermediate (I), and 51.8% resistance (R) to meropenem (MIC50 = 16 mg/L). As for imipenem (MIC5 = 32 mg/L), there were 39.3% S, 2.5% I, and 58.2% R. Of the 252 imipenem-resistant isolates, 23 (9.1%) were susceptible and 10 (4%) were intermediate to meropenem. Of the 224 meropenem-resistant isolates, 3 (1.3%) were susceptible and 2 (0.9%) were intermediate to imipenem.
Conclusions: Although decreased susceptibility rates were observed for both carbapenems, meropenem resistance rate was lower than the detected for imipenem. Furthermore, some imipenem-resistant isolates were still susceptible to meropenem, while the opposite was less frequent. However, biased sample cannot be ruled out, since centres are carbapenems users by study design requirements and isolates could have been overvalued due to their resistance patterns.

R1978

Activity of meropenem against Gram-negative isolates from the intensive care unit – Part of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Programme, 1997–2004

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Objective: The antimicrobial resistance of nosocomial isolates, especially from intensive care units (ICUs), is increasing worldwide. The aim of this analysis was to assess the in vitro activity of meropenem (MEM) and eight other antibiotics against Gram-negative isolates from a paediatric ICU.
Methods: Eight hundred and eighty Gram-negative isolates were obtained from clinical specimens of children hospitalized in ICU during 1997–2004. The isolates were identified using conventional methods. The Minimum Inhibitory Concentrations (MICs) of MEM, imipenem (IMP), piperacillin + tazobactam (TAZ), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPE), gentamicin (GM), tobramycin (TM) and ciprofloxacin (CIP) were determined using the NCCLS agar dilution method.

Results: The collection of Gram-negative isolates included *Escherichia coli* (n = 127), *Enterobacter cloacae* (n = 173), *Klebsiella oxytoca* (n = 71), *Klebsiella pneumoniae* (n = 151), *Serratia marcescens* (n = 37), *Acinetobacter baumanii* (n = 79), *Pseudomonas aeruginosa* (n = 178) and other species (n = 64). MEM, IMP and CIP were most active (>90% susceptibility) against the tested isolates. The overall order of activity of beta-lactam antibiotics was MEM >IPM > CPE > TAZ > CAZ > CTX. The MIC90 (mg/L) of MEM was nearly identical in 1997 and 2004. It was equal to 0.06 for Enterobacteriaceae, 1.0 for *A. baumanii* and 8.0 for *P. aeruginosa*. The MIC90 (mg/L) of IMP was equal to 0.25 for Enterobacteriaceae, 1.0 for *A. baumanii* and 16 for *P. aeruginosa*. During 1997–2004, among Enterobacteriaceae were only found three isolates (two *E. cloacae* and one *E. amnigenus*) resistant to carbapenems. Among IPM-resistant *P. aeruginosa* isolates no metallo-beta-lactamase producing strains were found. Susceptibility to GM and TM characterized 63.7% and 61.8% of the tested isolates, respectively. The incidence of extended-spectrum beta-lactamases and AmpC beta-lactamases producing isolates among Enterobacteriaceae decreased from 68.5% in 1997 to 35.4% in 2004.
Conclusions: MEM, IMP and CIP were the most active antibiotics (>90% susceptibility) against the tested strains, with no observed reduction in activity over 8 years. A greater proportion of *P. aeruginosa* isolates were susceptible to MEM compared to IPM.

R1979

Comparison of in vitro activity of cefepime alone or in combination with sulbactam against clinical strains of multi-drug resistant *Acinetobacter* spp.

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Objectives: With the increase in multi-drug resistant *Acinetobacter* spp. (MDR-A) there is an urgency for unique and effective antimicrobial regimens. The objective of this study was to determine the effect of cefepime (C) alone and in combination with sulbactam (S) against MDR-A isolates in an in vitro pharmacodynamic model.
Methods: Three clinical strains of MDR-A: 15-11270A; 86-3473 and 15-2016 were used. A previously described in vitro PD model was used to evaluate a variety of antibiotic regimens using a starting inoculum of 1×106 CFU/ml over 72 h. C dosing was simulated at 2 g per dose, where S was given as a 2:1 or 1:1 ratio concentration. Regimens evaluated included dosing every 8 or 12 h, along with continuous infusion (CI) individually or combined. When combined, the activity was evaluated for drug ratios oft both 2:1 and 1:1 of C to S.
Results: MIC testing against C and S for 15-11270A, 86-3473 and 15-2013 were 32, 32 and 4 μg/ml and 2, 8, and 2 μg/ml, respectively. Simulations utilizing single agents resulted in an initial drop in inoculum, however, regrowth up to the original organism density was demonstrated irregardless of dosing regimen. Combination regimens of C+S resulted in a greater kill at 72 h compared to individual agents. The pharmacodynamics in combination appeared to increase as a function of the dosing interval. When compared to the most effective single regimen, CI(1:1) against 15-11270A showed additivity (>1.5 log kill), whereas C+S every 8 h against 86-3473 resulted in synergistic killing (>2.5 log kill). Resistance at either 2 or $4 \times$ MIC was demonstrated throughout these models irregardless of regimen or drug, however with few exceptions, individual MICs did not vary at 72 h compared to baseline.
Conclusion: Combination of C and S demonstrated additivity or synergy against MDR-A. Improved activity appeared to be a function of the dosing interval, for instance the shorter intervals such as every 8 h or CI were more effective. Further investigation of C + S combination treatment for MDR-A is warranted.

R1980

Comparative in vitro activity of tigecycline and 14 other antimicrobial agents against 205 clinical isolates from a university general hospital in Athens, Greece

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Background: Continuous rise of resistance rates among nosocomial isolates create an urgent need for new antimicrobial agents potent against multiresistant pathogens. Tigecycline (TIG) is a novel glycylcycline with an expanded spectrum of activity against both Gram-negative and Gram-positive bacteria resistant to many other compounds. We compared the activity of TIG to that of 14 other broad-spectrum agents against a variety of pathogens isolated in the Infectious Diseases Research Laboratory in University General Hospital ''Attikon'' in Athens, Greece.
Methods: A total of 144 consecutive clinical isolates collected between January and October 2004 were included in the study. Only one isolate per species per patient was permitted. Isolates derived from various clinical sources such as blood, pus, sputum or bronchial secretions, bone and urine. MIC's were determined by the microdilution method according to NCCLS. Microtiter plates were provided by Wyeth Pharmaceuticals and were inoculated using a RENOK system, as instructed by the manufacturer. Methicillin resistance was determined by the cefoxitin disk diameter, as recommended by the NCCLS. For another collection of 61 multiresistant Gram-negative strains isolated the past 6 months from ICU patients, susceptibility to TIG was determined by the disk diffusion method and interpreted according to the manufacturer's recommendations.
Results: MIC90 (mcg/ml) of some of the tested compounds were as follows: Of 61 ICU isolates 26 (42.6%) were resistant to all currently available agents except colimycin. Eighteen of these strains (69.3%) were susceptible to TIG. TIG was active against isolates resistant to all other available agents.

Organism (n)	TIG	A/C	PTZ	LEVO	CAZ	FEP	IMI	VA
MRSA (18)	0.25	NA	NA	4	NA	NA	NA	1
Enterococcus spp (14)	0.12	>8	>16	32	NA	NA	>16	2
E.coli (19)	0.5	16	4	>8	<8	8	0.25	NA
Enterobacter spp. (24)	4	>32	128	8	>32	32	4	NA
Klebsiella spp. (20)	2	>32	>128	>8	>32	>32	8	NA
Serratia spp. (10)	2	>32	4	2	<8	0.4	1	NA
Acinetobacter spp. (20)	1	>32	>128	>8	>32	>32	>16	NA
P.aeruginosa (19)	>16	NA	>128	>8	>32	>32	>16	NA

TIG; Tigecycline, A/C; Amoxycillin/Clavulanic acid, PTZ; Piperacillin/Tazobactam, LEVO; Levofloxacin, CAZ; Ceftazidime, FEP; Cefepime, IMI; Imipenem, VA; Vancomycin, NA; Not applicable.

Conclusions: TIG is a potent compound with a wide spectrum of activity against Gram-positive and Gram-negative problematic bacteria, with the exception of *P. aeruginosa*. It could be an alternative to colimycin against multiresistant isolates causing infections in ICU patients.

Mechanisms of action and of resistance of antimicrobial drugs

R1981

The antibiotic resistance and metallo-β-lactamase production of carbapenem-resistant pseudomonas and acinetobacter

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Objectives: The betalactamase resistance mechanism are, outer membrane protein mutation, efflux pumbs and metallo-betalactamases (MBL). MBL enzymes which uses Zinc ion can hydrolyse betalactamase antibiotics and are usually found in Enterobactericeae ve and especially in *Pseudomonas aeruginosa* like nonfermentative bacteria. The genes encoding MBL are named as IMP, VIM, SPM and GIM, usually transmitted via plasmids. Their importance are that they hydrolyse all betalactam and betalactam inhibitor combinations except aztreonam. Especially the abundance of strains producing MBL in hospitals must be evaluated with care.
Methods: In our study carbapenem resistant 13 *Pseudomonas aeruginosa* and 10 *Acinetobacter* spp. strains isolated from various clinical samples sent to our clinical microbiology laboratory were tested for MBL production by MBL E test and for their susceptibilities for ciprofloxacin (CIP), gentamicin, amicasin, aztreonam, piperacillin-tazobactam (TZP), ve cephaperazon-sulbactam by disk diffusion test.
Results: Out of Carbapenem resistant 23 strains 21 were found MBL positive with E Test method. 23 strains were sensitive 29% for TZP, 28% for CIP, 17% for gentamicin, 19% for amikacin and 18% for cephaperazon sulbactam. Carbapenem resistant strains were mostly resistant also for the other antibiotics.
Conclusion: As a result carbapenem resistance in Gram negative aerob and anaerobe bacteria due to MBL is a popular but difficult problem to solve.

R1982

Emerging resistance of bacterial strains causing complicated and uncomplicated urinary tract infections to current antimicrobials

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Objective: The aim of the current study was to highlight some of the susceptibility patterns of bacterial pathogens responsible for complicated and uncomplicated urinary tract infection (UTI) and to assess the prevalence of antimicrobial resistance among such uropathogens.
Methods: Ninty-nine patients were divided into two groups: 58 with complicated and 41 with uncomplicated UTI. All urine specimens revealed significant bacteruria. Gram negative and positive isolates were identified to the species level. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion for all isolates. Detection of extending spectrum betalactamases (ESBLs) producing organisms was performed by screening test and oxoid combination test (CDO2). Also detection of methicillin (oxacillin) resistant (MR) Staphylococci was performed by oxacillin disc diffusion assay. Resistance was confirmed by measuring minimal inhibitory concentrations (MIC)by E test.
Results: *Escherichia coli* (*E. coli*) recorded a significant higher rate of isolation among uncomplicated UTI group (53.7%) than that of complicated group (24.1%). Gram negative isolates other than *E. coli* was 41.3% of the complicated UTI. Gram positive isolates were 32.3% with higher percentage among complicated UTI (34.4%). *E. coli*, *Enterobacter* and *Citrobacter* spp. isolates of uncomplicated UTI group were susceptible to commonly used drugs as amoxacillin/clavulanate, nitrofurantoin, aminoglycosides, cepholosporins and quinolones. While *Klebsiella* and *Enterobacter* spp. of complicated group were absolutely resistant to trimethoprim/sulfamethazole (MIC 16–32 μg/ml) and recorded low rate of sensitivity to amoxacillin/clavulanate and ampicillin/sulbactam (MIC > 256 μg/ml). ESBLs-producing organisms were 23.8%, 75% of which belong to complicated UTI. All *S. aureus* of both groups were susceptible to ampicillin, beta-lactamase inhibitors, aminoglycosides and cephalosporins (75%). Among Staphylococcus isolates 36.4% were MR *S. aureus* (MRSA) and MR *S. epidermidis*. Imipenem and meropenem remained the most potent antibiotic (100%) to Gram negative and positive isolates.
Conclusion: Antibiotics commonly used in UTI are still effective particularly in community acquired uncomplicated UTI. Antimicrobial resistance is more common when complicated factors are present. So, it is crucial to have regular monitoring of such resistance to make reliable information available for optimal empirical therapy for patients with UTI.

R1983

High frequency of mutator strains in multiresistant *Pseudomonas aeruginosa* in cystic fibrosis patients from Germany

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Objectives: *Pseudomonas aeruginosa* infections are the major cause of morbidity and mortality in cystic fibrosis (CF) patients. Rates of multiresistance among *Pseudomonas aeruginosa* strains isolated from CF patients are substantially higher than among *Pseudomonas aeruginosa* strains from other settings. Mutator strains are characterized by increased mutation rates mainly due to defects in genes involved in the DNA mismatch repair system. In a previous study a link between high resistance rates in CF patients and the presence of a high portion of mutator *Pseudomonas aeruginosa* strains was found. The objective of our study was to investigate the proportion of mutators among multiresistant *Pseudomonas aeruginosa* from CF patients in Germany.
Methods: Multiresistant *Pseudomonas aeruginosa* strains from CF patients were collected in a German hospital in 2004. Only one isolate per patient was permitted. Multiresistance was defined as reduced susceptibility (intermediate or resistant according to DIN guidelines) to at least four of the following class representatives: imipenem, ceftazidime, piperacillin, ciprofloxacin and gentamicin. The detection of mutators was performed by disk diffusion. Mutation frequencies were determined in triplicate on selective rifampicin agar plates. As previously defined, strains were designated hypermutable when the mutation frequency was at least 20-fold higher than that of the wildtype strain PA01.
Results: A total of 26 multiresistant *Pseudomonas aeruginosa* strains was collected. Of those 12 (46%) had increased mutation

rates ranging from 5.85×10^{-7} to 1.43×10^{-5}. Eight mutator strains showed reduced susceptibility to all five and four mutator strains to four class representatives.
Conclusions: Comparable to a previous study, in Germany the rate of mutator strains is also extremely high among multiresistant *Pseudomonas aeruginosa* isolates from CF patients. Our data underlines the link between hypermutation and the evolution of antibiotic resistance in CF patients.

R1984

Mechanisms involved in carbapenems resistance among isolates of *Pseudomonas aeruginosa* strains

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Objectives: The aim of the study was to determine the role of the permeability as well as metalloenzyme production in carbapenems resistant *P. aeruginosa* strains.
Method: Thirty strains of *P. aeruginosa* determined as resistant to one or two carbapenems (imipenem, meropenem) by disc diffusion (NCCLS) and E-test (AB Biodisk) methods were enrolled in this study. MICs of imipenem (IPM), meropenem (MEM), erythromycin (E), gentamicin (GM), cefoperazone (CFP), norfloxacin (NOR), piperacillin (PIP), tetracycline (T), chloramphenicol (C), EDTA, sodium hexametaphosphate (NaHMP) were determined by agar dilution method according to NCCLS. To estimate the role of the permeability MIC values were also determined in the presence of different concentrations of outer membrane (OM) permeabilizers: EDTA (0.1 mM, 1 mM, 2.5 mM) and NaHMP (100 mM, 200 mM, 300 mM). Detection of metallo-β-lactamases was performed by disc diffusion method using 2-mercaptopropionic acid and EDTA (as metalloenzyme inhibitors) as well as PCR using specific primers bla IMP1-A 5'- ACC GCA GCA GAG TCT TTG CC -3' and bla IMP1-B 5'- ACA ACC AGT TTT GCC TTA CC-3'.
Results: Among 30 *P. aeruginosa* studied strains 22 were resistant to two carbapenems while 8 were resistant only one of them. Twenty-seven strains were resistant to GM, 26 to T, 18 to NOR, 17 to CFP. EDTA and NaHMP decreased MIC of E from 2- to 32-fold and from 2- to 8-fold, respectively, MIC of GM from 2- to 16-fold and from 2- to 4-fold, MIC CFP from 2- to 64-fold and from 2- to 64-fold, NOR only 2- to 8-fold and 2- and 2-fold, MIC of PIP from 2- to 4-fold and from 2- to 4-fold, MIC of T from 2- to 8-fold and from 2- to 8-fold, MIC of C from 2- to 8-fold and from 2- to 16-fold. Only 1 strain of *P. aeruginosa* resistant to CFP, 2 strains resistant to PIP and 1 resistant to T became susceptible after treating with EDTA and/or NaHMP. Four of the 30 studied strains were detected to produce metallo-β-lactamases by disc diffusion method. PCR revealed that none of them was carring the bla IMP gene.
Conclusions: 1. Our results suggest that other metalloenzyme (VIM) are involved in carbapenem-resistance in studied strains. 2. Influence of EDTA and NaHMP on MIC decreased may indicate the role of permeability and efflux mechanisms in carbapenem resistance.

R1985

Incidence of inducible clindamycin resistance in staphylococci: first results from Turkey

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Objective: To determine the incidence of constitutive and inducible clindamycin resistance in a university hospital.
Methods: One hundred and five methicillin-resistant *Staphylococcus aureus* (MRSA), 111 methicillin-sensitive *S. aureus* (MSSA), 192 coagulase-negative staphylococci (CoNS) (94 methicillin-resistant) collected from consecutive clinical isolates were studied. Isolates that were clindamycin-sensitive (CL-S) but erythromycin-resistant (E-R) were tested for inducible resistance by the D-test. CL and E disks were placed 15 mm apart from centre to centre on Mueller–Hinton agar plates and the D-shaped inhibition zones seen on the plates were interpreted as D-test positive.
Results: Inducible clindamycin resistance was detected in 6 (5.7%) of 105 MRSA isolates, 4 (3.6%) of 111 MSSA isolates, 29 (30.8%) of 94 MR-CoNS isolates and 11 (11.2%) of 98 MS-CoNS isolates. All MRSA strains that have ER-R CL-S phenotype were D-test positive. The same results were obtained by using azithromycin instead of erythromycin. All 408 staphylococcal isolates tested were found to be susceptible to quinupristin–dalfopristin.

Table 1. Incidence of MLSBi and MLSBc among staphylococcal isolates

Phenotype	No. (%) of MRSA isolates	No. (%) of MSSA isolates	No. (%) of MR- CoNS isolates	No. (%) of MS- CoNS isolates
ER-S CL-S	32 (30.4)	98 (88.2)	10 (10.6)	58 (59.1)
ER-R CL-R	67 (63.8)	5 (4.6)	43 (45.8)	14 (14.3)
ER-R CL-SD$^+$	6 (5.7)	4 (3.6)	29 (30.8)	11 (11.2)
ER-R CL-SD$^-$	0 (0)	4 (3.6)	12 (12.7)	15 (15.3)
Total	105	111	94	98

Conclusion: Macrolides, lincosamides and streptogramins are commonly used in the treatment of staphylococcal infections. Increasing frequency of MRSA infections and the changes in the antimicrobial susceptibility patterns have led to renewed interest in the use of clindamycin in the treatment of such infections. Clinical microbiology should report in vitro inducible clindamycin resistance (MLSBi type) in staphylococci to alert phyisicians about the potential treatment failures due to this resistance pattern.

R1986

Detection of gyr A gene mutations in quinolone-resistant clinical isolates of *Pseudomonas aeruginosa*

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Objectives: To investigate the frequency of resistance of *P. aeruginosa* to fluoroquinolones and to detect gyrA gene mutations in quinolone-resistant *P. aeruginosa* isolates obtained from discharge of sinuses, middle or external ears of patients.
Methods: The 126 isolated strains were tested for susceptibility to various antibiotics and those resistant to ofloxacin were tested for point mutations in the gyr A gene using single strand conformational polymorphism analysis (SSCP, and by sequencing in 2 selected strains.
Results: Twenty-eight (22.2%) out of the 126 isolates were resistant to ofloxacin. Point mutation in gyrA gene was not detected in any of the 28 quinolone resistant strains by SSCP. However, by direct sequencing of the PCR- amplified gyrA fragment from two clinical isolates, single mutation in which, threonine at position 83 was substituted by an isoleucine, was detected in both cases.

Conclusion: SSCP was not a perfect method in the detection of gyrA gene mutation (s) in clinical isolates of *P. aeruginosa*. Confirmation of SSCP by direct sequencing, or modification of the usual SSCP analysis ought to be considered in future studies.

R1987

Effects of a mixture of antibiotics on a resistant bacterial strain during sewage treatment

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Introduction: Recent research has shown that antibiotics are present in the influent of municipal sewage treatment plants (STP). Few data are available on elimination rates and effects of antibiotics on bacteria present in the aeration tanks of sewage treatment plants.
Methods: We investigated the fate and effects of antibiotics during biological sewage treatment under standardized conditions in laboratory scale sewage treatment plants (LSSTPs). The plants were fed with a mixture of antibiotics whose composition reflected the annual average input of antibiotics into German municipal STPs. Two different concentration levels were applied. In order to study its fate under antibiotic pressure, a multi-resistant strain of *Acinetobaer baumannii* was added in high concentration to some of the plants. The fate of the antibiotics and of the multi-resistant strain were monitored by chemical and microbiological means.
Results: The antibiotics were not fully eliminated in the LSSTPs. After 2 weeks, the multi-resistant strain of Acinetobacter was no longer detectable by classical microbiological, chemotaxonomical and PCR-based methods.

R1988

Evaluation of various conventional methods for detection of methicillin resistance in *Staphylococcus aureus*

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Methicillin resistant *Staphylococcus aureus* (MRSA), is a major cause of nosocomial and community-acquired infections throughout the world. Early detection of MRSA, is important for both the treatment of the infected patients and the institution of appropriate infection control measures. However, determination of methicillin resistance is often difficult because of the heterogenity of the resistance expression. In this study, various conventional methods were compared with polymerase chain reaction (PCR) for determination of oxacillin resistance. A total of 160 clinical isolates of *S. aureus* were tested for oxacillin resistance by disc diffusion, MRSA screen latex agglutination test (Denka Seiken Co., Ltd., Tokyo, Japan), and the ATB Staph System (bioMerieux, Marcy-l'Etoile/France). The MIC value of oxacillin was also determined with broth microdilution method in 115 isolates. MecA gene was detected in 87 (54.4%) of 160 strains and 73 (45.6%) isolates were mecA negative with PCR. Disc diffusion method failed to detect 1 mecA positive strain and identified 2 mecA negative strains as oxacillin resistant. MRSA screen test and the ATB Staph System detected 85 and 86 of the 87 mecA positive strains; respectively. They also identified 4 and 8 of mecA negative strains as oxacillin resistant; respectively. The oxacillin MIC values were ≥ 4 μg/ml in 57 of 58 mecA positive *S. aureus* isolates; and ≤ 2 μg/ml in 56 of 57 mecA negative isolates with broth microdilution method. The sensitivities of disc diffusion method, MRSA screen test, Staph ATB System and broth microdilution method were 98.8%, 97.7%, 98.8%, and 98.2%; and the spesicificities were 97.2%, 94.5%, 89%, and 98.2%; respectively against PCR as gold standard. The differences in sensitivities or specificities were not statistically significant ($p > 0.05$).

R1989

Development of antibiotic resistance and the role of differentiation in microbial biofilms

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Cells attached to surfaces in biofilms can be more tolerant to various anti-microbial agents than their planktonic counterparts. In biofilm environments the cells have the ability to differentiate and change phenotype leading to severe treatment problems in many cases of persistent or chronic infections. An approach addressing this issue identified specific resistant subpopulations in *Pseudomonas aeruginosa* biofilms and indicated that differentiation plays an important role in this context. Biofilms were established in flow-chambers, and their development and responses to addition of colistin (a polymyxin) were monitored by confocal scanning laser microscopy and time-lapse recording together with use of live/dead staining. Initial results indicate that modification of LPS is an important factor in relation to colistin tolerance development and may be the initiator of cell differentiation as pmr mutant biofilms of PAO1 result in sensitivity to colistin.Results indicated furthermore a regulatory connection between the composition of the cell envelope and cell motility (twitching, swarming) and in that way a correlation between motility and tolerance to anti-microbial agents. Tube biofilm experiments were set up for isolation of colistin treated and non treated biofilm material and further analysis of changes happening in the differentiating population by use of micro Array and LPS analysis.

Epidemiology of resistance, antibiotic usage

R1990

The evaluation of metallo-β-lactamase presence with E-test in five imipenem resistant five klebsiella strains

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Objectives: Metallo-β-lactamases (MBL), are responsible enzymes in resistance mechanisms of Enterobactericeae family and nonfermentative Gram negatives. This resistance phenotype spreading via plasmids, can be shown with E-test strips in which on end is Imipenem and the other end is EDTA+ Imipenem. If the MIC values for Imipenem are 8-fold more then the MIC values for EDTA+ Imipenem, it can be said that this strain is producing MBL.
Methods: The isolated Imipenem resistant strains obtained from various clinical materials are tested for MBL production via E-test method. The strains were isolated in four patients in

the Intensive Care Unit (ICU) and in one in the Internal Medicine Ward. The resistant strains were isolated one from blood in the patient in Internal Medicine Ward, two from tracheal aspirates and two from blood in the patients in ICU.
Results: All of these strains were ESBL positive with double disk synergy method. The resistant 1st, 3rd, 4th and 5th strains were also resistant or intermediate susceptible for *piperacillin tazobactam* and *cefopearazone sulbactam*. Only the 2nd strain which was metallo-β-lactamase negative was susceptible for *piperacillin tazobactam* and *cefoperazone sulbactam*. While four strains among five were positive for Metallo-β-lactamase presence.
Conclusion: In our country the presence of ESBL is an important problem. The use of carbapenems to solve this problem leaded to new problem, increase of carbapenem resistant Klebsiella strains. Due to of antibiotic usage politics, the carbapenem resistance due to MBL is now a popular problem which is yet not solved.

R1991

Point-in-time prevalence survey of utilisation of antibiotics in departments of surgical infection in Russia: HIT-04 study results

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Objectives: To assess trends of utilization of antibiotics in surgical infections' departments of multidisciplinary hospitals for evaluation of safety and efficiency of combinations.
Methods: Case histories of inpatients were evaluated from 23 August to 1 September 2004 in seven large Russian cities. Each department of surgical infection was screened within one day and all hospitalized patients were enrolled.
Results: Overall 300 case histories of hospitalized patients were analysed. Of them, 179 (59.7%) were males and 121 (40.3%) were females with age 51.8 + 17.2 years. Length of hospitalization at the moment of the study was 14.8 + 14.6 days. Of all patients 214 (71.3%) had undergone surgery. Surgical infection was marked in 228 (76%) of patients. Following infections were documented: skin and soft tissue 181 (79.4%), bone and joint 24 (10.5%) and intra-abdominal 23 (10.1%). Antibiotics were administered to 211 (70.3%) of patients. Monotherapy and combination therapy received 129 (61.1%) patients and 82 (38.9%), respectively. There were 309 cases of administration of antibiotics and 305 (98.7%) were given for the therapy and only 4 (1.3%) for the prophylaxis. Three most often prescribed classes of drugs for monotherapy were β-lactams 97 (75.6%), predominantly ampicillin 34 (26.4%) and oxacillin 26 (20.2%); followed by lincosamides (lincomycin) 17 (13.2%), aminoglycosides 8 (6.3%). To notice, aminoglycosides were prescribed to 51 (17.0%) of all patients who received antibiotics. Body weight was determined in 6 (11.8%) and serum creatinine was measured in 37 (72.5%) of patients receiving aminoglycosides. Among prevailing combinations were gentamicin plus oxacillin 12 (14.6%), lincomycin and oxacillin 11 (13.4%), metronidazole and ciprofloxacin 5 (6.1%), and ampicillin plus gentamicin 5 (6.1%) of cases. In total, aminoglycosides for a combination therapy were administered in 43 (52.4%) of patients.
Conclusion: Penicillins remain most often prescribed antibiotics for monotherapy and in combination with aminoglycosides. However surgeons often omit safety recommendations of administration of aminoglycosides. Further strategies to improve safety issues of antimicrobial therapy in surgery should be implemented.

R1992

Prolonged regional arterial antibiotics infusion in the management of severe acute pancreatitis

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Objectives: Development of the purulent-septic complications is the main cause of the death of patients with severe acute pancreatitis (SAP) after first week of the disease onset. The adequate antibiotics prophylactic is necessary part of the management of these patients. However, the disorders of microcirculation, which are typical for necrotizing pancreatitis, do not permit achieve the therapeutical concentration of antibiotics in inflamed pancreas. Due to that the continuous regional arterial infusion may have benefits in the management of patients with necrotizing pancreatitis.
Methods: Ninety-four patients with SAP were treated. In 25 patients the antibiotics (ceftriaxone, gatifloxacin and imipinem) administrated arterially. These patients compiled the first group and the rest patients – the control group.
Results: The Balthazar, Ranson (more than 3) score was not different in both groups at admission. The rate of contamination of the necrotic foci was compared. During the first week the contamination of the necrosis was noted in 5 (7.2%) patients of the control group. During the second week the purulent complications of SAP were noted in 15 (21.7%) of the control group and one patients (4.0%) of the first group, which received ceftriaxone.
Conclusion: The continuous intraarterial infusion obviously decreases the risk of contamination of the necrotic foci. Applying gatifloxacin in the complex management of SAP has a benefit in prevention of purulent complications.

R1993

Drug-resistance and drug-sensitiveness of bacterias causing urinary tract infections in women under general practice care in Silesian Province

I. Szymczyk, W. Lukas, J. Józwiak, M. Kidziolka, E. Mizgala *(Zabrze, PL)*

Objective: Estimation of etiological factors cousing UTI in women. Antibiogram analysis considering drugs used most frequently by GP. Optimalization of ' in vitro' bacterial disc choice, considering their usefulness in general practice.
Materials and methods: Two hundred and eighty six positive urine cultures in women > 18 years-old were analysed, in January–October 2004 period. Morning urine from central stream was sampled on Uromedium and was transported to own laboratory. Antibiograms with use of Becton-Dicinson discs for 23 antibiotics were performed.
Results: Most frequent pathogen of UTI was: *E. coli*-58%, *Staphylococcus aureus*-10%, *Proteus mirrabilis*-10%, *Pseudomonas aeruginosa*-3%. Remaining micro-organisms (Serratia, Morganella, Enterococcus, *Pseudomonas cepacia*, Citrobacter, Klebsiella, *Staphylococcus epidermidis* and *saprophyticus*) in 19% of cases were observed. The highest *E. coli* sensitivity for Ceftazidim-90%, Ciprofloxacin-88%, Amikacin-87%. Norfloxacin-85% and simultaneously highest resistance for Ampicilin-55%, Co-timoxazole-29%, Tobramicin-26% was observed. Highest *Staphylococcus aureus* sensitivity and resistance were observed for Neomicin-100%, Amoxicilin/clavulonic acid-84%, Nitrofurantoin-83% and Pipemidic acid-70%, Oxacilin-59%, Gentamicin-37%, Co-trimoxazole-37% respectively. Proteus mirabilis sensitivity for:

Amoxicilin/clavulonic acid-86%, Gentamicin-86%, Cefalotine-83% and resistance for: Azlocilin-64%, Nitrofurantoin-54%, Neomicin-52% were found. Taking under consideration all micro-organisms' drug-sensitivity we observed that most useful in general practice was Ciprofloxacin-78% of sensitivity, Norfloxacin –78% and Amoxicilin/clavulonic acid-68%.
Conclusions: In more than 50% of women under GP care the UTI etiological factor was *E. coli*. Fluoroquinolones and amoxicilin/clavulonic acid should be preferable drugs to use in GP, considering their wide-spectum activity. Considering costs, drug-sensitivity examination should be limited to antibiotics most frequently used in GP.

R1994

Extended spectrum β-lactamase producing *Escherichia coli* in a Kuwait hospital

A. Dashti, P. West, M. Johny, F. Habeeb, J. Kawaf *(Suleibikhat, Sharq, KWT)*

Objectives: Although initially found in *Klebsiella* spp. extended spectrum β-lactamases (ESBLs) are now far more frequently encountered in *Escherichia coli* in the Amiri Hospital, Kuwait. In this poster we present the results of our initial study of ESBL producing *E. coli*, including origin, antimicrobial susceptibility, PCR data for the detection of TEM and SHV β-lactamases and pulsed field gel electrophoresis (PFGE)results.
Methods: Clinical isolates were initially identified and tested for antimicrobial susceptibility using the Vitek 2. Isolates suggested by the expert system to harbour ESBLs were further tested by the disc approximation test and E-test using cefotaxime/clavulanate and ceftazidime/clavulanate. PCR was performed to detect the presence of TEM and/or SHV enzymes. *Xba*1 was used to restrict the chromosomal DNA.
Results: A total of seventy two isolates were tested. Sixty two strains were from 59 patients from the Amiri Hospital isolated between November 2003 and May 2004. Nine were from a neonatal ICU outbreak in another hospital in 1994, and one was a control strain. The 2003–2004 isolates originated from many different departments including casualty, outpatient, male, female and paediatric wards and the ICU, and 27/59 were patients ≥60 years. No outbreak was apparent during the collection period. Cefotaxime/clavulanate detected ESBLs in 71/72 (98.6%) of the isolates and cefipime performed best in the disc approximation test, detecting ESBLs in (46/72) 64%. Strains from 54/59 patients from 2003–2004 were resistant to ciprofloxacin, 41/59 to cotrimoxazole and 40/59 to gentamicin. All the 1994 isolates were ciprofloxacin and cotrimoxazole susceptible, but resistant to gentamicin. TEM enzymes were detected in 62/72 (86%) isolates, SHV in 36 (50%) and TEM and SHV in 25 (34.7%). PFGE of DNA fragments showed the presence of multiple genotype groups.
Conclusions: The epidemiology of ESBL producing *E. coli* in Kuwait is complex, with many apparently unrelated cases yielding different ESBL producing strains. The majority of ESBL producing strains are now resistant to ciprofloxacin. Sequencing studies will be required to determine the TEM and SHV types.

R1995

Emergence of fluoroquionolone-resistant clones of *Streptococcus pneumoniae* in far eastern Russia

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To assess the floroquimolones resistace in *S. pneumoniae* strains, further define molecular mechanisms of fluoroquinolones resistance in pneumococci strains at the territory of the Far East of Russia.
Methods: We identified and genetically characterized a library of clinical isolates (about 150 strains) of *S. pneumoniae* gained from 1999 to 2003 in Primorye. MICs of ciprofloxacin, moxifloxacin, gatifloxacin and levofloxacin were determined by the agar dilution method. PCRs were performed with appropriate primers.
Results: The overall per cent of fluoroquinolone resistant strains is approximately 6.0% (9 strains).The overall rank order of activity of the four fluoroquinolones examined in this study was moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin, in which moxifloxacin (MIC at which 90% of isolates are inhibited [MIC(90)], 0.25 μg/ml; modal MIC, 0.12 μg/ml) was twofold more active than gatifloxacin (MIC(90), 0.5 μg/ml; modal MIC, 0.25 μg/ml), which in turn was fourfold more active than either levofloxacin (MIC(90), 1 μg/ml; modal MIC, 1 μg/ml) or ciprofloxacin (MIC(90), 2 μg/ml; modal MIC, 1 μg/ml). The isolates were evaluated for reserpine-sensitive efflux of all tested fluoroquinolones. The isolates were typed using pulsed field gel electrophoresis. The majority of the isolates were genetically unrelated. Lower level fluoroquinolone resistance (ciprofloxacin MIC 2 μg/ml) was associated with amino acid substitutions in ParC, while higher level resistance (ciprofloxacin MIC > or = 16 μg/ml) was associated with amino acid substitutions in both ParC and GyrA. ParE substitutions were not associated with clinical resistance. Three of nine (30.0%) isolates demonstrated reserpine-sensitive efflux of ciprofloxacin. Efflux alone conferred low level ciprofloxacin resistance in 2 isolates.
Conclusions: The in vitro results suggest that recently developed fluoroquinolones are very effective against clinical isolates of *S. pneumoniae* isolates in Primorye. Nevertheless, emerging fluoroquinolone resistance should be acknowledged and clinicians alerted. Surveillance should be carried out to monitor any changes in antibiotic resistance of *S. pneumoniae*.

R1996

Italian survey of urinary tract infections: in vitro activity of prulifloxacin

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Objectives: During 2004 we performed a multicentric study in order to evaluate: a) the incidence of different uropathogens responsible of nosocomial, complicated and uncomplicate urinary tract infections (UTIs); b) the trend of antibiotic resistance of these uropathogens and c) the potency of a new fluoroquinolone, prulifloxacin, in comparison to ciprofloxacin and levofloxacin.
Methods: From February to May 2004, 9 microbiological laboratories thoroughly distributed in Italy collected up to 200 microorganisms from hospitalised patients (nosocomial UTIs), from patients in urology unit (complicated UTIs) and up to 40 microorganisms (20%) from ambulatory patients (uncomplicated UTIs). All the uropathogens collected were sent to Department of Microbiological Sciences of Catania (5 centres of south Italy) and Genoa (4 centres of north Italy). These reference laboratories determined the identity of the isolates and perform the antibiotic susceptibility tests by the agar dilution method, as described by NCCLS. ATCC references microorganisms were tested as quality control strains.
Results: A total of 1835 uropathogens were isolated. *E. coli* (37.0%) was the most isolated uropathogen from 1066 nosocomial UTIs, followed by *E. faecalis* (17.3%) and *P. aeruginosa*

(12.2%). *E. coli* (39.7%, 62.5%) was also the most isolated microorganisms from 358 complicated UTIs and 411 uncomplicated UTIs, respectively, followed by E. faecalis (20.1% and 8.0%). Against Enterobacteriaceae and Pseudomonadaceae, prulifloxacin showed an in vitro activity similar to that of ciprofloxacin and levofloxacin. However, prulifloxacin showed a better intrinsic activity (lower MIC values than other two fluoroquinolones). Whereas, the in vitro activity of prulifloxacin was equal to the other quinolones against Gram-positive uropathogens.
Conclusion: *E. coli* remain the principal uropathogen in all urinary pathologies and on the basis of in vitro activity, prulifloxacin could be an useful antibiotic in the treatment of any kind of UTIs.

R1997

Patterns of antimicrobial resistance in *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis* isolates from urinary tract infection: one-year study

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Objectives: To suggest empirical antibiotic treatment of urinary tract infections in children.
Methods: The sensitivity to antibiotics in 790 *E. coli*, 82 *Klebsiella* spp and 72 *P. mirabilis* strains isolated from urine cultures of hospitalized and non hospitalized children from August 2003 to July 2004 was evaluated. All strains were tested for sensitivity to ampicillin, amoxycillin/clavulanic acid 1st, 2nd and 3rd generation cephalosporins, imipenem, nitrofurantoin, co-trimoxazole and aminoglycosides by Kirby–Bauer methodology according to the NCCLS recommendations. The MICs of resistant strains were also tested with VITEK II automated system (Biomerieux).
Results: 362 (45.8%) *E. coli* isolates were found sensitive to all antibiotics tested, with the same prevalence in hospitalized and non hospitalized patients. 108 (13.7%) strains were found resistant only to ampicillin, 50 (6.3%) only to co-trimoxazole whereas 96 (12.2%) to ampicilline and co-trimoxazole together. 36 (4.6%) strains were resistant to amox/clavulanic acid. Cefalosporins of 2nd and/or 1st generation revealed a resistance rate of 19%. As for aminoglycosides, they were active in 97.7% and nitrofurantoin in 98.9%. 30 (3.8%) *E. coli* strains revealed extended spectrum β-lactamase (ESBL) resistance. 24/30 (80%) of these strains, were isolated from hospitalized children in neonate, intensive care and oncology units or from children under antimicrobial prophylaxis suffering from urinary tract abnormalities. Multiresistant strains in more than three antibiotics were found to be 48 (2.3%). Among ESBL resistant strains, 9 (30%) were multiresistant. *Klebsiella* spp isolates were resistant to co-trimoxazole in 11/82 (13.4%), to amox/clavulanic acid in 6 (7.3%) and to cephalosporins of 2nd and/or 1st generation in 28 (34.1%). ESBL resistance was found to 16 (19.5%) strains derived mainly from hospitalized patients. 28 (34.1%) of *Klebsiella* spp strains were multiresistant. *P. mirabilis* isolates were resistant to ampicillin in 30/72 (41.7%), to co-trimoxazole in 9 (12.5%), to amox/clavulanic acid in 2 (2.8%) and to cephalosporins of 2nd and/or 1st generation in 7 (9.7%). Only 2 (2.8%) strains were multiresistant. No *E. coli*, *Klebsiella* spp and *P. mirabilis* strain was found resistant to imipenem.
Conclusions: Simple uncomplicated urinary tract infections may be empirically treated with amoxicillin/clavulanic acid (4.7% resistance) or co-trimoxazole (7.4% resistance). Aminoglycosides (2.7% resistance) may be added for complicated, serious cases.

R1998

Klebsiella pneumoniae harbouring CTX-M-15 extended-spectrum β-lactamase in Hungary

Á. Tóth, I. Damjanova, M. Gacs, J. Pászti, M. Füzi *(Budapest, HUN)*

Objectives: Since the 1990s the CTX-M type β-lactamases have constituted one of the most rapidly spreading extended-spectrum β-lactamase (ESBL) families. They have been detected in many countries of the world. The aim of this study was to genetically characterise the phenotypically ceftazidime susceptible and cefotaxime resistant ESBL-producing *Klebsiella pneumoniae* isolates.
Methods: Species identification was performed with BBL Crystal E/NF, and ESBL expression was confirmed with E test ESBL. CTX-M genes were amplified with PCR using consensus CTX-M primers. The conjugation experiments were performed using *Escherichia coli* J53-2 (rifampin resistant). The CTX-M genes in the transconjugants was detected with PCR. The amplifield products were analysed with RFLP, assigned to distinct groups on the basis of RFLP profiles and further analysed using specific primers. The amplified products were sequenced. The isolates were also investigated by phagetyping, genomic fingerprinting by ERIC-PCR and PFGE and plasmid profile analysis.
Results: Among 158 ESBL-producing *K. pneumoniae* strains submitted to National Center for Epidemiology over one-year period twenty strains were selected for further analysis on the basis of phenotypic results. They were isolated in nine geographically distinct hospitals across Hungary. By PCR amplification the CTX-M encoding genes were detected in all strains and they had identical RFLP pattern. Sequencing of the corresponding genes confirmed CTX-M-15 in selected isolates. Of 20 strains, 15 and 5 belonged to XIA and IA26 phage type, respectively. ERIC-PCR and PFGE analysis revealed clonal relatedness among all CTX-M-producers. A plasmid of about 130 kb was obtained from all transconjugants by plasmid extraction and CTX-M harbouring was confirmed by PCR.
Conclusions: This is the first description of *K. pneumoniae* harbouring CTX-M-15 β-lactamase in Hungary. Epidemiological typing suggested inter- and intrahospital clonal dissemination of these Hungarian CTX-M-producing *K. pneumoniae* strains.

R1999

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

R. Reynolds, M. Colman *on behalf of the BSAC Working Party on Bacteraemia Resistance Surveillance*

Objective: To monitor prevalence of established forms of resistance, detect emerging forms, and assess the activity of newer antimicrobial agents.
Methods: Bacteraemia isolates were collected from 25 laboratories in the UK and Ireland in 2003, excluding duplicates isolated within one week. MICs were determined centrally using the BSAC agar dilution method and interpreted by BSAC criteria. Results were compared with those from similar surveillance in 2001 and 2002.
Results: Among 235 *S. aureus*, 40% were methicillin-resistant (MRSA), similar to previous years. Of these MRSA, 98% were resistant to ciprofloxacin and 90% resistant to erythromycin. Of 212 coagulase-negative staphylococci, 58% were methicillin-resistant (MRCoNS), a significant reduction from 80% in 2001 and 72% in 2002. MRCoNS were also commonly resistant to ciprofloxacin (62%), erythromycin (98%) and trimethoprim

(77%). Overall, 10% of 245 enterococci were vancomycin-resistant (VAN-R), comprising 4% of 156 *E. faecalis* and 24% of 74 *E. faecium*, like earlier years. VAN-R *E. faecium* were often highly resistant to gentamicin (67%) and had high ciprofloxacin and erythromycin MICs. Penicillin non-susceptibility (PEN-NS) occurred in 8% of 239 *S. pneumoniae*, but, as in previous years, no MICs > 1 mg/L were seen. PEN-NS isolates were more commonly resistant to erythromycin (37%) and tetracycline (32%). Two were of serotype 35B, a serogroup not covered in current vaccines, making its first reported appearance in the UK. Prevalence of tetracycline (and minocycline) resistance in 68 group *B. streptococci* remained very high at 82%, but these isolates were highly susceptible to β-lactams, and tigecycline MICs were not raised. Activity of new agents against resistant isolates is shown in the table by mode and maximum MIC; all these distributions were unimodal.

MIC summaries, mg/L Resistant species	Ceftobiprole		Daptomycin		Linezolid		Tigecycline	
	Mode	Max	Mode	Max	Mode	Max	Mode	Max
MRSA, n = 95	2	4	0.5	1	2	4	0.25	2
MRCoNS, n = 122	1	4	0.5	1	1	2	0.25	2
PEN-NS *S.pneumoniae*, n = 19	not tested		0.25	0.25	1	2	0.06	0.25
VAN-R *E. faecium*. *n* = 18	not tested		1	2	2	2	0.12	0.25

Conclusion: Major 'headline' resistance rates have changed little since 2001, apart from a reduction in MR-CONS. The tested new agents had good activity against multi-resistant isolates. The arrival of PEN-NS *S. pneumoniae* of a non-vaccine serogroup (35B) could herald troublesome serotype replacement.

R2000

Setting up a surveillance system of antimicrobial resistance and use in a low-resource setting: West Delhi, India

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Objectives: To set up a surveillance system for antimicrobial resistance and use of antimicrobials in the community in a low resource setting using *E. coli* as an indictor organism.
Methods: The study area was 'Blocks' in a 10 Km. radius of Sir Ganga Ram Hospital, New Delhi, India. Microbiology Surveillance: MSU's were collected from all cases not taking antimicrobials and without suspected complicated UTI attending the OPD from designated region. Once *E. coli* (pathogenic as well as contaminants) were isolated and identified using conventional biochemical reactions its susceptibility was determined using Kirby Baur disc diffusion method on Muller Hinton agar. Antimicrobial use surveillance: Monthly purchase of all antimicrobial drugs from 30 private retail pharmacies was collected and converted to DDD. The denominator for the drug use was the population of the administrative areas in which these pharmacies are based. In addition, exit interviews (prescription auditing) were done at each pharmacy to have information on 15–25 consecutive prescriptions per month having any antimicrobial agent. Data on use is presented as DDD's per total number of patients/prescriptions.
Results: During the study period (11/03–9/04) 3590 MSU samples were collected and 377 *E. coli* were isolated. 85% of the strains were resistant to ampicillin, and more than 50% were resistant to quinolones (60%), tetracycline, third generation cephalosporins, cotrimoxazole and ampicillin + sulbactam. Purchase data and exit interview data was analysed for 9 months and the results showed that fluoroquinolones are the most frequently sold antimicrobials. Exit interview data revealed that DDD/1000 prescriptions/patients/month for fluoroquinolones; penicillins, cephalosporins and macrolides are 2972,1296, 1034,884 respectively.
Conclusions: The extensive usage of quinolones and penicillins are reflected in the antimicrobial resistance. A decrease in resistance to cephalexin from 85% to 41% could be noticed during the study period. The data collected on antimicrobial drug use and antimicrobial resistance in *E. coli* isolated from antenatal cases seems suitable for collection of information on the resistance and use in the community, valuable for planning targeted interventions. This is an on going project for one year.
Funding agency: WHO, Geneva.

R2001

Resistance to telithromycin in *Streptococcus pneumoniae* is rare and associated with reductions in bacterial fitness

D.J. Farrell, J.G. Hurdle, I. Chopra *(London, Leeds, UK)*

Objectives: PROTEKT - a global, longitudinal study of the antibiotic susceptibility of bacterial respiratory tract pathogens - has now completed its fourth year. The objectives of this study were to determine the global rates of bacterial resistance to telithromycin, the first ketolide antibiotic, and to evaluate the fitness of two telithromycin-resistant *Streptococcus pneumoniae* isolates relative to their putative isogenic telithromycin-susceptible counterparts.
Methods: Minimum inhibitory concentrations (MICs) for telithromycin were determined by the NCCLS broth microdilution method and interpreted using NCCLS breakpoints. The isogenic relationships between telithromycin-susceptible and -resistant isolates (two independent sets) were determined by serotyping and multi-locus sequence typing. The relative competitive fitness (W) of telithromycin-resistant strains was evaluated by established procedures involving mixed culture competition between isogenic pairs of telithromycin-resistant and -susceptible strains.
Results: Of the 20 750 *S. pneumoniae* isolates collected between 1999 and 2003 in the PROTEKT study, 0.1% exhibited low-level resistance to telithromycin (MIC ≥ 4 mg/L), with the highest MIC being 8 mg/L. This low rate of telithromycin resistance is observed across the globe, ranging from 0% in Australasia, the Middle East and North America to 0.22% in Central Europe. The development of resistance to telithromycin was accompanied by moderate reductions in W - 32% in one strain and 25% in the other.
Conclusions: Clinical resistance to telithromycin among *S. pneumoniae* is rare and low level, with a global prevalence of around 0.1%. In the two sets of isogenic strains examined, resistance to telithromycin was associated with moderate fitness cost (25–32%). These data suggest that the potential for *S. pneumoniae* to develop clinically relevant resistance to telithromycin may be limited. However, further studies are needed to examine the mechanisms of telithromycin resistance and the potential for genetic adaptation, which might reinstate bacterial fitness in telithromycin-resistant strains.

R2002

Administration of gentamicin in a Dublin teaching hospital

E. O'Neill, H. Humphreys, E. Smyth *(Dublin, IRL)*

Objectives: Appropriate use of antimicrobial therapy improves patient outcome and prevents the spread of antimicrobial resistance. It has been established that gentamicin should be administered at a dose of 5mg/kg in patients with normal renal

function and for a shorter duration of therapy to improve bactericidal killing and post-antibiotic effect whilst reducing the risk of toxicity associated with prolonged treatment. We performed a prospective audit of gentamicin usage in our institution over a one month period.
Methods: Patients with normal renal function receiving gentamicin were identified from Clinical Microbiology team ward rounds, review of drug prescription charts, consultation with staff nurses and gentamicin assays done in the microbiology department. A member of the Clinical Microbiology team reviewed patients on a daily basis. Details including patient demographics, indications for treatment, dose administered and duration of treatment were obtained.
Results: A total of 86 patients in our institution were on gentamicin therapy over the four week period with hospital acquired pneumonia (24.4%), intra-abdominal infection (23.2%) and neutropaenic sepsis (16.3%) accounting for the most common indications for therapy. 75% of patients were administered an inappropriate dose of gentamicin, of which 95% were being underdosed. One third of patients received a prolonged course of gentamicin therapy (i.e. longer than one week) in the absence of clinical or microbiological requirements.
Conclusions: This audit highlighted the high number of patients in our centre on inappropriate gentamicin therapy. In conjunction with the Microbiology and Pharmacy departments education sessions have been arranged for health care professionals to optimise gentamicin therapy and allow feedback on the findings of this audit.

R2003

Antibiotics susceptibility patterns of commonly isolated pathogens in a Greek university hospital

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Objective: Our aim was to assess the sensitivity to antibiotics of the most commonly isolated pathogens in blood cultures from patients admitted in a tertiary care hospital.
Materials and methods: We analysed the results of 858 positive blood cultures, during last year from patients admitted in Ahepa University hospital, focusing on their susceptibility to antibiotics. Identification of bacterial isolates and their antibiotic susceptibilities were performed by Vitek 2 analyzer of Bio-Merieux.
Results: The most frequent isolate was coagulase-negative staphylococci (CNS) (29%) followed by *Staphylococcus aureus* (16,9%), *Acinetobacter calcoaceticus* (12,8%), *Pseudomonas aeruginosa* (9,6%), *E. coli* (9,4%), *Klebsiella pneumoniae* (5,9%), and *Proteus* spp (3,5%). *Klebsiella* spp and *Proteus* spp. isolates were not significantly resistant to the antibiotics used. E. coli isolates were resistant as followed: 64% to ampicilline, 36 % to trimethoprime /sulfamethoxazole, 28% to amoxicillin/clavulanic acid (A/CA), 7% to cefuroxime, 19% to nitrofurantoin and 6% to ciprofloxacin (CIP). There was no resistance to ceftazidime (CFZ) and imipenem (IMI). Among *Pseudomonas aeruginosa* isolates 26% were resistant to CFZ, 28% to CIP, 22 % to IMI, 24% to tobramucin (TO) and there was no resistance to piperacillin/tazobactam (P/T). In terms of sites of isolation *Acinetobacter* spp were mainly isolated from surgical wards while *Staphylococcus epidermidis* spp from other clinics. 36% of *Acinetobacter* spp isolates examined showed resistance to ampicillin/sulbactame, 77% to IMI , 79 % to meropenem, 72% to P/T and 97% to CIP. 89% of CONS strains and 67% of *S. aureus* isolates were resistant to oxacillin. As far as glycopeptide resistance is concerned 88% of CONS isolates and 100% of *S. aureus* were sensitive to teicoplanin, while no VRSA or VRSE strains were isolated.
Conclusions: One third of the *S. aureus* isolated in our hospital were methicillin resistant (33% MRSA). Compared with previous results there has been observed a significant increase in resistance rates of *pseudomonas aeruginosa* to all antibiotics except ceftazidime, as well as in *Acinetobacter* spp to piperacillin/tazobactam.

R2004

Resistance to antibiotics and heavy metal in different species of *Aeromonas* collected from the Loa River in Chile

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Objectives: The Loa river, are the longest in Chile, and has been dramatically contaminated with heavy metals several times in recent years. The objective of this study was to determine the possible resistance to heavy metals as effect of these contaminations, as well as, the antibiotic resistance levels in *Aeromonas* spp. present in the Loa River.
Methods: Samples of waters and sediments were collected by duplicated from 22 stations through the river. The samples were analysed by the membrane filtration technique and the filters were incubated on ampicillin-dextrine agar (ADA), a selective medium for Aeromonas, at 30°C for 24 hours. Yellows colonies from the ADA and oxidase positive were presumed to be Aeromonas colonies. Eight of these colonies were typed by the PhP-AE rapid screening plate for *Aeromonas*. Selected PhP types were identified by biochemical test and typed for PFGE. The MIC for several antibiotics and heavy metals was done by a dilution agar technique.
Results: A total of 186 Aeromonas isolates belonging to 7 different species were recovered from the Loa River, including *A. hydrophila* (29.5%), *A. veronii* (22.4%), *A. caviae* (12.2%), *A. media* (11.5%), *A. sobria* (10.9%). The incidence of antimicrobial resistance in this Aeromonas isolates was relatively low, showing less than 10% of the isolates resistance to nalidixic acid, ciprofloxacino, cloranfenicol or tetracilina. However, all isolates were resistant to ampicillin. A high percentage of resistance to the heavy metals was observed. The MIC90 values for Lead and Arsenic were 3200 mcg/ml (ranges from 800 to 3200 mcg/ml). For Copper, the complete range observed was 200–1600 mcg/ml, and the MIC90 value was 1600 mcg/ml. In the case of Cadmium the population was distributed from 25 to >400 mcg/m, being the MIC90 400 mcg/ml. Genetic typing by PFGE-*Xba*I show high heterogeneity in the isolates. No differences were detected among the isolates recovered from the beginning and through the all way of the river.
Conclusions: The high metallic contamination could been contributed to the resistance to heavy metal of Aeromonas strains that habit in the Loa river.

Community-acquired infections

R2005

What factor will influence fever in community-acquired pneumonia?

D. Genné, R. Sommer, R. Rakotoarimanana, D. Lew *(La Chaux-de-Fonds, CH)*

Objectives: Although fever (> 38 °C) accounts for one of the major clinical signs of community-acquired pneumonia (CAP), it is not well studied in that disease.
Methods: We conducted a prospective observational study involving 228 adults patients hospitalized for CAP (diagnosed on IDSA guidelines) and measured their axillary body temperature 3 times a day until discharge. The causal pathogen, the empirical antibiotic choice and other factors that could influence the follow-up of fever were investigated.
Results: 162 (71%) patients requiring a hospitalisation for a CAP presented with fever. Although the level of fever decreased with age, it was not statistically significant. The mean duration of fever was 3 days (1–28). Except the presence, in one instance, of influenza virus which shortened the duration of fever, there were no differences related to the causal agent. A multivariate analysis showed that the presence of empyema and the empirical use of the quinolone trovafloxacin prolonged the duration of fever of 3 days (CI 95% 1.5–4.7, $p < 0.001$) and 0.7 days (CI 95% 0.09–1.30, $p = 0.024$) respectively.
Conclusions: Fever was absent in a significant proportion of patients (29%) hospitalised for a CAP. It should last for about 3 days for appropriately treated patients. For those with a febrile period exceeding 5 days, the presence of empyema should actively be searched.

R2006

Peripheral T-cell lymphoma masquerading as cellulitis

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Objectives: Primary cutaneous non-Hodgkin lymphomas (NHLs) encompass many subtypes of lymphoma, namely mycosis fungoides, lymphoblastic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, anaplastic large cell lymphoma and peripheral T cell lymphoma not otherwise specified. However, there are no reports of cellulitis due to lymphoma.
Methods: Description of our experience with a patient with peripheral T-cell lymphoma masquerading as cellulitis.
Results: A 28-year-old Greek-American male presented with extensive cellulitis of the left sub-axillary area. The patient had been evaluated in two hospitals abroad where he was advised to receive antibiotics, which did not lead to any appreciable improvement. A supraclavicular mass, thought clinically to be an enlarged lymph node, was removed at the second hospital. The biopsy showed inflammatory changes without any evidence for malignancy. Biopsy of a mass from the left axillary fossa, removed at our hospital, showed conclusive evidence for peripheral T-cell lymphoma.
Conclusion: A significant delay in the correct diagnosis was the result of a rare manifestation of lymphoma and limitations of the diagnostic work up, including biopsy of removed tissue. Clinicians should be aware that lymphoma may present with typical cellulitis-like lesions.

R2007

Regression of skin lesions of Kyrle's disease with clindamycin: implications for an infectious component in the aetiology of the disease

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Background: Kyrle's disease was first described in a 22-year-old woman with diabetes mellitus by J. Kyrle in 1916, under the name of hyperkeratosis follicularis et parafollicularis in cutem penetrans. It refers to a disorder of keratinization, which usually appears as scattered or grouped keratotic papules on the extremities and the trunk. Kyrle's disease belongs to a group of skin disorders called 'perforating skin dermatoses'. This group consists of four skin diseases: (a) elastosis perforans serpignosa (b) reactive perforating collagenosis (c) perforating folliculitis and (d) Kyrle's disease.
Methods: We describe a patient with Kyrle's disease who was successfully treated with clindamycin.
Results: We report our experience with the management of a 44-year-old man with manifestations of Kyrle's disease. The patient presented with skin lesions of two different types on his face and upper and lower extremities. The old lesions were large hyperkeratotic plaques with burrows without signs of inflammation. The newer lesions were also hyperkeratotic plaques but they clearly had signs of inflammation. The patient was successfully managed with combined surgical and medical management. The hyperkeratotic old and large skin lesions, which did not have manifestations of ongoing inflammation, were surgically removed. In addition, antimicrobial therapy with oral clindamycin was administered for one month, which led to disappearance of the small newer lesions, which had findings of ongoing inflammation and were clearly part of Kyrle's disease. No recurrence was observed during 8 months of follow up.
Conclusion: Although clindamycin may have some anti-inflammatory effects unrelated to its antimicrobial action, the observed control of the inflammation and the gradual regression of the newer lesions with clindamycin, suggest that microorganisms (probably anaerobic bacteria) play a role in the pathogenesis of the progression of Kyrle's disease at least in the initial stages of the skin lesions.

R2008

A prospective study of neck mass excluding thyroid nodules in one hospital

M. Zangeneh *(Tehran, IR)*

Introduction: A mass in the neck is a common clinical finding that presents in patients of all age groups. The differential diagnosis may be extremely broad, and although most masses are due to benign processes, malignant disease must not be overlooked. Therefore, it is important for physicians to develop a systemic approach for developing a working diagnosis and management plan for the patient.
Objective: The aim of this study is to evaluate neck masses and their etiology except thyroid nodules.
Materials and Methods: This is a Cross-Sectional Prospective study on 155 patients who has been referred to adult infectious diseases clinic with neck mass from 1999/March/20 to 2003/March/19. In this study correlation between neck mass and age, sex, initial time, size, number, position, sign, symptom, and etiology has been evaluated.

Results: Thirty-two patients did not accept more evaluation, the findings of 123 patient left include: 47/3% men, 52/7% women, 43/6% single, 56/4% married; 8% less than 10-years-old, 28% 10–19-years-old, 22% 20–29-years-old, 7/4% 30–39-years-old, 10/2% 40–49-years-old, 10/2% 50–59-years-old, 6/6% more than 60-years-old; time of neck mass in 24/1% less than one month, 29/9% 2–3 months, 21/8% 4–6 months, 10/3% 7–12 months, 13/8% more than 12 months; mass position 42/8% on left side of neck, 29/7% on right side of neck, 24/1% on both sides, 2/8% on midline, 0/7% on occiput; number of mass 59/4% one mass, 6/3% 2 masses, 34/3% 3 or more than 3 masses; also size of masses was evaluated. 30% have signs and symptom, the more common signs are lymphadenopathy on the axilar and hepatomegaly, 13/6% have past history. Etiology: 35% toxoplasmosis, 14/6% tuberculosis, 13% cancer, 8/5% bacterial lymphadenitis, 6/3% viral infections, 4/8% lymph node hyperplasia with unknown ethiology, 4/8% salivary glands infections, 1/6% thyroiditis and ectopic thyroid, 1/6% infected cyst, 1/6% brucellosis, 0/8% lypoma.
Conclusion: According to the results that TB and cancer are more common in the adult, it is important that in any patient with neck mass physician to develop a systemic approach for developing a working diagnosis and management plan for the patient.Supported by Azad Islamic University

R2009

Hormone replacement therapy and prevalence of bacterial vaginosis

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Objectives: The purpose of this study was to evaluate the effect of oral estroprogestinic hormone replacement therapy (HRT) on the bacterial vaginosis (BV) prevalence in postmenopausal women.
Methods: One hundred thirty eight women, aged 45–69 years were enrolled in this study. All of them had a natural termination of menstruation in at least 12 months prior the study. Fifty eight (group A) were currently received oral HRT (tibolone), whereas the other 80 (group B) had not received HRT, orally or transdermally at least in the past six months. All vaginal speciments were collected from the posterior fornix or lateral vaginal wall. Diagnosis of BV mainstayed on the Nugent score method (a score of 7–10 was considered indicative of BV) as well as the presence of 'clue cells' on Gram stained vaginal smears.
Results: The mean age ± SD of women in group A and the average number of postmenopausal years was 56.4 ± 3.9 years (range, 51–63 years) and 6.8 ± 3.1 (range, 4–13 years). While in group B was 55 ± 5.2 (range, 45–69 years) and 6 ± 3.2 (range, 1–18 years) respectively. Prevalence of BV in group A was slightly lower (12.5 %) than among women in group B (13.3 %), but the difference was not statistically significant ($P > 0.05$). Absence of lactobacilli from vaginal smears without any symptoms of vulvovaginitis was found in 36.6% of women in group B and 11% of women in group A. High number of lactobacillus colonization was observed in only 10% of women who did not received HRT.
Conclusions: Treatment of postmenopausal women with tibolone has no statistically significant effect on 'full BV' prevalence. However, it did seem to affect lactobacillus colonization of the vagina, altering the flora to premenopausal profile.

R2010

Bacterial pathogens associated with acute diarrhoea on the island of Crete, Greece, and their resistance to antibiotics

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Objective: The aim of the present study was to determine the prevalence of bacterial pathogens associated with diarrhoea on the island of Crete, Greece, and their resistance to commonly used antimicrobial agents.
Methods: From January 1999 to December 2003, stool samples from 6.814 diarrhoeal patients were examined for bacterial pathogens. Cultures and identification of the isolates were done using standard microbiological methods. Susceptibility testing to antibiotics was performed by using the disc diffusion method following the recommendations of the NCCLS.
Results: Bacterial pathogens were identified in 681 (10%) patients. *Salmonella enterica* were the most commonly isolated bacteria (58.7%), followed by *Campylobacter* spp. (29.7%), enteropathogenic *Escherichia coli* (EPEC) (4.4%) and *Yersinia enterocolitica* (4.1%). *Aeromonas* spp. (1.6%) and *Shigella* spp. (1.5%), were less frequently isolated. Resistance to ampicillin was observed in 10.7% of the *Salmonella*, 50% of the *Shigella*, and 33.3% of the EPEC isolates. Resistance to cotrimoxazole was observed in 4% of the *Salmonella*, 50% of the *Shigella*, and 16.7% of the EPEC isolates. High percentages of resistance to fluoroquinolones (37%), were detected among *Campylobacter* isolates, while resistance to erythromycin was observed in 14.4% of them.
Conclusion: The knowledge of the bacterial agents of diarrhoea and their local patterns of antimicrobial resistance are of utmost importance for the selection of the appropriate treatment.

R2011

Eosinophilic cellulitis (Wells' syndrome) due to tick bite

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Objective: To present a case of Wells' Syndrome due to tick bite.
Case report: A case of Wells' syndrome possibly due to tick bite is presented. Eosinophilic cellulitis (Wells' Syndrome) is characterized by skin eruptions with edema, flame figures and marked infiltrate of eosinophils in the dermis. Hypersensitivity to arthropod bites, cutaneous viral infections (HSV-2), cutaneous parasitic infestations (toxocariasis), ascariasis and drug hypersensitivity has been reported as etiologic factors. Sixty-year-old woman was referred to our clinic with the suspect of erythema chronicum migrans and erythema annulare centrifugium. The patient complained from widespread reddish elevations since 5 months after tick bite. Lesions had first appeared in scalp and face, then had spread to the body. She was hospitalized in the local hospital; skin biopsy was consistent with nonspecific chronic dermatitis and anti-Borrelia IgM and IgG were positive. Terbinafine (systemic and local), systemic tetracycline, amoxicilline clavulanate was administered but no response was observed. The patient was hospitalized in our clinic with the initial diagnosis of eosinophilic cellulitis, erythema chronicum migrans and erythema annulare centrifugium. Dermatologic examination revealed widespread elevated erythematous papules and plaques of 1–10 cm diameter formed by coalescing papules, with central sparing.

Histopathological examination of punch skin biopsies showed eosinophilic infiltration in the dermis, but no flame figures were observed. Borrelia burgdorferi IgG was positive but IgM was negative. Marked eosinophilia (11%) was noted in the peripheral blood sample, total IgE was elevated (275 mg/dl) other biochemical parameters were normal. No other signs of systemic Borreliosis was found. The patient was treated with dapsone (150 mg/day) and methylprednisolone (80 mg/day) with the diagnosis of eosinophilic cellulitis. Lesions had tended to resolve by the 2nd month of the therapy and complete resolution without sequele was observed on the 6th month.

Conclusions: Tick bite has been reported as a causal factor for Wells' syndrome. Moreover, arthropod born infectious agents like *Borrelia burgdorferi* may also be a triggering factor. Dapsone combined with corticosteroids may be an effective treatment in this situation. The role of antibiotherapy in the acute phase of the infection is controversial.

R2012

Rabdomyolysis complicating *Shigella flexneri* acute gastroenteritis

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Objectives: To report a case of acute rabdomyolysis associated with *Shigella flexneri* acute gastroenteritis.

Methods: Stool specimens cultured on the appropriate media for isolation of the enteropathogenic bacteria and direct smears were examined. The enteropathogenic bacterium was identified by standard methods, API 32E system (bioMerieux) and serotyping with antiserum reagents. Myoglobin was detected by biochemical method. Susceptibility testing was carried out by disc diffusion method.

Case report: A male patient aged 71 years was admitted to the hospital because of acute gastroenteritis with watery, bloody stools associated with abdominal pain and myalgias. He was suffering from coronary artery disease and diabetes. The patient was pyrexial (380°C), dehydrated, hypotensive (blood pressure: 90/70 mmHg) and had generalized weakness. Abundant leukocytes and erythrocytes were seen on direct smears of stools. Admission laboratory data included WBC count 18.250/μl (neutrophils 84.6%), Ht 45.1%, Hb 15.9 g/dl, urea 64 mg/dl, creatinine 1.5 mg/dl, glucose 192 mg/dl, Ê 3.6 mEq/l, Na 134 mEq/l, CPK 11840 IU/L (normal value < 170 IU/L), CK-MB 124 IU/L (normal value < 6%), LDH 543 IU/L, SGOT 265 IU/L, SGPT 81 IU/L, ã-GT 21 IU/L, albumin 3.2 g/dl and myoglobinuria. From the clinical and laboratory findings rabdomyolysis was diagnosed. The patient was treated with parenteral hydration and ciprofloxacin 400 mg tid iv. From the stool culture *S. flexneri* isolated. The strain was resistant to trimethoprime/sulphamethoxazole, ampicillin, amoxycillin/clavulanic acid, tetracycline, chloramphenicol and susceptible to ciprofloxacin, cefotaxime, piperacillin. His condition showed gradual improvement. At the next days he was presented gradual improvement. CPK on the 2nd and 3rd hospital day was 4700 IU/L 900 IU/L respectively and had returned to within normal by the sixth hospital day. The patient was discharged on day 7.

Conclusions: Rabdomyolysis complicating *S. flexneri* gastroenteritis is very rare. The present case is only the second found in the English literature. The possibility of rabdomyolysis must be considered in all cases of severe gastroenteritis so that prevention and timely treatment of complications are made possible.

R2013

Corynebacterium urealyticum encrusted cystitis: a new case and review of literature

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Encrusted alkaline cystitis (EC) is an unusual process, almost forgotten by urologists. It tends to appear complicating an underlying cystopathy. EC consists of a vesical mucosal inflammation with encrustation of amonium magnesium phosphates in the urothelium. Their genesis requires pre-existing mucosal damage, urinary infection by urolithic microorganisms and alkaline urine. A 50-years-old man was victim of a traffic accident with multiple injuries. He was admitted in our ICU hospital and urinary catheterisation was perfomed for 20 days. Because of hematuriae and abdominal pain urine samples were collected. *Corynebacterium urealyticum* grown in the urine, and some foci of malakoplakia were found in the area of encrustation and endoscopically excised. Antibiotic treatment with teicoplanin and ciprofloxacin was fulfilled during 60 days. After 30 days of treatment, endoscopical resection of bladder wall was achieved for bacteriological data. Few cases of encrusted cystitis were reported this past years. We propose literature review and therapeutic options in this communication.

R2014

Differences in aetiology according to Fine score in adult patients with hospital admitted CAP in Spain

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Objectives: Despite the overlapping aetiology of CAP regardless of severity, there exists a trend towards a higher implication of *S. pneumoniae* as severity increases. Here we present the relative involvement of different pathogens in a large series of adults with CAP admitted to hospital in Spain.

Methods: Retrospective review of the microbiological requests and results of patients admitted to hospital with the diagnosis of CAP over a 1-year-period (1Nov2001 to 31Oct2002) in 10 geographically scattered hospitals in Spain. Data were available from 3233 patients. Of those patients only 1722 had recorded the necessary information to calculate the Fine score, and this was our target population.

Results: The prevalence (%) of the main respiratory pathogens by Fine score is shown in the Table 1.The composite prevalence of *S. pneumoniae* in patients with low risk Fine score (groups 1–3) was 58.5% vs. 69.0% in those with high risk Fine score (groups IV–V), p = 0.025.

Fine score	Biologic diagnosis	S. *pneumoniae*	*Legionella* spp.	*H. influenzae*	*M. pneumoniae*	Others
1	25.3	52.9	11.8	3.9	2.0	29.4
2	24.7	65.1	7.9	4.7	7.9	14.3
3	26.9	57.3	9.4	8.3	6.3	18.8
4	23.4	66.4	8.2	2.7	4.0	18.6
5	22.4	76.7	4.7	3.1	0.0	15.6

Conclusions: (1) Mean etiologic diagnosis was established in around 25% of patients. (2) *S. pneumoniae* remained as the most important responsible in any of the Fine groups. (3) Contrary to the literature, in our series *Legionella* was more common in low

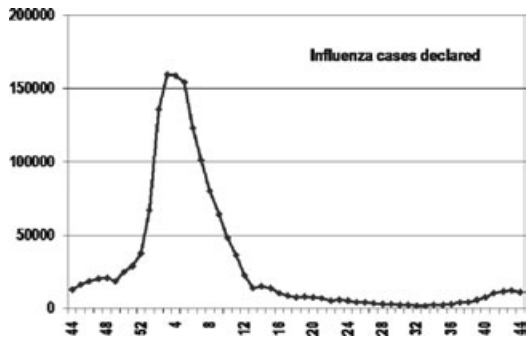
risk than in high risk Fine groups. (4) However, *S. pneumoniae* was significantly more common as severity increased. (5) *H. influenzae* did not show clustering in any group. (6) *M. pneumoniae* was more common in less severe cases.

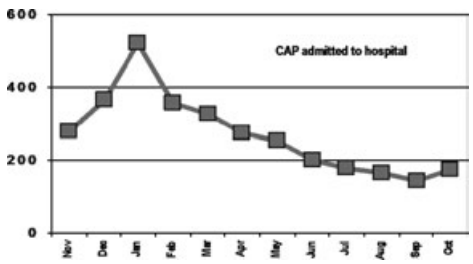
R2015

Influence of influenza cases on the number of hospital admissions of CAP in Spain

J.M. Nogueira, J. Torres, F. Baquero, J.E. Martín-Herrero, R. Dal-Ré *on behalf of the NACER Group*

Objectives: The processing of microbiological samples is known to yield different results depending on many external factors many of them are of logistic nature. We wanted to assess whether this statement stands in the microbiology of samples from patients with CAP admitted to 10 Spanish hospitals.
Methods: Retrospective review of the charts of patients admitted to the hospital with the diagnosis of CAP over a 1-year period (from 1 Nov 2001 to 31 Oct 02) in 10 geographically scattered hospitals in Spain. Data were available from 3233 patients. Concomitantly, we represent the number of declared cases of influenza as provided by the Spanish Institute for Health Carlos III from the same time period.
Results: Number of CAP admissions and influenza cases declared are shown in Figures.





Conclusions: (1) The number of CAP admitted to hospital increased from the beginning of the study period peaking abruptly in January and decreasing at a steady pace afterwards to increase again from September onwards. (2) Likewise, the number of influenza cases had a mirroring increase during the last two months of 2001 with a parallel, although much larger magnitude, bursting increase in the first weeks of 2002. (3) However, the decrease in the notification of influenza cases was faster than the decrease in hospital admission due to CAP.

R2016

Prevalence of complicated urinary tract infections in Italy among patients attending the urologist's office

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Background: Few data are available on the prevalence of complicated urinary tract infections (cUTIs) in Italy. We report here the major results of a large prospectively planned survey carried out on the prevalence and disease management of cUTIs.
Methods: Some 111 urologists throughout Italy recorded all outpatient visits. Each case of cUTIs, according to stated clinical definitions was comprehensively evaluated. All the urologists were specifically trained on the protocol and case report-form compilation.
Results: A total of 13,030 patients were observed in the study timeframe. Approximately 20% of visits were audited. Overall, the prevalence of cUTIs was 10.8% (n = 1405). The mean age of the patients population was 60.9 years (sd 15.7). cUTIs diagnoses were mainly associated with prostatic hypertrophy (40.7%), presence of stones in bladder or urinary tract (19.7%) and recent urologic surgery (12.8%). Diabetes was the more frequently observed co-morbidity (17.5%). The cUTIs clinical picture included: prostatic hypertrophy (42.9%) suprapubic pain (40.6%), flank pain (13.4%), etc. The first cUTIs episode was observed in 51% of the patients, while 34% and 10.3% presented a re-infection or a recurrent episode, respectively. The more frequently encountered pathogens were *E. coli* (39.2%), *Proteus* (8%), *Klebsiella* (4%), *Enterococcus* (3.9%), and *Pseudomonas* (3%). Nearly 20% of patients declared absence from work for the current episode (mean of 6.6 days ds 5.2; range 1–35). No significant geografical variations among all the major items were detected.
Conclusion: cUTIs are a significant clinical entity among Italian outpatients in terms of morbidity, quality of life and social costs.

R2017

Group B *streptococcal* arthritis in an immunocompetent adult

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Objectives: Group B *streptococci* (GBS) are causes of lifethreatening infections in neonates, infants and pregnants ,but they are possible causes of invasive infections especially in immunocompromised adults. GBS arthritis has been rarely described in immunocompetent adults, usually associated with other risk factors as neoplasms, cirrhosis, diabetes. We present a GBS arthritis in an immunocompetent male patient.
Case report: A 65-year-old white man, presented with a 2-day history of fever and chills, abdominal pain and a 1-day history of torticollis and pain of the right hip. Remarkable physical findings included toxic appearance, temperature of 38.5 °C, neck stiffness. His past medical history was unremarkable, except arterial hypertension under treatment. Admission laboratory tests revealed WBCs 17000 cells/mm^3 (87% neutrophils), hematocrit 47.7%, normal platelets. Coagulation tests were normal. ESR was 125 mm/1st hour and CRP qualitive measurement was positive. Transaminase levels were normal. Three sets of blood culture and a urine one were obtained. Blood cultures were carried out by mini Vital system (BioMerieux). The patient began receiving ciprofloxacin and dicloxacillin, thought to be a septic arthritis. At 72 hours, three bottles of blood culture grew group B *streptococcus*. Species identification was performed by classic methods.

Susceptibility test was performed by disc diffusion method (according to NCCLS).The strain was resistant to aminoglycosides. Antibiotic therapy was changed to intravenous penicillin. Whole body bone scan, with Tc99m-MDP revealed arthritis of the right acetabulum. 8 days later, the patient presented increased mobility of the right hip and he finally recovered without sequelae.

Conclusions: This case report tries to increase the awareness of clinicians to GBS as a potential cause of septic arthritis. Although GBS disease is frequent in adults with asplenia-organic or functional-, chronic liver disease, diabetes and congestive heart failure, and although urinary tract infection, pneumonia and soft tissue infections are the commonest presentations, GBS has not to be underestimated in the differential diagnosis of septic arthritis even in patients without predisposing factors.

R2018

Severe complications in patients with *Brucella melitensis* infection

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Brucellosis can present with a variety of symptoms and can affect virtually every organ and tissue of the body. We report here complications in 15(17%) out of 88 patients with Brucellosis that were diagnosed and treated at our Hospital in a period of 7 years. These complications were of clinical significance because they lead to diagnostic and therapeutic dilemmas. Complications that lead to diagnostic dilemmas because of the broad list of differential diagnosis included skin rash in 3 patients, orchitis/epipidymitis in 3 patients, severe pancytopenia in 2 patients and single lineage cytpenias in another 3, and finally stiff neck with CSF pleocytosis in one patient.Therapeutic dilemmas emerged in 3 patients who developed arthritis (spondylitis, sacro-ileiitis and spondylitis, and right ankle arthritis, respectively) while they were receiving treatment for Brucellosis. Finally, it was only after a detailed diagnostic work up that a patient who presented with recurrent fever and arthritis while on treatment for Brucellosis for 8 weeks was diagnosed with endocarditis of an artificial aortic valve. Successful management required prolonged treatment with a combination of antibiotics.

Conclusion: The diagnosis and treatment of Brucellosis are usually simple. However, the successful management of its complications that can be severe, may require detailed diagnostic work up and prolonged treatment.

R2019

Therapeutical problems and effective antimicrobial therapy in post-traumatic patients with Streptococcal cellulitis and Streptococcal toxic shock syndrome

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Objective: We observe an increasing number of invasive soft tissue infections caused by *Streptococcus pyogenes* in recent years among patients following trauma hospitalized in The Dpt. of Trauma Surgery of the Medical University of Gdañsk. GAS may cause cellulitis, necrotizing fasciitis, myositis and myonecrosis. The highest rate of mortality (30–70%) occurs in cases with Streptococcal Toxic Shock Syndrome (STSS) related with soft tissue infections in 40–50% cases.

Methods and materials: We analysed records of patients clinical materials from recent 2 years. We present 4 posttraumatic patients with severe GAS infections of soft tissues with cellulitis and STSS. All patients developed infectious complications following injuries. All cases required quick and aggressive surgical procedures- large incisions and removal of necrotic tissues; one of the patients needed multiple surgical interventions. Three patients improved in spite of severe course of infection; one patient died. Two patients suffered from head injury, two patients had injuries to the extremities complicated by extended tissue necrosis. Amputations of the limbs were not necessary. The course of infections was rapidly progressive with fever, extensive soft tissue necrosis and marked systemic toxicity. All cases required broad spectrum antimicrobial therapy- patients were treated with lincosamids, mainly with clindamycin given intravenously in large doses from 1800 to 3600 mg/day. In all patients microbiological procedures were performed: cultures of the wound and blood were obtained and sent for routine and anareobic cultures and for direct microscopic examination. Suitable diagnostic materials were: exsudate from the wound and fragments of necrotic tissues.

Patient	Clinical diagnosis	Microorganism/ biologic material	Treatment	Final result
No 1 Female Age 68	Suppurated head wound	*Streptococcus pyogenes*/ wound exsudate	Clindamycin 3 × 600 mg Metronidazole 3 × 100 mg	Death (13 day)
No 2 Male Age 30	Abscesses of the ankle and elbow regions	*Streptococcus pyogenes*/ wound exsudate	Clindamycin 3 × 1200 mg (3 days) 3 × 900 mg	Recovery
No 3 Age 52 Female	Diffuse phlegmone of the hand	*Streptococcus pyogenes*/ wound exsudate	Clindamycin 3 × 600 mg	Recovery
No 4 Male Age 50	Suppurated head wound	*Streptococcus pyogenes, Staphylococcus aureus*/ wounds exudate *Streptococcus pyogenes, Staphylococcus aureus*/ tissue fragments	Lincomycin 3 × 600 mg	Recovery

Conclusion: GAS infections are severe and life threatening. According to our findings clindamycin in high doses improves the prognosis and should be the drug of choice in Streptococcal cellulitis and STSS.

R2020

Demographic and microbiologic features of urinary tract infections in young and adult patients

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Background: Urinary tract infection (UTI) is one of the most common bacterial infections. Since antibiotics are often administered empirically, it is necessary to evaluate the spectrum and susceptibility of the etiological agents in the various clinical settings.

Objectives: To evaluate some demographic and microbiologic features of patients with UTI and some risk factors associated with the resistance of *E. coli* to the most common oral antimicrobials.

Methods: All consecutive young and adult patients (16–50 years of age) attending the Urinary Tract Infection Clinic at the Pisa General Hospital from January 1997 to October 2004 were evaluated.Results: During the study period, 460 (375 females and 85 males) pts with UTI were enrolled. *E. coli* was isolated in 62.1% of the women and in the 45.0% of the males; the difference however was not statistically significant. *Pseudomonas* spp was more frequently isolated in males than in females

(10% vs 0.8%, $p < 0.001$). *E. coli* showed similar resistance rate in males and females to ampicillin, cotrimoxazole, nitrofurantoin and ciprofloxacin. The isolation rate of *E. coli* was lower (54.4% vs 64.2% , p < NS). in women treated with antimicrobials during the 2 months prior their enrolment than in those with no previous treatment. The resistance rate of *E. coli* to the antimicrobials in previously treated and non treated women was the following respectively : cotrimoxazole 22.6% vs 8.3%, $p = 0.07$; ampicillin 41.9% vs 22.6%, p NS; ciprofloxacin 9.7% vs 0.4%, p NS ; nitrofurantoin 9.7% vs 8.8%, p NS).

Conclusions: In young and adult pts (16–50 years of age), *E. coli* is the causative agent of UTI more frequently in females than in males. In men, other microorganisms (expecially *Pseudomonas*) play a significant role. In females, a previous antimicrobial treatment increases the risk of *E. coli* resistance to ampicillin and cotrimozazole.

R2021

Complications of acute brucellosis among adults in Diyarbakir, Turkey

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Objective: Brucella infection is endemic in our region Diyarbakir in particular. We describe here the clinical results and the complication of the infection in the hospitalized patients.

Methods: This retrospective study was conducted on patients with acute brucellosis in department of infectious diseases in Diyarbakir from 2003 to 2004. The complications and laboratory test results were noted.

Results: The study included 34 patients (20 females, 14 males) and their mean age was 34.8 ± 14.9 (range, 16–74) years old. Fever (91%) and sweating (91%) were the most common clinical findings which were determined in all patients. Arthralgy (85%) and headache (35%) were the other findings in patients. The diagnosis was established according to the clinical findings and serologic markers in all patients. Rosebengals test was positive in 29 patients. *Brucella* spp. was grown from blood cultures in 12 patients (35.3%). Osteoarticular involvement in 16 patients (47%), epididimoorchid in five patients (14.7%), Central nervous system (CNS) involvement in two patients (5.9%), spinal abscess and liver involvement in a patient (2.9%) were developed. Combination antibiotherapies of duo or trio (rifampicin, doxocycline/streptomycin and/or cotrimoxazole/streptomycin/ceftriaxone) were given to patients for 2–6 months period.

Conclusion: Osteoarticular involvement is by far the most common focal complication of *Brucella* infection. Osteoarticular involvement (47%) was the most complication in this study too. Because of this reason patients which were came with Osteoarticular complains, were researched about *Brucella* infection.

R2022

Microbiology facts in cystic fibrosis patients in Greece

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Objectives: Serious respiratory infections, due to *P. aeruginosa* and *B. cepacia* consist a major problem in the life of Cystic Fibrosis (CF) patients. In Greece, the *B. cepacia* isolation in CF is considered rather rare. The aim of our study was to investigate the presence of *B. cepacia* in respiratory infections of CF patients of 'Sotiria' Chest Diseases Hospital of Athens.

Methods: The selective medium *B. cepacia* agar (Oxoid) was used, in addition to conventional media, for quantitatively culturing sputum samples of CF patients, admitted to our Hospital, for the period 2003–2004. Identification methods included API 20E, API NE, and Vitek 2 (Biomerieux). All Gram (-), oxidase (+) isolates were also molecularly identified initially by multiplex PCR, in order to identify two bacterial species *P. aeruginosa* and *P. cepacia*. The multiplex PCR included primers specific for the 520 bp fragment of the ribosomal 16S gene, identifying the five main gemovars of *B. cepacia*. Included in the multiplex PCR was a universal bacterial primer pair targeting the 16S rRNA to act as internal control (233 bp). Samples giving results for the 520 and/or 463 bp fragments were further tested for the identification of the bacterial strain with specific PCR. The recA gene PCR is a useful assay, which discriminates between the *B. cepacia* complex and *B. cepacia* like microorganisms.

Results: None of the isolates was identified as belonging to *B. cepacia* complex, either by selective medium or PCR. A high level of correspondence was noted between selective *B. cepacia* medium and molecular identification of isolates.

Conclusions: There is evidence that *B. cepacia* participation in CF respiratory infections is rather unusual in Greece.

R2023

Influence of proper antimicrobial prescribing on outcome of acute sinusitis

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Objective: The aim of the study was to evaluate correlation between antimicrobial (AM) prescribing for outpatient adults with acute sinusitis (AS) and outcome of the disease.

Methods: Case histories of outpatients with the diagnosis of AS were consecutively selected in outpatients' departments in 8 regions of Russia for retrospective analysis. AM were classified using ATC-codes. All collected data were analysed with specially designed software 'Pharmatherapeutical Data Analysis' (IAC, Smolensk, Russia). Outcomes were classified as 'success' (cure or improvement) and 'failure' (hospitalization or prescription of an additional AM). Patient's age and frequency of sinus puncture (SP) as factors potentially influencing outcome of AS were also analysed. Ages were compared with Wilkokson–Mann–Whitney test. Outcomes and frequency of SP for the treatment with different AM were compared using weighted pairwise Fisher criterion.

Results: A total of 1529 case histories of outpatients aged from 16 to 81 (539 males, 990 females, average age 37.1 ± 13.2 years) were analysed. 10 most popular AM (82.7% from all AM) used for initial monotherapy including ampicillin (AMP), amoxicillin (AMX), amoxicillin/clavulanate (AMC), erythromycin (ERY), midecamycin (MID), gentamicin (GEN), lincomycin (LIN), doxycycline (DOX), ciprofloxacin (CIP), co-trimoxazole (CTX), were prescribed to 1029 (67.3%) patients. All groups were similar according to age. AMX group was associated with significant less frequency of SP than LIN, CIP, GEN ($p < 0.001$) and MID, AMC ($p < 0.05$) groups. Initial prescription of CIP or LIN was found to have higher rates of SP compared to AMP, ERY and DOX ($p < 0.05$). Rates of treatment success and failure are presented in the table. Treatment with AMX was statistically more successful than with other AM ($p < 0.05$) except AMC and MID. There was no significant difference in outcomes between other groups except MID, which had better outcome than CIP and DOX ($p < 0.05$).

Antimicrobial	Outcome	
	Success, N (%)	Failure, N (%)
Amoxicillin	183 (96.8)	6 (3.2)
Midecamycin	77 (92.8)	6 (7.2)
Amoxicillin/clavulanate	33 (89.2)	4 (10.8)
Erythromycin	27 (87.1)	4 (12.9)
Lincomycin	181 (87.0)	27 (13.0)
Ampicillin	122 (86.5)	19 (13.5)
Gentamycin	58 (85.3)	10 (14.7)
Ciprofloxacin	128 (83.1)	26 (16.9)
Doxycycline	69 (81.2)	16 (18.8)
Co-trimoxazole	27 (81.8)	6 (18.2)

Conclusion: proper AM prescribing (AMX) for initial treatment of AS led to better outcome. AMX start-up therapy was associated with less frequent SP.

R2024

Antimicrobials prescription patterns in outpatient adults with community-acquired pneumonia in Russia: 2003 vs 1998

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Objective: A number of guidelines for CAP management in adults have been published since 2000. The present survey evaluates the patterns of antimicrobial (AM) prescribing to adults with CAP in Russian outpatient departments in 2003, as compared to 1998.
Methods: Case histories of outpatients with CAP who had not required hospitalization were randomly selected in fifteen regions of Russia for retrospective analysis and were compared with the data obtained in 1998. The system of distance double data entry was used for data collection.Results: Altogether, 2174 case histories of patients from 16 to 91-years-old (1140 males, 1034 females, average age 47.0 + 16.4) were included in the study in 2003 vs. 778 case histories of patients from 16 to 88-years-old (383 males, 395 females, average age 47.1 + 15.2) in 1998. A total of 35 AM were prescribed in 2003 compared to 36 in 1998. The most common drugs for initial monotherapy in 2003 were amoxicillin (24.0%), ciprofloxacin (14.6%), midecamycin (10.9%), cefazolin (10.1%), amoxicillin/clavulanic acid (6.5%) vs. gentamicin (15.6%), ampicillin (12.1%), ciprofloxacin (10.5%), co-trimoxazole (8.0%) in 1998. About 8% of patients in 2003 vs. 16% in 1998 received combinations of 2–3 AM as initial therapy. Amoxicillin + gentamicin (14.7%), ampicillin + gentamicin (9.2%), ciprofloxacin + gentamicin (5.5%) were the most frequently prescribed combinations in 2003 vs. ampicillin + co-trimoxazole (26.1%), gentamicin + co-trimoxazole (9.2%), ampicillin + gentamicin (6.7%) in 1998. In 72.5% of cases AM were administered orally (vs. 71.6% in 1998), in 24.7% – intramuscularly (vs. 28% in 1998), 0.5% – intravenously, 2.3% – route was not indicated.
Conclusion: Numerous AM were used for CAP treatment in 2003 as well as in 1998, but prescribing habits have improved. An increased rate of amoxicillin and amoxicillin/clavulanic acid and a decreased gentamicin and co-trimoxazole usage were observed. The very low rate of 'new' macrolide usage and an unacceptably large number of ciprofloxacin and cefazolin prescriptions should be noted.

R2025

Isolation of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in non-gonococcal urethritis

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Objectives: *Haemophilus* spp. have been suggested as a potential pathogen in urethritis. We report four cases in which *Haemophilus* spp. were isolated in men with urethritis as only pathogen present.
Methods: Samples from 97 males with sexual risk behaviors (homosexuality), were processed for bacterial and fungi culture. *Chlamydia trachomatis* and *Mycoplasma urealyticum* were performed by specific request and microscopic examination for *Trichomonas vaginalis* 21.64% of patients had symptoms of urethritis and 79.36% were asymtomatic
Results: *Haemophilus* spp. was isolated in 24 patients (24.7 %): *H. influenzae* en 2 cases (8.3%) and *H. parainfluenzae* in 22 cases (91.7%) *Haemophilus* spp. was isolated as only pathogen in four men with urethritis: 1 *H. influenzae* and 3 *H. parainfluenzae*.

	Haemophilus spp.	*Chlamydia trachomatis*	*Neisseria gonorrhoea*	*Candida albicans*	*Streptococcus agalactiae*	*Ureaplasma urealyticum*	Normal flora
Asymptomatic	20	1	2	1	2	0	50
Symptomatic	4	0	0	3	1	1	11

Conclusions: *Haemophilus* spp. was isolated at a hight frequency in our studied group.The pathogenic role of *Haemophilus* spp. was suggested when it was isolated as an only pathogen.

Nosocomial infections, infection control

R2026

Cure of multidrug-resistant (MDR) *Acinetobacter baumannii* bacteraemia with continuous intravenous infusion of colistin

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Background: Although intermittent intravenous bolus dosing of antimicrobial agents is the standard way of administration of antibiotics in clinical practice, the last few years, there is renewed interest among clinicians and researchers in examining the benefits of continuous mode of treatment. Methods: Description of a patient with multidrug-resistant *Acinetobacter baumannii* bacteraemia who was successfully managed with continuous intravenous infusion of colistin.
Results: A 41-year-old white man was admitted to the ICU of our hospital because of sudden cervico-occipital headache accompanied by vomiting. A CT of the brain revealed extensive intraventricular haemorrhage originating from the basal ganglia. During his long-standing hospitalization to our hospital several

events of clinical significance occurred. On day 84 of his hospitalization, the patient developed pneumonia due to *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. He was managed with intravenous antimicrobial agents including vancomycin, gentamicin, and piperacillin/tazobactam, which led to clinical improvement. However, on day 96, the above-mentioned isolates grew again from cultures of bronchial secretions specimens. Gentamicin was discontinued, and intravenous ciprofloxacin and rifampin were started. On day 117, the patient developed an extensive maculopapular rash on the trunk and the extremities. In addition, ocular and perioral swelling was observed. All antimicrobial agents were discontinued. On day 119, he developed bacteraemia due to *Klebsiella pneumoniae* strain. Intravenous gentamicin was started but it was discontinued one day later due to the flare up of the rash. Finally, on day 127, a blood specimen culture grew a MDR *Acinetobacter baumannii* strain, sensitive only to colistin. A continuous intravenous infusion regimen with colistin 2,000,000 units per 24 hours was initiated. The clinical condition of the patient gradually improved, and the rash gradually diminished.
Conclusion: Continuous intravenous administration of colistin may be associated with fewer hypersensitivity (adverse) reactions and may be as effective as the traditional intermittent way of infusion.

R2027

Colonisation and antimicrobial resistance in tracheal aspirates of hospitalised patients in intensive care units of a tertiary medical care centre in Kosova

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Objective: To determine colonization rate of the nosocomial pathogens in tracheal aspirates of patients hospitalized in intensive care units of University Clinical Center of Kosova and to investigate their in vitro antimicrobial susceptibility pattern.
Methods: A total of 324 isolates from ICU patients received between November 2003 to November 2004, were processed in the Department of Microbiology within the National Institute of Public Health of Kosova. Standard microbiologic methods were used for microorganisms identification. The antimicrobial resistance was determined with disk diffusion methods according to NCCLS guidelines.
Results: Of 324 tracheal aspirates 259 (79.9%) specimen yielded growth of microorganisms. 75.3% of isolates were recovered from adult intensive care units. Of all positive isolates, 6.8% were polymicrobic. Gram negative bacilli accounted for 75.3% of all microorganisms. *Pseudomonas aeruginosa* was the most common aetiologic agent isolated (29.5%), followed by *Acinetobacter baumanni* (16.9%), *Klebsiella pneumoniae* (9.9%) and *Citrobacter freundii* (5.4%). *Staphylococcus aureus* predominate among gram positive bacteria with overall presence of 9.8% accompanied by *Staphylococcus epidermidis* (5.35%). Ampicillin, gentamycine, ceftriaxone and imipenem global resistance rates were 93.8%, 87.6%, 78.1% and 25.9%, respectively. 75.4% of *Staphylococcus aureus* isolates were resistant to oxacillin Resistance rate of *Pseudomonas aeruginosa* to third generation cephalosporins was 85.7%. All isolates of Acinetobacter were multidrug resistant.
Conlcusion: High frequency colonization of multidrug resistant nosocomial pathogens in tracheal tube of ICU hospitalized patients suggest that infection control measures should be implemented with prudent use of antimicrobial agents.

R2028

Changing pattern of neonatal bacteraemia: microbiology and antibiotics resistance

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Objective: The work presented here sought to determine the most important causative agents of bacteraemia in a neonatal intensive care unit (NICU), their changing distribution and their antibiotic susceptibility patterns over a five years period from 1997 to 2001 in the Tripoli Medical Center (TMC).
Methods: This study was performed between January 1997 and January 2001 at the Microbiology section in Tripoli Medical Center. During this period 1431 Oxoid Signal Blood Cultures sets were obtained from 1092 Neonatal Intensive Care Unit (NICU) with suspected bacteraemia. Conventional methods, API 20E and API 20NE (bioMerieux) were used to identify the isolated bacteria. The Kirby-Bauer disk diffusion method was performed to assess their antibiotic susceptibilities in accordance with NCCLS standard.
Results: Over the period of the study 801 sets out of the total 1431 blood cultures were positive for microbial growth, which represented 648 cases of neonatal bacteraemia from a total of 1092 cases. From the total number of isolates the members of *enterobacteriaceae Serratia*, *Klesiella* and *Enterobacter* spp were the most common cause of bacteraemia. The coagulase negative and positive *Staphylococci* were also frequently isolated. A changing pattern of causative pathogen was observed during this study between members of the three leading genera of Enterobacteriaceae. Antibiotic susceptibility testing showed a high level of resistance among the most common pathogens. Resistace to Aztreonam, Imipenem, Ciprofloxacin and piperacillin/tazobactam was less frequently encountered. Staphylococcus resistance to anti-staphylococcal antibiotic and due to hyperproduction of penicillinase enzyme was also high and all isolates were remained sensitive to vancomycin.
Conclusion: Gram-negative bacteria especially members of the enterobacteriaceae are important causes of bacteraemia in neonatal intensive care units. Although most isolates remain sensitive to the new antibiotics, emergence of resistant strains can not be excluded in the future. For that reason new strategies and continuous surveillance are required to monitor the changing epidemiology of pathogens, antibiotic susceptibilities and antibiotic use needed to overcome the increasing incidence of resistance to conventional drugs.

R2029

Phagocytic activity of human peripheral blood polymorphnuclear leucocytes to methicillin-sensitive and resistant coagulase-negative *Staphylococci*

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Objectives: Two types of white blood cells, the polymorphnuclears (PMN) and monocytes are important in resistance to infections. Phagocytes play a significant part as the first line of defence against bacteria, viruses and fungi in the human host. Methicillin-resistant *Staphylococci* have emerged as significant nosocomial pathogens and express certain virulence factors. The aim of the present study was to evaluate the host defence against methicillin-resistant *Staphylococci* in comparison with methicillin-sensitive strains.
Methods: The study was conducted during 2002–2003 in the Hospital of Traumatology and Orthopedics, Riga, Latvia. During this period, 31 strains of coagulase-negative *Staphylococci* were studied. From them – 10 strains isolated from

healthy persons (control strains, additionally ATCC 12208 were used as the control), 15 methicillin-resistant strains (MRCoNS) and 5 methicillin-sensitive (MSCoNS) isolated from clinical specimens were studied. The phagocytic capacities of human peripheral blood polymorphnuclears of 11 volunteers (against the above-mentioned strains) were evaluated. The percentage of active cells and the phagocytic index were measured, as well as the percentage of digested cells in 3 hours.

Results: Using the control strains of *Staphylococci*, 80% of active phagocytes with the phagocytic index 7.8 was observed. In 3 hours, 100% of *Staphylococci* was digested. In experiments with MSCoNS, the following results were registered: 77% of active cells with the phagocytic index 4.36 and 78.2% of digested cells. In experiments with MRCoNS, there was 67.75% of active phagocytes with the phagocytic index 3.71 and 68.4% of digested cells in 3 hours.

Conclusions: Methicillin-resistant strains of CoNS are more virulent than MSCoNS and are not destroyed by phagocytes as actively as MSCoNS.

R2030

Colonisation of nasopharynx by Gram-negative rods in patients with resectable lung cancer during short-term hospitalisation

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Objectives: The aim of this study was to assess the frequency of colonization of nasopharynx by Gram-negative rods in patients with lung cancer undergoing thoracic surgery, who routinely receive antimicrobial prophylaxis (piperacillin, cefuroxime alone or in combination with amikacin). Antimicrobial agent susceptibility of isolated strains was also defined.

Methods: Sixty-three patients with resectable lung cancer were included in the study. Throat and nasal specimens were taken two times: on the day of hospital admission (examination I) and on the fourth day after surgery (examination II). Samples were routinely cultured under aerobic conditions. The strains were identified by using API 20E or API 20NE. Susceptibility to selected antimicrobial agents was detected by the disc diffusion method according to NCCLS recommendations.

Results: 24 strains of Gram-negative rods, 17 belonging to *Enterobacteriaceae* family and 7 – to non-fermenting rods, were found in 28 samples: in 8 patients in examination I (12.7%) and in 16 patients in examination II (25.4%). Double increase of frequency of these bacteria on mucous membranes of nasopharynx in examination II was observed (χ^2 test, $p = 0.0695$). The strains of *Enterobacteriaceae* were highly susceptible to antimicrobial agents, whereas the most of non-fermenting rods were classified as multidrug resistant organisms. Among the antibiotics used in this study, cefepime and ticarcillin/clavulonic acid were the most active in vitro against the isolated Gram-negative rods. We found that 1 strain produced extended-spectrum β-lactamases and 4 strains – inducible β-lactamases.

Conclusion: During hospitalization, in patients with lung cancer undergoing thoracic surgery and preoperative prophylaxis, qualitative and quantitative changes in Gram-negative rods colonizing nasopharynx were observed. In the samples taken on the 4th day after surgery increased prevalence of non-fermenting rods (mostly multidrug resistant) was found. Obtained results confirm the need to conduct analyses of microflora of upper respiratory tract in patients with lung cancer to monitor potential etiologic factors in nosocomial respiratory tract infections.

R2031

Outbreaks of *Serratia marcescens* bacteriuria in a neurosurgical intensive care unit of a tertiary care teaching hospital: a clinical, epidemiologic, and laboratory perspective

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Objectives: The aims of this study were (1) to identify the risk factors associated with the development of *Serratia marcescens* bacteriuria in neurosurgical intensive care units (NSICU) (2) to genotype the pathogens in order to determine a source of infection (3) to compare these results with the antibiogram and (4) to implement the appropriate control measures.

Methods: A retrospective case-control study of the epidemiological data, the surveillance of environmental cultures and the genotyping of strains using arbitrarily primed polymerase chain reaction (AP-PCR) were performed in a 750-bed, tertiary care teaching hospital. Seventy-four bacteriuria patients were compared with 74-age-gender-matched control-patients in the NSICU between March 2002 and March 2004. The factors assessed included the patients' demographics, the duration of the hospital stay and the indwelling catheter before and during admission to the NSICU, a chronic underlying illness (diabetes mellitus, cardiovascular disease, malignancy), other sites of infection, a history of trauma, exposure to a nasogastric tube, mechanical ventilation, urinary catheterization, central venous catheterization, surgical drainage, tracheostomy, or brain or spine surgery, and thereceipt of total parenteral nutrition (TPN), antimicrobials (β-lactams, aminoglycosides, quinolones, carbapenems, vancomycins) or steroids.

Results: Patients with *S. marcescens* bacteriuria were more likely to have a longer NSICU stay and other sites of infection. Environmental surveillance showed the handling of urine jugs to be a point source of contamination. Genotyping and an antibiogram of 14 patients were the same except for two patients.

Conclusions: The patient-related risk factors were identified, and a rapid identification of the organism was made. Altering the method for handling urine jugs provided a focus for the heightened surveillance, infection control measures, and empirical therapy, terminating outbreaks.

R2032

Spondylodiscitis after facet joint steroid injection

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Background: Reported infections following facet joint injections are rare and include paraspinal and epidural abscess formation and septic facet joint arthritis.

Methods: We describe a case of *Pseudomonas aeruginosa* spondylodiscitis after facet joint steroid injection.

Results: A 78-year-old male was presented to his local orthopedic surgeon for low back pain. The patient had no neurologic deficits, and with the presumptive diagnosis of degenerative spondylosis, he has been treated with non-steroidal anti-inflammatory agents and physiotherapy for 3 months. Three months later, the patient had worsening of his back pain. Plain radiographs, computed tomography (CT) and magnetic resonance imaging (MRI) of the lumbar spine showed osteoarthritis of the lower lumbar facet joints. Facet joints injection was done using methyl-prednisolone acetate and bupivacaine hydrochloride 0.5%. The patient had temporary relief of his symptoms for 2 days followed by deterioration of his low back pain and acute

onset of low-grade fever. Laboratory investigation revealed increased white blood cell count (16.010/ìl), erythrocyte sedimentation rate (83 mm/1st hour), and C-reactive protein (185 mg/l). Spine infection was suspected and ciprofloxacin was administered (500 mg p.o. q12h) for four weeks. However, this treatment led to minimal improvement of the clinical symptoms. At the time of his admission to our institution, his main signs and symptoms included low back pain and tenderness, and increased body temperature. MRI, fine needle aspiration and cultures of the affected area confirmed the diagnosis of *Pseudomonas aeruginosa* spondylodiscitis at the L2-L3 and L3-L4 levels. The patient was treated with intravenous administration of amikacin (500 mg q12h) and imipenem plus cilastatin (500 + 500 mg q8h), for four weeks, followed by oral administration of ciprofloxacin for 24 weeks. At the latest follow-up, 6 months after the completion of the antimicrobial therapy, MRI of the lumbar spine showed significant narrowing of the L2–L3 and L3–L4 intervertebral spaces with no signs of active infection.
Conclusion: The association of the facet injection with the deterioration of the patient's clinical condition, in addition to the absence of any obvious hematogenous mechanism of infection, suggests an iatrogenic cause of spondylodiscitis. This iatrogenic complication should be included in the risk-benefit analysis of clinicians. Sterile preparation prior to the procedure should be stressed.

R2033

Selected virulence factors and resistance to antimicrobials of nosocomial and carrier *S. aureus* strains

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Objectives: To detect the production of selected virulence factors and the resistance to antistaphylococcal therapeutics of *S. aureus* strains causing sepsis, wound and joint implants infections and to compare them with the carrier strains.
Methods: *S. aureus* strains were isolated from haemocultures (31 strains), surgery wounds (40 strains), prosthetic hip and knee joints infections (42 strains) of patients of Faculty Hospital, Bratislava, and from nasal swabs of carriers (31 strains). Production of hyaluronidase, alpha, beta, and delta-haemolysisns, adherence to glass surface, polysaccharidic extracellular adhesin (PIA) production (on Congo-red plates), and the dynamics of plasmacoagulase production were tested. Resistance to oxacillin, erythromycin, clindamycin, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazol, quinupristin/dalfopristin (Q-D), linezolid (LNZ) and vancomycin (VAN) was estimated by disk diffusion test according to NCCLS. Susceptibility to VAN was confirmed by E-test and PBP2a in methicillin-resistant strains (MRSA) was detected by latex agglutination test.
Results: The comparison of investigated groups of *S. aureus* strains showed, that all, except 2 patient strains, produced alpha-haemolysin – some of them in combination with beta and delta haemolysins. Plasmacoagulase production till the 1st hour of incubation was detected in the majority of patient strains (45 to 70%), while among the carrier strains only in 30%. The most active were strains isolated from joints prostheses infections - JPI (70%). Adherence to glass surface was detected in 93% of all tested strains. The most intensive production of PIA (+++) was detected in 25% strains from haemocultures and 20% from JPI, but only in 12.5 % strains from wounds and 6.5 % of carrier strains. Only 6 strains were found to be MRSA (2 from haemocultures and 4 from wound infections), and all 6 produced only alpha-toxin and PIA. 7 % of strains (from patient samples only) were resistant to gentamicin. All tested strains were susceptible to VAN, LNZ, and Q-D.
Conclusions: Patient *S. aureus* strains differ from the carrier strains by a more rapid production of plasmacoagulase. Strains from haemocultures and prosthetic joint infections are characteristic by a more intensive production of PIA, which differentiates them from wound and carrier strain groups. MRSA were found only in the group of haemoculture and wound infection strains. All strains were susceptible to VAN, LNZ, and Q-D.

R2034

Investigation of a possible nosocomial urinary tract infection outbreak due to ESBLS producing *E. coli*

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Aim: Multiresistant *E. coli* are very rare in our hospital. Thus the isolation of four *E. coli* isolates showing the same multiresistant resistance phenotype from the urine of four patients hospitalized in the same ward during a week period (14/06/2004–21/06/2004) urged us to investigate the possible epidemiological significance of this finding.
Material and Patients: The *E. coli* strains were isolated from equal number of inpatients, two men and two women, mean aged 75 years old who were hospitalized for infections other than UTI (n : 3) and immunosuppression (n : 1). All but one had pyuria (>50 leucocytes p. f), fever (>38°C) and were receiving extended spectrum antibiotics. Indwelling catheters were present in all four patients.
Methods: The resistance phenotype was performed by Kirby Bauer and microdilution methods according to NCCLS guidelines. ESBLS production was confirmed by Double Disk Test (DDT) and E-test : cefepime/cefepime–clavulanic (AB BIODISK, Solna, Sweden). Biotyping was based on Wider II (Francisco SORIA Melguizo, SA) automated system results. The ERIC II PCR was used for the molecular typing of the isolates.
Results: Although epidemiological data (time and space clustering) as well as phenotypic data (all strains showed the same resistant phenotype and the same biotype) were consistent with possible clonal spread of the same isolate in all patients, molecular typing revealed that only two out of the four *E. coli* ESBLS producing strains (isolated from patients who shared the same room) were similar and thus could be associated as part of the same outbreak. The remaining two strains had different molecular patterns.
Conclusion: This study supports the clue that classic microbiological studies (colonial morphology, biotype and resistance phenotype) as well as molecular typing methods are both necessary and must be performed to investigate and reveal an outbreak of infection in a hospital setting.

R2035

A prospectic nosocomial infection – survey in a medical department in Italy

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Objectives: Measuring the incidence of nosocomial infections and microbial isolates in an Internal Medicine 23 bed-Unit in a 100-bed hospital.
Methods: Data were collected according to the NNIS protocol using a dedicated software from Epinfo program.

Results: From October 2002 to December 2003 (for a 14-month term), 1920 patients were enrolled (55% male and 45% female, median age: 76) for a total of 8530 patient-days; 78% of the patients had one or more comorbidities. The infections were 36 in 33 patients (1.71% of the total cases): 20 Urinary tract infections (UTI), 18 of which were catheter-related, 10 pneumonia, 5 blood stream infections (BSI), 1 of which Central Line Catheter-associated, 1 case of Pseudomembranous colitis. The device-utilization ratios were: 1.2% for CVC with an average catheterization of 7 days (103 central line-days); 19.9% for urinary catheter with an average catheterization of 5 days (1702 urinary catheter-days). A Central Line Catheter-associated BSI developed in 8% of patients exposed to CVC with a central line-associated BSI rate of 9.7 ‰. An UTI developed in 6.1% of patients exposed to a urinary catheter with an urinary catheter-associated UTI rate of 10.5 ‰. One patient died because of nosocomial pneumonia (0.9% attributable mortality), 7 patients died having been diagnosed a nosocomial infection. The microbial etiologies of UTIs were: Gram negative in 11 cases, Gram positive in 10 cases, *Candida albicans* in 3 cases (4 patients showed two contemporary pathogens isolated); BSIs *Staphilococci* in 3 cases, Gram negative in 1 case, 1 case of Clinical Sepsis.
Conclusions: Our study, comparing NNIS data, shows that nosocomial infections may be a problem also in Hospital Units not usually considered at high risk, probably due to advanced age and multiple comorbidities of the patients admitted. Despite a urinary catheter-utilization ratio within the norm, we observed a high incidence of UTI, which however showed an approximate 10% decrease during the period of our research.

R2036

Epidemiological aspects of hospital-acquired pneumonia in intensive care units in the far east of Russia

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Objectives: Surveillance of nosocomial infection in the intensive care unit (ICU) received a high level of attention and outcome indicators are now used in benchmarking the quality of patient care.
Methods: Continuous prospective data collection was conducted on 4602 patients admitted to at the intensive care unit of the Second State Vladivostok Hospital in 2000–2003.
Results: The most numerous group consists from 1377 (29.9%) patients with neurological diseases, 40.8% of them were after 50 years and 7.7% (106) were children. The 73.1% of patients among adult ones were males and 68% of patients among children were boys. Children and age patients with chronic infections, severe conditions were the main groups of risk. There were 580 patients (42.1%) including 19 children with lethal outcome. The main reason of lethal outcome in adults were severe combined and skull-brain traumas (75.9%), acute disturbance of brain blood circulation, and infectious disease (5.5%). The severe combined and skull-brain traumas also have taken the leading positions among children. During last 3 years 15 children (79%) have died of this reason, 10.5% of children have died of the infectious disease, and the others have died of the acute disturbance of brain blood circulation and tumors. The 25% of adults and 47.4% of children died of the traumas during the first hours. About 53% of adults and 86.7% of children spent in the ICU more than 5 days. The main causative pathogens of hospital-acquired pneumonias were *E. gergovia*, *S. aureus*, *P. aeruginosa*. The most strains of *E. gergovia* was resistant to amikacin, ampicillin, amoxicillin, gentmycin, tobramycin, carbenicillin, cefalotin, cefalexin.The most frequent causative pathogen in children was *S. aureus* which was resistant to penicillin, ceftazidim. But also it was sensitive to cefobite, oxacillin, amoxicillin, gentomycin, lincomycin, chloramphenicol, erythromycin, doxicyclin. *P. aeruginosa* takes the second place in the ethiological structure of the causative pathogens of hospital-acquired infections which was resistant to cefalexin, cefalotin, carbenicillin, tetracycline, tobramycin, levoflaxacin.
Conclusions: There are some problems in registration of laboratory results of anaerobic infections which are connected with absence of specialists. Also it leads that there is the large group of undiagnosed infections which complicates treatment and increases the frequency of the lethal outcomes.

R2037

Unusual bacteraemia-associated pathogens: a 12-year experience

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During the period 1992–2004, Microbiology Dept of 'Sotiria' Chest Diseases Hospital of Athens had an interesting experience of several not widely known bacteraemia-associated isolates. These were as following:

- *Achromobacter xylosoxidans*, following an interventive cardiologic examination. Outcome: death
- *Pasteurella multocida*, in one patient with COPD and in one patient with myelosclerosis. Outcome of both cases: death
- *Burkholderia pseudomallei*, in a patient traveling from S. Asia, on the grounds of pneumonia caused by the same pathogen. Outcome: favorable
- *Cardiobacterium hominis*, in a patient with endocarditis. Outcome: favorable
- *Yersinia enterocolitica*, in a multi-transfused patient. Outcome: favorable
- Beta-haemolytic streptococcus group B, in one patient with endocarditis, in a second patient with lung cancer and in a third patient with diabetes mellitus. Outcome: favorable for the first two cases, death for the third
- Beta-haemolytic streptococcus group A, in an intravenous drug-abusing patient. The patient left hospital latently. Outcome: unknown
- *Serpulina pilosicoli*, in a critically ill patient with gastroenteritis. Outcome: death
- *Cryptococcus neoformans*, in a chronic lymphocyte leukaemia patient, and in a second patient with sarcoidosis. Outcome: death for the first, and favorable for the second patient
- *Radiobacter rhizobium,* in a patient with myelosclerosis. Outcome: death
- Pseudomonas testosteroni, in one patient with cardiac valve insufficiency and in a second patient with acute abdomen. Outcome: favorable in the first case, death in the second Given the fact that new pathogens emerge as causative factors of bacteraemia, the microbiology laboratory should be alert and able to detect, isolate and identify them.

R2038

Pseudomonas aeruginosa colonisation in adult patients with cystic fibrosis: antimicrobial susceptibility pattern

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Objectives: To evaluate the prevalence of infection and/or air way colonization by *Pseudomonas aeruginosa* from September 2003 to September 2004, in cystic fibrosis (CF) diagnosed patients, and to determine the susceptibility in respiratory isolates.

Methods: Forty-two patients with cystic fibrosis, 21 male and 21 female, with an average age of 26.60 years. 78.6% with pancreatic insufficiency. The patients came from the monographic unit of adults with C.F. The sputum samples were cultured following standard microbiological procedures. Colonies quantification in OFPBL agar, saboureaud chloramphenicol agar, blood agar, chocolate agar, chocolate agar with bacitracin, MacConkey agar, mannitol salt agar and Gram stain was performed. Isolates were identified by conventional test and an automated system (Microscan, Dade-Behring) The antimicrobial susceptibility was determined by an automatic system (Micro-scan) and disk diffusion method according to NCCLS recommendations.
Results: The prevalence of infection by *Pseudomonas aeruginosa* was 47.6%. It was isolated in 20 patients, 10 females and 10 males, with an average age of 23.75 yrs. The number of *Pseudomonas aeruginosa* isolated in the 20 patients during the study period was 81. 35% of patients were also colonizated by *Aspergillus fumigatus*, 30% by *S. aureus* methicillin sensitive, 15% by *S. aureus* methicillin resistant and 15% by *Haemophillus* sp. The antimicrobial susceptibility obtained is shown in the table.

	Sensitive		Intermediate		Resistant	
Antimicrobial agent	Number	%	Number	%	Number	%
Gentamicin	34	42	10	12	37	46
Tobramycin	58	72	14	17	9	11
Amikacin	48	59	2	2	31	38
Piperacillin	64	79	3	4	14	17
Piperacillin/tazobactam	65	80	7	9	9	11
Ciprofloxacin	47	58	11	14	23	28
Ofloxacin	39	48	9	11	33	41
Ceftazidime	63	78	1	1	17	21
Cefepime	51	63	13	16	17	21
Aztreonam	54	67	8	10	19	23
Imipenem	59	73	5	8	17	21
Colistin	100	100	–	0	–	0

Conclusions: A high frequency of *Pseudomonas aeruginosa* colonization was found in patients with cystic fibrosis. The 100% of *Pseudomonas aeruginosa* strains were susceptible to colistin in vitro. The highest resistance rate was shown by gentamicin.

R2039

Adhesion to catheters and slime production of *Staphylococcus haemolyticus* isolated from hospitalised patient occurrence of antibiotic susceptibility

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Objectives: Last ten years studies showed participation of Coagulase Negative Staphylococci (CNS) in pathogenicity of many diseases. The reason of growing interest about CNS as the ethiological infectious factors can be seen in increasing number of patients from high risk group and use of the invasing diagnostic and clinical methods. The most exposed to the CNS infections except patients with artificial implants. The best characterized CNS pathogenic factors are ability to tissue colonization, adhesion to the synthetic surfaces and evasion. There is a fundamental stage in the pathogenicity of CNS infections. These properties are caused by the cell wall ingredients (teichoic acid), and also by integral substances associated with bacterial cell such as extracellular polysaccharide (slime). The study have been done on *S. haemolyticus* strains isolated from patient hospitalized on Surgical Unit. Aim of the study was to determine pathogenic traits of *S. haemolyticus*: slime producing, adhesion to biomaterials, antibiotics susceptibility.
Methods: Slime production has been examinated by means of two methods according Christiansen. Adhesion of *S. haemolyticus* strains was analysed according to Richards method assessing the value of substrate TTC reduced to isolube red formazan. Susceptibility to antibiotics was determined using the disc-diffusion method.
Results: Among 44 *S. haemolyticus* strains, in the test-tube method, there have been 38% labeled as slime producing and 62% as non-producing. In the plate method at 48% slime production was noticed, while 52% strains did not produce slime. 7% of analysed strains were found to have TTC reduction of 3+, 77% of 2+ and 14% of 1+. Among these 2% of the assessed strains did not reduce TTC.
Conclusions: It is quite significant that all strains which have an ability to slime produce, that was proved by means of two methods (test-tube and plate), show a high level of TTC's reduction to formazan. The analysis of resistance to antibiotics in relation to slime production demonstrated more frequent antibiotic resistance of the slime-producing strains. A detailed study into the mechanism and adhesion factors of CNS staphylococci can help prevent infections caused by these microorganism.
Acknowledgments: This study was supported by the Polish Committee of Scientific Studies (KBN Grant No 2 P05D 134 26).

R2040

Follow up of MRSA (methicillin-resistant *Staphylococcus aureus*) after discharge from the hospital

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Objective: In 2004 the VU medical centre started active follow-up of patients known with MRSA contamination in the past. The MRSA status was determined and if necessary treatment was offered. MRSA carriership in the past is a reason for strict isolation measures. With this programme we hope to reduce the impact of MRSA on the environment and future hospital admissions.
Method: Of each patient a file was made with following items: general data, case history, risk factors, results of culture, results of molecular typing, agreements made with the treating doctor or general practitioner. For each patient was decided which approach was best to be followed: contacting the patient at policlinic visit, an approach via the general practitioner or via other hospitals. Signalling of MRSA positive patients was started by a hospital monitoring system. Entering the system with the patient's identification results in a warning concerning the MRSA status.
Results: Of 66 MRSA patients admitted since 1998 the current MRSA status was unknown in 2004. From March 2004 a total of 12 patients have been declared MRSA-free. For 12 patients the follow up is still in progress. 3 patients were known to be deceased. 27 could not be traced; reasons could be that they lived abroad and were admitted in our hospital only once or changed general practitioner or were possibly deceased. The general practitioners of 15 patients were asked to contact the patients. Nine of them agreed to do so. Three patients have cooperated and could be declared MRSA-free. They are included in the 12 mentioned above. The other patients refused to participate in the programme.
Conclusions: An active follow-up system is time consuming, but will eventually reduce isolation measures and thereby costs. Furthermore, It will be less aggravating for the patients. A monitoring system can simplify the tracking down of patients. The approach by means of the general practitioner takes a lot of

effort and is relatively unsuccessful. Hence, it is important that at the time of detecting MRSA, a clear route of follow up is agreed with the patient and the treating doctor. The general practitioner should also be involved. Moreover, it is important to give the patient enough information in order to be certain of good cooperation.

R2041

Molecular characterisation of MRSA outbreaks in a children's clinical university hospital

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Objectives: To characterize MRSA isolates by PCR- based methods.
Methods: Antimicrobial susceptibility of the MRSA isolates was determined according to NCCLS standards by disc-diffusion method. Presence of the mecA, clfA and lukS-lukF genes as well as the type of SCCmec were determined by PCR. MRSA strains were typed by restriction fragment length polymorphism (RFLP).
Results: MRSA isolates (n = 20) from 17 patients were collected at Children's Clinical University Hospital from November 2003 to September 2004. Eight isolates originated from intensive care unit and 4 from neonatal unit. All but one MRSA isolates carried type III SCCmec. The 11861-122 clone possessed type IV SCCmec. None of them, including 11861-122, was PVL positive. RFLP typing revealed two evident groups of 11 isolates from 9 patients in one and 5 isolates from 4 patients in the other, respectively.
Conclusions: Molecular data strongly suggest that two separate outbreaks could have occurred over the study period.

R2042

Outcome of coagulase-negative staphylococci bacteraemia in an intensive care unit

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Objectives: The aims of this study were to determine the prevalence of coagulase- negative staphylococci bacteraemia (CNSB), their clinical characteristics and prognosis in critically ill patients in an intensive care unit.
Material and methods: From 1996 to 2004, 226 patients with nosocomial bacteraemia admitted in an intensive care unit were prospectively evaluated. Clinical characteristics and global and related mortality of cases produced by CNE are presented. A multivariate analysis was performed to determine the influence of CNE on related mortality.
Results: Thirty-eight (16.8%) of 226 nosocomial bacteraemias were due to CNB. The mean age of patients with CNSB was 67.4 ± 9.8 years and the relation between men/women was 1.9. Thirty-six (94.7%) of CNSB were considered primary bacteraemias (11 cases related to catheter infections and 25 of unknown origin); the origins of the other 2 cases were: 1 abdominal and 1 cutaneous. The mean APACHE II score at the onset of bacteraemia was 15.4 ± 6.8. Severe sepsis or septic shock was present in 57.8%. The incidence of inadequate empirical antibiotic treatment was 42.1%, and the related mortality rate for CNSB was 15.7% (not significantly different from the mean of mortality by other significant microorganisms, 23.4%). By multivariate analysis the factors related to mortality were septic shock, inadequate empirical antimicrobial treatment, Staphylococcus aureus bacteraemia and Apache II, but not CNSB.
Conclusions: CNSB is a significant cause of mortality in critically ill patients with nosocomial bacteraemia. Almost all cases were considered primary bacteraemias, probably related to intravascular invasive procedures. Frequently, patients with CNSB are given inadequate empirical antimicrobial treatment.

R2043

Susceptibilities of isolates of *Enterobacter cloacae* and *Citrobacter freundii* to seven broad-spectrum antibacterial agents: results of the Antimicrobial Resistance Surveillance Study of the Paul-Ehrlich-Society for Chemotherapy, 2001

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Objectives: *Enterobacter cloacae* and *Citrobacter freundii* frequently cause nosocomial infections. Both species produce chromosomal AmpC beta-lactamases. In addition, strains may become resistant to other antimicrobial drug classes. The objective of this study was to evaluate the in vitro susceptibilities of clinical isolates of *E. cloacae* and *C. freundii* to various frequently used broad-spectrum antibacterial agents.
Methods: In November 2001, a total of 234 isolates of E. cloacae and 73 isolates of *C. freundii* were prospectively collected from 26 microbiology laboratories distributed throughout three Central European countries (Austria, Germany, and Switzerland). Minimal inhibitory concentrations of ceftazidime, cefepime, imipenem, meropenem, piperacillin-tazobactam, ciprofloxacin, and tobramycin were determined by the broth microdilution method according to the standard of the German DIN.
Results: Ninety-three and 187 isolates were collected from patients in intensive care units (ICUs) and non-ICU inpatient areas, respectively. The remainder were either from outpatients or data were not available. Susceptibility patterns of *E. cloacae* isolates showed the highest susceptibility to meropenem (99.6%) followed by imipenem (98.7%), cefepime (95.3%), and tobramycin (94.4%). The susceptibility to ciprofloxacin was 89.7%, whereas susceptibilities to ceftazidime and piperacillin-tazobactam were each below 70%. Meropenem (100%), imipenem (98.6%), and cefepim (93.2%) were also the most active compounds against *C. freundii*. The susceptibilities to ciprofloxacin and tobramycin were 91.8% and 89.0%, respectively, and again susceptibilities to ceftazidime and piperacillin-tazobactam were each below 70%. Rates of resistance (%) were as follows: *E. cloacae* - ceftazidime 20.9, cefepime 2.6, imipenem 0.4, meropenem 0.4, piperacillin-tazobactam 12.0, ciprofloxacin 7.7, and tobramycin 3.0; C. freundii - ceftazidime 31.5, cefepime 2.7, imipenem 0, meropenem 0, piperacillin-tazobactam 17.8, ciprofloxacin 5.5, and tobramycin 5.5. For the two species combined, susceptibilities to ceftazidime and piperacillin-tazobactam were significantly lower ($p < 0.05$) among isolates from ICU patients (59.1% and 55.9%) than among isolates from non-ICU inpatients (71.1% and 70.1%).
Conclusion: Carbapenems and cefepime seem to be the most active antimicrobial agents against isolates of *E. cloacae* and *C. freundii* recovered from patients in hospitals located in Germany, Austria, and Switzerland.

R2044

blaVIM-2 *Pseudomonas aeruginosa* isolates in a newly established intensive care unit

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Metallo-β-lactamases are emerging worldwide as acquired resistance determinants in nosocomial strains of Gram(–)

bacteria. The results of the present study suggest the risk factors for VIM appearance in an Intensive Care Unit.

Material and methods: We identified the first 35 patients (20 male, 15 female), who were admitted in a newly established ICU during 3 month period. Tracheobronchial aspirates and urine cultures, were obtained every day during the first three days, and then, every second day after their admission to the ICU. Identification was performed with the PASCO system used according to the manufacturer's instructions. MICs of antipseudomonal drugs, except carbapenems, were determined with the PASCO microdilutionsystem and applying the criteria prescribed by the NCCLS. Susceptibility to imipenem and meropenem was determined by agar disk diffusion in accordance with NCCLS recommendations. A blaIMP, blaVIM specific product was amplified by PCR with crude DNA extracts using published primers and conditions. Qualitative data were expressed as percentages and compared by chi-square test. We considered $p < 0.05$ to be statistical significant. The p values are based on 2-tailed test results. Statistic analyses were performed using logistic regression (SPSS).

Results: We isolated totally 43 multiresistant strains. 33 of those was *P. aeruginosa* strains,5 was *K. pneumoniae* and 5 *A. Baumannii*. All the *P. aeruginosa* strains were imipenem resistant, and among those only nine (27%), were blaVIM-2 positive. All the strains of *K. pneumoniae* and *A. Baumannii*, were blaVIM-2 negative by PCR. Among the total of 9 blaVIM-2 positive *P. aeruginosa* isolates collected, 22% were from urines and 78% were from bronchial secretions.

Discussion: Risk factors for VIM appearance include: (A) Carbapenems administration (n = 0.002), fact that may be due to the augmentation in selections of the above genes under the press of the specific drug. (B) Administration of several β-lactam derivatives (n = 0.005), aminoglycosides (n = 0.039), and combinations between carbapenems-aminoglycosides (n = 0.026). The statistical significant results of aminoglycoside's administration, may be due, to the augmentation of selection press (the same integron contain the blaVIM gene and three different aminoglycoside resistance determinants, which carried on mobile gene cassettes (aphA15 aacA29A, aacA29). (C) Long length of ICU stay, is a risk factor for blaVIM-2 positive strains, appearance (n = 0.015).

R2045

Urinary tract infections in patients with spinal cord injury

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Objectives: Urinary tract infections in patients with spìnal cord injury (SCI) is the most frequent complication due to vesical neurogenic alteration. It is enhanced by the use of permanent urethral catheters with deficient vesical voiding and frequent hospitalization. The objective of this study is the update of ethiology and antimicrobial susceptibility in urinary tract infections in this group of patients.

Methods: Over a six year-period (1998–2003) a total of 176 866 urine specimens were cultured. About 8013 corresponded to patients of the SCI unit. Antimicrobial susceptibility was performed by authomatized microdilution and in special strains by disk diffusion method.

Results: In the SCI patients 56.5% urine specimens were positive and 43.5% negative or contaminated. Bacterial species most frequent were *E. coli* (35.0%), *P. aeruginosa* (12.6%), *Enterococcus* spp. (9.8%), *P. mirabilis* (9.8%), *Klebsiella* spp. (7.9%), *P. stuartii* (5.7%) and *A. baumanii* (5.4%). Isolation of Enterobacteriaceae with extended spectrum beta-lactamase (EEBL) in SCI patients has increased considerably (47 patients in the last three years), being the hospital unit with the highest number.

Conclusions: 1. Positive results were considerably higher in SCI patients (56.5%) compared with the rest of patients (21.3%). 2. Percentage of *E. coli* isolates is lower than in other patients (35.0% vs. 57.2%), due to the increment of urinary infections by nosocomial species. 3. The elevated percentage of *P. aeruginosa* can be due to the use of urethral catheters. 4. *P. stuartii* is an important urinary pathogen in SCI patiens, with a degree of isolation 20 fold higher than in the rest of patients and with antimicrobial multiresistence. It is the second species with EEBL, after *E. coli*. 5. Persistence of EEBL strains in these patients is a reservoir difficult to erradicate. 6. SCI patients present a high degree of colonization by *A. baumanii* and *S. aureus* meticillin resistant. 7. Risky sport activities and traffic accidents are increasing the number of SCI patients, affecting mostly young people. Patients cooperation is essential in the prevention of urinary tract infections.

R2046

Serum amyloid A and brucellosis

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Objectives: Brucellosis remains an illness with a wide clinical spectrum and universal distribution; Greece, in particular, is one of the most endemic regions worldwide. Diagnostic procedure and laboratory evaluation is a challenge. Inflammation markers can be used to evaluate the clinical state and the outcome of the disease.

Methods: The study included patients admitted with suspected infection in the Emergency Department. Serological evaluation was performed using Brucella agglutination test (Wright test). A titre of 1:160 or higher was considered significant and titres of 1:40 to 1:80 suspicious despite the endemic characteristics of our area. Blood cultures were obtained independently of STA titres. Clinical manifestations were recorded and evaluated. Any organ involvement was referred as localized disease. Serum Amyloid A (SAA) measurements were performed using nephelometric assays (Dade-Behring) and values higher than 6.0 mg/L were considered indicative of high inflammatory state.

Results: SAA was measured in 26 patients with culture-proven brucellosis. 23 patients (88%) had values >6.0 mg/L. Average positive value 232.59 mg/L (SD 299.51 mg/L Confidence interval 95%). Patients with anemia presented with values >294.59 mg/L and patients with osteoarticular involvement >258 mg/L. Any localized disease was associated with higher levels of Serum Amyloid A while febrile illness alone did not present with such values.

Conclusions: Although our sample represents a pilot study measurement, SAA seems to be an interesting inflammatory marker in brucellosis and can be associated with localized infection and hematopoiesis disorders in terms of anemia. Such findings may explain anemia of brucellosis, involving other cytokine production and modulating bone marrow hematopoetic progenitor cells. Further study is required in order to elucidate this enigmatic complication.

R2047

Accessing quality in infection control surveillance

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Objectives: To evaluate adequacy in activities executed by health care professionals in a 1,200-bed teaching Brazilian hospital.
Methods: Study performed during the period of June 2003 to October 2003 designed to evaluate the level of adequacy related to four main procedures: urinary catheter care, intravenous catheter care, and dressings and health care facilities care. Adherence to barrier precautions was also evaluated. This study was composed of two parts. The first part was an observational study. In this phase, performed by the infection control nurses, the observations were registered in a standardized case report form. The second component of the study was an intervention: the results were presented to assistant professionals, who were also trained. After that, new observations were taken.
Results: The proportion of non-conformity was 59.0%, 75.6%, 68.4%, and 71.4% for procedures involving urinary catheters, intravenous catheters, catheter dressings and material cleaning, respectively. After training, the global level of adequacy increased to 66.6%. At this moment, trained health care professionals are evaluating the quality of assistance and making new observations, and their work is being audited by infection control nurses.
Conclusions: As infection control professionals do not provide direct care to patients, the value of their practice must be demonstrated in other ways, such as stressing quality and economy. These professionals can not be seen as a merely 'data-gatherers'. Demonstrating value in infection control programmes has become a very important issue; according to this statement, the acquisition of new skills should be one of the priorities. It is important to consider how to help optimize costs, serve the customer better, improve productivity, and innovate, mainly in areas of limited resources.

R2048

Five-year surveillance of nosocomial infections in a training and research hospital in Ankara

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Objective: To assess the rate of nosocomial infections, frequency of nosocomial pathogens and antimicrobial susceptibility changes in our hospital in a 5-year period.
Methods: Laboratory based nosocomial infection surveillance was performed in 530-bed hospital between 1999 and 2003. Results of the cultures taken from hospitalized patients evaluated daily by bed-side visits. Nosocomial infections were defined according to the CDC definitions for nosocomial infections and nosocomial surgical site infections. Nosocomial infection rate was calculated by number of nosocomial infections per number of hospitalized patients for every year. Antimicrobial susceptibility tests were performed according to NCCLS.
Results: Nosocomial infection rates were between 1.4 and 2.4%. High nosocomial infection rates were observed in neurology, neurosurgery, pediatry and dermathology clinics in changing orders. The most common infections were urinary tract infections, surgical site infections and primary bacteraemia in every year of surveillance.The most frequent pathogens were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* spp, and *Staphylococcus aureus*. Carbapenems were the most effective antimicrobials against Enterobacteriaceae isolates and more than 609% of *E. coli* and *K. pneumoniae* isolates were susceptible to aminoglycosides, quinolones and cephalosporins with some slight decline in years. Methicillin resistance of *S. aureus* isolates were less than 50% and all *S. aureus* and *Enterococcus* spp. isolates were susceptible to glycopeptides except one glycopeptide resistant *E. faecium* isolate identified in 2003. Chloramphenicol was the second most effective agent after glycopeptides against *S. aureus* isolates.
Conclusion: Nosocomial infection surveillance provides tracing the hospital specific health care for hospitalized patients and supplies data regarding which precautions should be taken. Our low percentage of nosocomial infection rates was due to our method of surveillance. However, following the data by the same method provided both comparisons between years and the changes occurred recently. Nosocomial pathogen isolation and antimicrobial susceptibility tests provided guidance to empirical antimicrobial therapy of nosocomial infections and determining outbreaks.

R2049

Outbreak of glycopeptides *Enterococcus faecium* infection in a university teaching hospital in Clermont-Ferrand, France, 2004

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Objectives: To report the first outbreak of glycopeptides-resistant *Enterococcus faecium* (GR-*E. faecium*) infection in a university tertiary-care hospital, Clermont-Ferrand, France, and to identify risk factors for GR-E. faecium infection/colonization.
Methods: Patients were identified through the hospital surveillance system focused on nosocomial and/or multi-resistant bacterial infections. Resistance to glycopeptides was defined by the laboratory of Bacteriology (MIC vancomycin > 256 mg/l and MIC teïcoplanin at 32–64 mg/l). A nested case–control study was restricted to 16 patients hospitalised at the St Vincent ward including the infectious diseases and haematology unit and who were diagnosed with GR-*E. faecium* colonization or infection from the 1st of January to the 31 of July 2004. The control group was derived from a randomized sample of patients who were hospitalized in the ward during the same period. All strains were sent to the National Laboratory Centre for Streptococci to identify the PFGE patterns.
Results: 30 patients were identified in 14 different wards between the first of January 2004 and the 31st of October 2004: the epidemic curve peaked in April and in September. GR-*E. faecium* was isolated from blood in 6 patients (2 pts died), from urines in 16 pts (11 with a urinary catheter, 5 without catheter), from peritoneal fluid (2 pts), from IV catheter (1 pt), from wounds (2 pts), from drainage tubes (3 pts). All GR-*E. faecium* infections were hospital-acquired. Multivariate analysis, controlling for time at risk and the Charlson Index of Co morbidity, identified prior exposure to IV 3rdG cephalosporin (OR = 10; 95%CI: 1.5–64; $p = 0.02$), antimicrobial regimen with a beta-lactamase inhibitor (OR = 5; 95%CI: 0.9–28; $p = 0.07$) and urinary catheter (OR =20; 95%CI: 1.3–291; $p = 0.03$) to be independently predictive for GR-*E. faecium* infection or carriage. Clinical isolates were also resistant to amoxicillin, macrolides and clindamycin, but susceptible to linezolid, vibramycine and intermediate to aminoglycosides, quinupristin-dalfopristin. All PFGE patterns, but one, were clonally related.

Conclusion: Clonal emergence and diffusion of GR-*E. faecium* is likely to be a result of antibiotic pressure and cross-transmission. As a result, strict recommendations regarding isolation of the patients with multi-resistant bacterial infections and restrictions in the prescription of broad-spectrum antibiotics have been implemented in our hospital.

R2050

Genotypic analysis and occurrence of extended spectrum beta-lactamases (ESBL+) producing *Klebsiella* spp. strains in a neonatology unit – 1 year study

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Objectives: To determine the occurrence of ESBL+ *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated from newborns and the genetic relatedness between strains.
Material and methods: A total of 28 *Klebsiella pneumoniae* (K.pn.) and 28 *Klebsiella oxytoca* (K.ox.) was isolated from various material (blood, urine, swabs from nose, throat, anus) from newborns hospitalized in Intensive Care Unit and Neonatology Unit between April 2003 and March 2004 were tested for detection of ESBL – double disc test. Strains of *Klebsiella* sp. were submitted to a molecular characterisation by pulsed-field gel electophoresis (PFGE) with using the enzyme Xba I.
Results: 50% of K.pn. and 60% of K.ox. strains produced ESBL. For K.pn. three clonal epidemic strains were found: type A included 9 strains (recovered from 7 newborns), type B – 6 strains (5 newborns), type C – 4 strains (3 newborns), type D – 2 strains (2 newborns) and 8 unique unrelated strains. For K.ox. described three types: type A included 21 strains (15 newborns), type B – 2 strains (2 newborns), type C – 2 strains (2 newborns) and 3 strains unrelated.Type A of K.pn. was isolated between July 2003 and March 2004, type B was isolated between June 2003 and February 2004, type C was recovered from newborns between November 2003 and January 2004, type D was isolated once in August and once in September 2003. Type A of K.ox. was isolated between July 2003 and March 2004, type B was isolated in September 2003 and type C was isolated in July 2003 and in November 2003. All clonal epidemic strains of K.pn. and K.ox. produced ESBL opposite to unrelated strains. Clonal strains were isolated from both Intensive Care Unit and Neonatology Unit. Some of them were responsible for infection and colonization also.
Conclusion: Isolation of the same genetic types from different newborns and units during 1 year proves spreading and remaining of the *Klebsiella* clones in Neonatology Unit. This epidemiological situation should be under full control of hospital infection committee.

R2051

Mechanisms of nosocomial *S. aureus* resistance to beta-lactam antibiotics

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Introduction: Of the various mechanisms of acquired *S. aureus* resistance to (β-lactams, resistance due to (β-lactamases is the most prevalent. Alterations in the preexisting penicillin-binding proteins (PBPs), acquisition of a novel PBP insensitive to (β-lactams can also confer resistance to methicillin and (β-lactams. These changes in the outer membrane of proteins include production of supplement PBP (PBP2a or PBP 2') that are encoded by a chromosomal mec gene (mecA, mecB).
Objective: The study of both resistance mechanisms to α-lactams of nosocomial strains of *S. aureus*.
Methods: Over a period of 3 years 340 strains have been tested. All the tests have been performed with the following methods: (1) oxascreen agar (Bio-Merieux), a Muller–Hinton agar with NaCl 4% and oxacillin 6 mg/ml, for detection of the resistance to oxacillin. (2) MRSA test (Bio-Merieux), an agglutination test for detection of PBP 2'. (3) Cefinase discs (Bio-Merieux), for detection of (β-lactamase production. (4) Etest (Solna-Sweden), for detection of penicillin and oxacillin MICs.
Results: Out of 340 strains of *S. aureus*, 248 (72.09%) were resistant to (β-lactam antibiotics.Out of the latter, 85 strains (34.27%) have shown resistance, which was due to the production of (β-lactamase. Furthermore,134 strains (54.03%) have shown resistance which was due of the alterations in penicillin-binding proteins. Finally, 29 strains (11.69%) have shown resistance which was due to both mechanisms.
Conclusions: (1) High percentage of nosocomial *S. aureus* strains was resistant to (β-lactams.
(2) In this study, the resistance mechanism of change of the PBPs proteins to PBP2' was the most frequent.

R2052

Computerised surveillance of nosocomial infections at a university hospital

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Objectives: Computerized surveillance of ventilator associated pneumonia (VAP), catheter related bloodstream infections (BSIs) and surgical site infections (SSIs) by treating physicians at a German university hospital: implementation, quality assurance and time as well as cost related aspects.
Methods: Two computer-based surveillance input masks were developed with the Department for Clinical and Administrative Data Processing of the Giessen University Hospital (AKAD), one for VAP and BSIs and one for SSIs. The VAP and nosocomial BSIs were protocoled and identified by the ward physicians and surgical sites were assessed upon dismissal under surveillance of the infection control unit. This was accomplished for six intensive care units and seven surgical departments. Indicator operations were chosen according to the operations given by the National Reference Center for surveillance of nosocomial infections (NRZ). The input masks were based on the criteria of the Centers for Disease Control and Prevention (CDC) and the OP-KISS (Krankehaus Infection Surveillance System) module of the NRZ. For both input masks, several data components could automatically be taken out of other computer programs. The infection control staff examined the documentation via intranet. The results were discussed with the departments during surveillance ward rounds. Work time was evaluated using log-tables and an external quality control was performed during a routine phase for 15 weeks.
Results: Nearly complete documentation was achieved by the physicians and/or the medical documentation officers. Type and infections rates of VAP, BSIs and SSIs concerning indicator operations will be given and compared to NRZ data. Also data on work time and personnel costs as well as quality assurance will be presented.
Conclusion: Due to the simple and computerized data assessment, personnel time concerning surveillance is very low compared to the non computer assisted surveillance and showed comparable infection rates with national data.

R2053

Prevalence of extended-spectrum beta-lactamases in a university hospital

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Objective: Nowadays resistance against the drug is growing problem in all over the world. In this study we aimed to determine the prevalence of extended spectrum beta-lactamases (ESBL) which responsible of nosocomial infections at the Dicle University Hospital which is the main hospital based in South Eastern of Turkey.
Methods: A total of 182 g negative bacterial which were isolated from clinical specimens to 2 years according to Center for Disease Control (CDC) criteria were included the study for to determine prevalence of ESBL as nosocomial infection at the Dicle University Hospital. ESBL were determined in hospital based gram negative bactericea (GNB) which resistant at least against on of ceftriaxone, ceftazidime and cefotaxime, by E-test.
Results: A total of 182 GNB were studied by *E*-test and 62 (34%) were found to be ESBL producers. ESBL was detected in 24 of *Klebsiella* spp., 14 of *Escherichia coli*, 13 of *Pseudomonans* spp., 4 of *Enterobacter cloaca*, 4 of *Acinetobacter* spp., 2 of *Proteus mirabilis* and 1 of *Serratia marcescens*.
Conclusion: This is the second study on ESBL production at the Dicle University Hospital and 34% GNB were confirmed to be ESBL producers. In the first study this ratio were reported to be 31%. Infections with ESBL producing organisms can pose a therapeutic challenge; leading to treatment failure in wrong class of antibiotics is used. As a result it is very important that each hospital should create their rational antibiotic management strategies.

R2054

P. aeruginosa susceptibility differences among specialised hospital units: MYSTIC Program Brazil 2004

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Objective: To compare the susceptibility patterns of *P. aeruginosa* clinical isolates for meropenem, cefepime, amikacin, and ciprofloxacin in different specialized hospital units in the MYSTIC Program Brazil 2004.
Methods: Nineteen specialized hospital units in Brazil participated in the 2004 programme edition. Centres were intensive care (ICUs) (12), neutropenic patients (2) and general ward (5) units. Minimum inhibitory concentrations (MICs) were determined in 471 *P. aeruginosa* clinical isolates to meropenem, cefepime, amikacin, and ciprofloxacin, by Etest methodology according to manufacturer's instructions. Interpretive criteria used were described by NCCLS document M100-S14. Susceptibility patterns of all isolates from ICUs (309), neutropenic patients (37) and general ward units (125) were determined and described. A chi-square test (Altman, 1999) was applied to identify differences in the susceptibility rates among the three unit types studied. p values below 0.05 were considered significant.
Results: Unit differences among the 471 *P. aeruginosa* clinical isolates are described. Overall resistance rates (%*R*) were 50.1% to meropenem, 50.7% to amikacin, 56.9% to ciprofloxacin, and 60.7% to cefepime. Higher resistance rates were observed in ICUs for all antimicrobials, except for amikacin. However, differences among resistance rates in specialized units were only significant for meropenem ($p = 0.03$).

Unit differences among resistance rates (%R) of 471 *P. aeruginosa* isolates MYSTIC Program Brazil 2004

	Resistant n (%)			
	ICU (n = 309)	Neutropenic (n = 37)	Ward (n = 125)	*P* value
Beta-lactems				
Meropenem	168 (54.5)	16 (43.2)	52 (41.6)	0.03
Cefepime	199 (64.4)	20 (54.1)	67 (53.6)	0.08
Aminoglycoside				
Amikacin	156 (50.5)	18 (48.6)	65 (52)	0.9
Fluoroquinolone				
Ciprofloxacin	179 (57.9)	18 (48.6)	71 (56.8)	0.6

Conclusions: Elevated resistance rates were observed to all antimicrobials, with higher overall susceptibility to meropenem. Meropenem resistance rates were higher among ICU isolates. This could be due to higher antimicrobial use or biased sample, since clonal spread or overvalued resistant clinical isolates could not be ruled out so far.

R2055

Predictors of outcome in patients with blood-stream infections

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Objective: To evaluate the epidemiology and risk factors of bloodstream infections (BSI), to determine the mortality associated BSIs and identify independent predictors of mortality.
Methods: This study was performed retrospectively, at the hospital from April 2002 to November 2002.
Results: 324 patients with BSI were enrolled to the study. Mean age was 41.7 ± 23.1 years old, and 196 (60.5%) were male. During study period 407 episodes occurred, of which 47.4% were nosocomial bacteraemia, 25.8% were community acquired bacteraemia, 22.2% represented contamination and 4.6% represented transient bacteraemia. Gram positive microorganisms were the most commonly isolated microorganisms (66.5%). Nosocomial BSI occurred in 134 (41.3%) patients. The most common pathogens nosocomial BSI group were coagulase negative *Staphylococcus* (29.2%), *S. aureus* (23.5%), *E. coli* (11.9%), *Acinetobacter* spp. (6.4%), *P. aeruginosa* (5.6%) and *Klebsiella* spp. (5.1%). Methicillin resistance was detected in 58% of staphylococcus isolates in nosocomial BSI group. 18.7% of the patients died. Mortality rate associated with bacteraemia obtained as 14.8%. Mortality rate in nosocomial BSI group obtained as 33.4%. Multivariate analysis revealed nosocomial acquisition, age > 60 years, hospital stay more then 7 days, stay in intensive care unit as factors associated with mortality rate.
Conclusion: These results indicate that eliminate factors influencing the outcome of bacteraemia especially in nosocomial bacteraemia. The prevention of nosocomial infections and appropriate antibiotic treatment can improve the prognosis of patients.

R2056

Antibiotic susceptibility patterns and serotypes of Streptococcus pneumoniae isolated from invasive and other infections at a university hospital in Turkey

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Objectives: To determine the antibiotic susceptibility and serotype prevalence of clinically significant S. pneumoniae isolated from a teaching hospital in Turkey.

Methods: The antimicrobial susceptibilities to nine antimicrobial agents of 61 clinical isolates of Streptococcus pneumoniae recovered from patients with invasive and other clinically significant pneumococcal infections from 2002 to 2004 were studied using E-test and disk diffusion methods following the NCCLS guidelines. Of the 61 isolates, 25% (n = 15) were from blood, 20% (n = 12) from bronchoalveolar-lavage, 16% (n = 10) from tracheal aspirate, 10% (n = 6) from cerebrospinal fluid, 8% (n = 5) from pleural fluid, 7% (n = 4) from endophthalmitis, 5% (n = 3) from peritoneal fluid, 3% (n = 2) from sinus aspirate, 3% (n = 2) from middle ear fluid, 2% (n = 1) from catheter, 2% (n = 1) from fistula specimen. Serogrouping of the pneumococcal isolates was performed using the Quellung technique (Statens Serum institute, Copenhagen, Denmark).
Results: Antibiotic susceptibilities and serotypes were shown in Table. Capsular types of 46 pneumococcal isolates belonged to types 23 (24%), 19(15%), 9(15%), 6(13%), 18(11%), 7(11%), 1(7%) and 3(4%).

Antibiotic susceptibilities of 61 isolates of *S. pneumoniae*

Antibiotics	Method	Susceptible no (%)	Intermediate no (%)	Resistant no (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Levofloxacin	Disk	51 (98)		1 (2)		
Meropenem	E-test	58 (95)	1 (2)	2 (3)	0.004	0.125
Cefotaxime	E-test	56 (92)	2 (3)	3 (5)	0.023	0.75
Chloramphenicol	Disk	51 (88)		7 (12)		
Clindamycin	Disk	46 (79)		12 (21)		
Tetracycline	Disk	45 (78)		13 (22)		
Erithromycin	E-test	44 (72)	2 (3)	15 (25)	0.032	3
Penicillin-G	E-test	35 (57)	22 (36)	4 (7)	0.047	0.75
Trimeth-sulph	Disk	26 (45)		32 (55)		

*In the disk diffusion method, intermediate-susceptible isolates were interpreted as resistant.

Conclusions: Antibiotic resistance was common in pneumococcal isolates in our region. Vaccine formulations can prevent the majority of pneumococcal infections.

R2057

Clostridium difficile infection in hospital: active surveillance

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Objectives: *Clostridium difficile* is a Gram positive anaerobic spore-forming rod. It is present in intestinal flora, in soil and it circulates in hospitals. *C. difficile* produces different potential virulence factors: a 308 kDa enterotoxin (toxin A) and a 270 kDa cytotoxin (toxin B). Toxin A is the major cause of enteric disease. Colitis can be determined by endogenous or exogenous causes and antibiotic treatment is the most important endogenous cause. The exogenous origin of the infection is referred to orofaecal contamination due to colonised patients. In 1997 an epidemic took place in a surgery division and this fact led to the formulation of an operative protocol for *C. difficile* associated infection. The operative protocol starts from CDC guidelines and is based on the enteric isolation of *C. difficile*. The protocol defines the hygienic procedures to be adopted in case of *C. difficile* associated infection. The target of the protocol is the prompt identification of the symptomatic patient and the subsequent application of procedures to avoid the pathogen's transmission.
Methods: Since May 1997, a surveillance programme is active for *C. difficile* associated infection and in 2004 the protocol's review was planned. Case definition: *C. difficile* associated infection is suspected in a patient with diarrhoea. In this case, the patients is investigated for presence of *C. difficile* and toxin A in stool: if one or both analyses are positive, the division is alerted to implement the protocol's directions and the epidemiological nurse is alerted too.
Results: We analysed the positive results for *C. difficile* infection related analysis from 2000 to October 2004.The study of the time-infection in the different divisions of the hospital showed that no new epidemic occurred and the positive cases are referred to isolated infections. Only in 2004 a small cluster in Geriatrics Division was reported, in occasion of the moving of the division.

Year	Positive results for *C. difficile* toxin A
2000	85
2001	31
2002	32
2003	39
Ocotober 2004	55

Conclusions: *Clostridium difficile* monitoring infections performed in hospital during last years and after the introduction of a dedicated protocol, confirmed the surveillance's effectiveness in infection's control. We believe that management of antibiotic related *C. difficile* infections based on a dedicated protocol, can avoid further epidemics.

R2058

G-control Charts. A novel method for monitoring contamination rates of blood cultures

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Objectives: Blood culture (BC) is the criterion standard for identifying patients with bacteraemia. However, elevated false-positive rates are common and are associated with substantial health care costs. We had monitored numbers of BC without Coagulase-negative *Staphylococci* (CNS) between blood cultures with CNS in a g-type control chart. Periods with deviation from the protocols for skin disinfection and for specimen's collection were compared with periods without deviations.
Methods: Results of blood cultures from tree hospitals had been exported from the laboratory system. The protocols for skin disinfections and for specimen's collection and inoculation are standardized for all hospitals. Periods with deviation from the protocols:
Amager Hospital (AH): From June 2000 to November 2001 alcohol swabs in separate packing were replaced with alcohol swabs in an open container, from witch alcohol evaporated.
Hvidovre Hospital (HH): From February to September 2004 alcohol swabs were replaced with iodine and gloves were recommended at blood culture sampling.
Bispebjerg Hospital (BBH): Needle holders were thermal disinfected between every blood sampling.
g-Control chart: The centre line CL illustrate the central tendency of number of BC without CNS between BC with CNS. The upper control limit (UCL) is CL + 3 x standard deviation (the lower control limit is zero). The most important criteria for lack of statistical control are control values outside the UCL, eight consecutive values on the same side of CL and any 12 of 14 consecutive control values on the same side of CL.
Results: When alcohol swaps from an open container were used for skin disinfection at AH, CNS were isolated from 9% of BC versus 2.8% of BC when separate packing alcohol swabs were used. The rate of CNS in BC was not reduced with use of iodine and glows (HH) or thermal disinfection of needle holders between BC sampling (BBH). In the container swabs period the AH

g-control chart showed lack of statistical control with up to 100 control values below the centre line through months. G-contol charts from HH and BBH had several out of control periods.
Conclusion: A g-control chart was found to be a valuable statistical tools allowing quick identification of an increasing CNS contaminations rate of blood culture. Sufficient skin disinfection think to bee the most important factor for minimizing blood culture contaminants.

R2059

Gastric fluid cultures of neonates

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Objectives: To estimate the type of microorganisms that are usually isolated from gastric fluid cultures of neonates, as soon as possible after their birth in order to determine the potentially pathogenic microorganisms that colonize the neonate during its passage from genital channel.
Methods: We analysed retrospectively the neonates' gastric fluid cultures that turned to positive during the 2003 and first half-year period of 2004. The reception of gastric fluid usually followed immediately birth. The identification and sensitivity test against antimicrobial agents was performed by the Vitek system (Biomerieux).
Results: 230 neonates' gastric fluid cultures turned to positive during the 2003 and first half-year period of 2004. Microorganisms that isolated were: 95 Coag negative *Staphylococci* (41%), 72 *E. coli* (31%), 16 *Klebsiella pneumoniae* (7%), 14 *Enterococcus faecalis* (6%), 8 *S.aureus* (3.5%), 6 *Streptococcus bovis* (2.5%), 6 *Streptococcus agalactiae* (2.5%), 4 *Serratia marcescens* , 4 *Pseudomonas aeruginosa* and 5 *Candida* spp. All Coag negative *Staphylococci* strains were sensitive to Vancomycin but only 62.1% were sensitive to Oxacillin. *E. coli* strains were 66.7% sensitive to Ampicillin and there were not ESBL positive strains.
Conclusions: Gastric fluid culture of neonates is a useful method to determine the microorganisms that colonized the neonate during birth.

R2060

Culture negative infective endocarditis: two decades experience at tertiary care hospital

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Objectives: 1. To review all culture negative Infective Endocarditis (IE), presentation and outcome. 2. To compare medical VS surgical culture negative IE.
Methods: Retrospective study of all Echocardiography (echo) positive vegetations and all intraoperative findings suggestion of endocarditis. Patients confined at King Faisal Specialist Hospital and Research Center from 1985 to 2003
Results: Thirty-one patients satisfied the inclusion criteria. Of the 31 cases, 17 cases were identified to be native valve IE while 14 cases were prosthetic valve IE. Fifty per cent of the studied patients were male patients. Fifty-nine per cent of the cases were having rheumatic valvular diseases. The most common affected valves were mitral (71.9%), aortic (50%), and tricuspid (9.4%). Prior valvular diseases (37.5%), Prosthetic valve, (9.4%), and dental procedure (9.4%) were highly attributed risk factors to IE. Sign and symptoms available at presentation were fever (71%), new murmur (50%), Clubbing (46.9%), microscopic hematuria (18.8%), heart failure (59.4%), joint pain (25%), and splenomegaly (15.6%). Of all cases, the ECG findings were remarkable for heart block (34.4%). Echo, as TTE, was done in (90%) of the cases, in which vegetation was present in (37.5%) of the identified cases, while, valve lesion and abscess were identified in (50%) and (12.5%) of the cases, respectively. Eighty-one per cent of all patients were managed medically and surgically, while only (15%) were managed medically. Of the identified patients, (87.5%) survived IE, while (9.4%) of them died. Of interest, there was no recurrence of IE after surgery.
Conclusion: This study shows that mitral valve was the most affected valve in culture negative IE patients. One third of patients showed heart block. Most of the patients were managed both medically and surgically. Interestingly, of the 31 IE patients, mortality rate was shown in almost 10% of all patients.

Infection in the immunocompromised host (except HIV)

R2061

Cefepime combined with a short (four days) or long course of isepamicin for empirical therapy of high-risk febrile neutropenic haematologic cancer patients: a prospective, randomised, multicentre study

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Objectives: To compare the efficacy and safety of cefepime plus either a short course (SC) (four days) or a long course (LC) of once daily isepamicin in the empirical treatment of febrile neutropenia.
Methods: Between Feb 2002 and Feb 2003, a total of 134 adult neutropenic patients with fever and hematologic malignancies were randomized to receive cefepime (2 g tid) in combination with isepamicin (15 mg/kg/o.d.) either four days or longer from 10 different centres all over Turkey. Classification of febrile episodes and evaluation of response were designed in accordance with the guidelines published by Immunocompromised Host Society. Duration of the SC arm was selected as 4 days to avoid early unnecessary modifications of empirical treatment.
Results: A total of 119 patients were assessable for efficacy (58 SC, 61 LC). Underlying diseases were AML (86), ALL (22), BMT (2), lymphoma (7), other hematological malignancies (2). The median duration of isepamicin therapy was 10 (4–21) days in the LC arm. Infection was microbiologically and clinically documented in 33 episodes (28%) and in 7 episodes (6%), respectively. The overall success rate without modification was 50% in SC arm, 49% in the LC arm. The mean time to defervescence was 3.8 + 2.5 days in the SC arm and 4.1 + 3.1 days in the LC arm ($p > 0.05$). Modification of the initial regimen with antivirals and/or antifungals raised the success rate to 60% in the SC arm, 57% in the LC arm. Without a change in antibiotics, the response rates were 5/7 in the SC arm and 5/6 in the LC arm in patients with single-organism gram-negative bacteraemia. Overall side-effects were observed in 19 patients (29%) in SC arm, 22 (32%) in the LC arm in the intent to treat analysis. The nephrotoxicity were rare and similar in both arms (4 SC, 2 LC, $p = 0.31$). Mortality due to infection with or without hemorrhage occurred in a total of 9 patients (4 SC, 5 LC).

Conclusion: Cefepime combined with a short (four days)course of isepamicin seems to be as effective and safe as a longer duration of isepamicin in febrile high-risk neutropenic patients.

R2062

Clinical-evolutive aspects of varicella in immunocompromised patients

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Immunocompromised patients represent a high risk group for a large number of viral and bacterial infections, requiring a strict clinical and therapeutic surveillance.

Objectives: The study of clinical-evolutive aspects of varicella in adult immunocompromised patients.

Methods: The authors have studied a group of 24 patients from the Clinic of Infectious Diseases in Timisoara suffering from varicella occurring on known chronic affections, evolving on immunocompromised field. Five of the patients were known to have insulin-dependent diabetes mellitus, 4 patients with chronic renal insufficiency (hemodialysis) 3 patients with chronic lymphatic leukaemia, 2 patients with chronic myelogenous leukaemia, 8 patients with tuberculosis, one patient with breast cancer and one patient with Hodkin disease. The diagnosis of varicella was based on the clinical-evolutive symptoms (fever, general itching rash consisting of maculas, papulas and vesicles, affecting both the skin and the mouth, anal–genital, and conjunctiva mucosa occurring in 3–4 waves) and epidemiological elements (contact with patients suffering from varicella or herpes zoster). All patients followed treatment with acyclovir, 2 g/day for 8–10 days, C vitamin, calcium, antihistamines, sedatives, adequate diet and the treatment of the basic disease.

Results: The duration of the skin rash was of 4 weeks in 18 patients and of 5 weeks in 6 patients; the rash was intensively expressed in 4 waves in 20 patients; the following complications were observed: interstitial pneumonias in 15 patients, acute congestive anginas in 5 patients, mouth Candida in 8 patients, cerebellum ataxia in 3 patients, viral meningitis in 2 patients, bacterial skin over-infections in 10 patients, 1 patient with erysipelas, 4 patients with bacterial conjunctivitis; antibiotic treatment with claritromicine in 5 cases, amoxicillin + clavulanic acid in 4 cases and cotrimoxazol in 6 cases was performed. No decease was recorded.

Conclusions: Adult immunocompromised patients develop prolonged clinical forms of varicella with the possibility of complications that impose a clinical and therapeutically strict surveillance.

R2063

Microbial flora of the peritoneal catheter exit site in patients on continuous ambulatory peritoneal dialysis

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Objectives: The study of the microbial flora of the peritoneal catheters exit site in continuous ambulatory peritoneal dialysis (CAPD) patients and its relation to exit site infection and peritonitis episodes.

Patients and methods: During 24 months (2002–2003) peritoneal catheter exit site cultures were examined independently of the presence exit site infection in 18/28 (64.3%) CAPD patients. The specimens were cultured on the appropriate media. The identification of the microorganisms was performed by standard methods and API systems (bioMerieux). Susceptibility testing was carried out by disc diffusion method.

Results: During these 2 years exit site infection was noticed in 11/18 patients (61.1%). No peritoneal catheter tunnel infection was noticed. From the exit site infection episodes Gram (+) microbes were isolated in 57.9% of the strains (CNS in 42.1% and *Staphylococcus aureus* 15.8%), Gram (–) bacteria in 36.8%, and *Candida crusei* 5.3%. Resistant colonization by the same strain, was noticed in 45.5% of the patients with exit site infection (colonization time 3–14 months). Production of extended spectrum (-lactamase (ESBL) was observed in 14.3% of isolated Gram (–) bacteria. CNS strains were 100% resistant to oxacillin. 54.5% of the patients were treated by systemic antimicrobials, 18.2% by local antimicrobials and 18.2% by local antiseptic (povidone iodine). In 26 specimens from 11 patients without exit site infection, CNS were isolated in 75% of the strains, Gram (–) bacteria in 17.6% and *Candida* spp. in 7.1%. CNS strains were 90.4% resistant to oxacillin, 57.1% to erythromycin, 66.7% to gentamicin, 52.4% to ciprofloxacin, 66.7% to trimethoprime/sulphamethoxazole and 61.1% to fucidic acid. Production of ESBL was observed in 40% of isolated Gram (–) bacteria. Peritonitis episodes from microbes which have been isolated from the exit site (colonization or inflammation) were noticed in 4/18 (22.2%) patients.

Conclusions: Multiresistant CNS were isolated from the exit site in the majority of CAPD patients. 50% of the Gram (-) bacteria were unfermented, and 25% were producing ESBL. Cultures from normal exit site are useful for prevention in CAPD patients. Clinical evaluation of exit site appearance is essential for the administration of treatment dependent of isolated microorganisms.

R2064

Infections in patients with malignancies

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Objectives: This study was conducted in order to assess the prevalence of infections and pathogens in critically ill patients with underlying malignancy in our hospital.

Methods: We studied 164 cancer patients presenting to our hospital with signs and symptoms of infections. Cultures were performed adequately in accord with the infection site. Identification of strains and antibiotic susceptibility testing were performed with the VITEK System, ATB Expression (BioMerieux, France).

Results: Out of the 164 patients there were 88 (53.7%) men (mean age 60.8 ± 11.5 years) and 76 (46.3%) women (mean age 62.5 ± 13.2 years). Overall mortality was 17.9%. The sites of primary malignancy were: lung 25.0%, digestive tract 17.1%, breast 12.2%, urinary tract 11.6%, gynecologic 10.9%, prostate 9.7%, head–neck 5.5%, haematologic 4.3% and others 3.7%. Ninety-four (57.3%) patients were diagnosed with metastatic cancer. In particular, the sites were: bone 50.0%, liver 22.3%, lung 9.6% and others 18.1%. There were 186 concomitant infection sites, of which the most common were urinary tract (33.4%), bacteraemia (24.7%), pulmonary (21.5%), skin or soft tissue (14.5%) and abdominal infections (5.9%). We observed a high prevalence of multiresistant strains among the pathogens isolated. In particular, the most commonly isolated pathogens and their multiresistance rate were as follows: in the urinary tract, *Escherichia coli* (27%), *Pseudomonas aeruginosa* (60%) and *Enterococcus faecalis* (58%), in bacteraemia, *Staphylococcus epidermidis* (58%), *E. faecalis* (43%) and *P. aeruginosa* (61%), in pulmonary infections, *Klebsiella pneumoniae* (67%) and *P, aeruginosa* (68%), in skin and soft tissue infections, *P. aeruginosa* (75%),

S. epidermidis (60%) and *Candida albicans*, while in abdominal infections *E. faecalis* (52%) and *S. epidermidis* (54%).
Conclusions: The most commonly encountered infections in patients with underlying malignancy were urinary tract infections, bacteraemias and respiratory tract infections. In our study there was a high percentage of multiresistant isolates. Gram-positive cocci were the leading cause of bacteraemia and abdominal infections. Of all Gram-negative rods *P. aeruginosa* was the most resistant.

R2065

Isolation of *Neisseria meningitidis* from sputum/bronchial secretions from immunocompromised patients

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Objectives: Two cases of immunocompromised patients in which *Neisseria meningitidis* strains were isolated from sputum/bronchial secretions are presented in this study.
Methods: Both sputum and bronchial secretions were cultured in blood/chocolate agar. Isolation and identification of *N. meningitidis* strains, were performed according to conventional microbiological procedures. To the isolated *N. meningitidis* strains, serogroup was performed by the slide agglutination test and serosubtyping by the use of monoclonal antibodies with Whole-cell ELISA. Simultaneous diagnostic approach was carried out by the use of PCR in whole blood samples of the two patients. The amplified gene was crgA specific for *N. meningitidis* and siaD specific for serogroups A, B, C and Y. The amplification products were 230 bp for *N. meningitidis* and 450 bp, 250 bp, 400 bp, and 120 bp for the serogroups B, C, A, and Y, respectively.
Results: In the first case although the blood cultures were negative, PCR in the whole blood was positive for *N. meningitidis* serogroup W-135, indicating meningococcal septicemia and the same strain was isolated from the sputum. In the second case, *N. meningitidis* serogroup C:4:P1.14 strain was isolated from the bronchial secretions although blood cultures and PCR was negative.
Conclusions: *N. meningitidis* in sputum or bronchial may cause severe bacteraemia or meningococcal disease especially in immunocompromised patients. PCR detecting genetic material of *N. meningitidis* in whole blood, play important role in laboratory diagnosis of the infection. In cases in which *N. meningitidis* is isolated from respiratory tract infections, this should be notified and chemoprophylaxis must be given to avoid complications of meningitis or septicaemia.

R2066

Bacteraemia in patients with hematological malignancies

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Objectives: To determine of predominant pathogens isolated from blood of patients with hematological malignancies.
Methods: A total of 309 strains were isolated from 1535 blood cultures taken from 239 patients with hematological malignancies between 1997 and 2003. BACTEC 9050 (Becton Dickinson Diagnostic instrument Systems) and Bact/ALERT 3D (Bio-Merieux) automated blood culture systems were used in our study. The clinical significance of isolates was assessed at the time of the positive culture by consultation with the patients' physician. Decisions were also made in accordance with the definitions published by the Centers for Disease Control and Prevention.
Results: 126 from 309 strains were determinated as contaminants (40.8%). Thus clinically significant strains accounted for 183 ones. Gram-positive cocci presented 49.2% (90), gram-negative bacilli – 31.1% (57/183), fungi – 14.8% (27/183). *S. aureus* was the leading pathogen (19.1%, 35/183), followed by coagulase-negative staphylococci (15.8%, 29/183), *Enterococcus* spp. (9.8%, 18/183), *Candida* spp. (9.8%, 18/183), *E. coli* (8.7%, 16/183), *Streptococcus* spp. (4.4%, 8/183), *Enterobacter* spp. (4.4%, 8/183), *Klebsiella* spp. (3.3%, 6/183), other gram-negative enteric bacilli (3.3%, 6/183), *P.aeruginosa* (4.4%, 8/183), other non-fermenting gram-negative bacilli (7.1%, 13/183), other yeasts (1.6%, 3/183), moulds (3.3%, 6/183), anaerobes (2.2 %, 4/183), *Corynebacterium* spp. (1.1%, 2/183), *Bacillus* spp. (0.5%, 1/183), *Listeria ivanovii* (0.5%, 1/183), *Stomatococcus mucilaginosus* (0.5%, 1/183).
Conclusion: Gram-positive cocci (mainly *S. aureus*) were predominant pathogens between 2000 and 2003. It is necessary to notice the changing landscape: *Listeria ivanovii, Stomatococcus mucilaginosus, Rhodotorula rubra, Nectria mauritiicola* – unusual pathogens for our hospital.

R2067

Blood stream infections in children with acute myeloid leukaemia: contribution of microbiological gum and gut monitoring

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Objectives: Blood stream infections (BSI) have an important impact on the clinical course and the outcome of patients with acute myeloid leukaemia (AML). Viridans group streptococci (VGS) are considered the main cause of severe sepsis in these patients (20–30%). The aim of this study was to evaluate (i) the frequency of BSI, (ii) the incidence of VGS sepsis and (iii) the contribution of microbiological monitoring of gum decontamination by vancomycin mouth-washing in the prevention of VGS sepsis, in our paediatric patients with AML between 1993 and 2003.
Methods: Medical records of 78 children, treated according to the EORTC 58921 clinical trial including 264 chemotherapy courses, were retrospectively analysed. For prevention of infection, patients were isolated in laminar air flow rooms, had gum decontamination (vancomycin mouth-wash), gut decontamination (per os aminoglycoside and amphotericin B), and received trimethoprim–sulfamethoxazole. Weekly quantitative microbiological analyses of gum and gut were performed to check the effectiveness of decontamination and the absence of emergence of resistant bacteria. In case of febrile episodes, empirical antibiotic treatment consisted of piperacillin, amikacin and vancomycin. Vancomycin was stopped 72 hours later if Gram-positive sepsis was absent.
Results: Overall, 58 BSIs occurred corresponding to a total of 59 microorganisms(49 Gram-positive, 8 Gram-negative and 2 yeasts). Only 4 VGS sepsis occurred in 3 patients. No patients died from BSIs and no patients with VGS sepsis required intensive care unit management. The weekly microbiological gum monitoring found a very low incidence of VGS. In those cases where VGS were detected, this enabled the mouthwash regime to be modulated accordingly, which no doubt further contributed to our very low rate of VGS sepsis (6.7% in our population versus 20–30% in other studies). Weekly microbiological gut monitoring found no vancomycin-resistant enterococci in spite of use of local antibiotics.

Conclusion: Weekly microbiological analyses enabled the effectiveness of vancomycin-mouth washing to be monitored and adjusted. Our procedures contributed to significantly decreasing the frequency of VGS sepsis in children with AML, without emergence of vancomycin-resistant enterococci.

R2068

Efficacy of meropenem versus imipenem-cilastatin for the treatment of diabetic patients with complicated skin and skin structure infections

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Objectives: A previous study has shown the broad spectrum carbapenem, meropenem (MEM), to be as effective as imipenem–cilastatin (IPM) in patients with complicated skin and skin structure infections (cSSSI). Skin infections in patients with diabetes may be more severe, more complicated and associated with Gram-negative and anaerobic pathogens. This study reports the results from a large subgroup of patients with and without diabetes mellitus.
Methods: This was a multicentre, double-blind, randomized trial comparing MEM with IPM (both 500 mg IV every 8 hours). Results from patients were analysed according to the presence or absence of diabetes mellitus. The primary efficacy endpoint was clinical outcome at follow-up, as determined by the investigator, in the clinically evaluable (CE) population (all patients meeting eligibility criteria receiving at least 1 dose of study drug, who received adequate therapy, did not receive concomitant antibiotics and who had an appropriate follow-up).
Results: Of the 1037 patients enrolled and treated in the study, 398 (38%) had a history of diabetes mellitus. Overall, pathogens were identified in 79% of the patients. There were a larger proportion of Gram-negative and anaerobic pathogens in patients with diabetes (42% of isolates) than in patients without diabetes (34%). Similarly, polymicrobial infections were seen in 44% of patients with diabetes and 34% of patients without the diagnosis. The most common pathogens were *Staphylococcus aureus* and *Enterobacteriaceae.* Clinical cure rates in the CE population were similar between patient and treatment groups: 85.6% (83/97) MEM and 72.4% (76/105) IPM for patients with diabetes, and 86.6% (142/164) MEM and 89.0% (162/182) IPM for patients without diabetes. In patients with infected diabetic ulcers, the clinical cure rates were 71.4% (10/14) for MEM and 55.6% (10/18) for IPM.
Conclusion: Patients with diabetes mellitus had a higher proportion of Gram-negative, anaerobic and polymicrobial infections than those without diabetes. MEM 500 mg IV every 8 hours was as effective as IPM irrespective of a diagnosis of diabetes. MEM was also at least as effective as IPM in patients with diabetic foot ulcers.

R2069

Does the granulocyte transfusion therapy have the potential to adversely affect the clinical outcome of subsequent haematopoietic stem cell transplantation?

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Objectives: Granulocyte transfusion therapy (GTx) has been done increasingly for the treatment of severe bacterial and fungal infections in patients with prolonged neutropenia. However, the effect of GTx on clinical outcome after subsequent hematopoietic stem cell transplantation (HSCT) have not been precisely defined to date. The aims of this study were to determine the effect of GTx on the point of engraftment, graft-versus-host disease (GVHD), neutropenic fever and CMV infection after subsequent HSCT.
Methods: Out of patients who received allogeneic HSCT at the Catholic HSCT Center from January 2001 to December 2002, patients who had previous history of GTx enrolled into the case group. Patients who had no history of GTx and matched at the aspect of age, sex, underlying disease, HSCT type, stem cell source, and GVHD prophylaxis regimen, enrolled into the control group.
Results: Fourty-one patients enrolled into the case group and 1:2 matched 82 patients enrolled into the control group. Mean age of the two groups were 34.2 and 34.8 years, respectively. Acute leukaemia (75.6%) was the most common underlying disease. Matched related transplantation was 75.6%. Median follow-up duration were 570 and 651 days after transplantation ($P = 0.15$). Engraftment failure was more frequent in the case group than in the control group (14.6% vs 1.2%, $P = 0.006$). However, duration of neutropenia was not different between the two groups. Neutropenic fever and invasive fungal infection developed more frequently in the case group ($P < 0.05$). Duration of neutropenic fever and use of intravenous antibiotics were longer in the case group ($P < 0.05$). Although the incidence of acute and chronic GVHD was not different, more extensive type of GVHD tended to occur in the case group ($P = 0.05$). CMV infection occurred more frequently in the case group, but there was no statistical significance. Whereas CMV disease developed more frequently in the case group (8.6% vs 1.2%, $P = 0.049$). Crude mortality during post-engraftment period had no significant difference between the two groups (45.7% vs 38.3%).
Conclusion: GTx has the potential to adversely affect the course and outcome of subsequent HSCT. So, GTx should not be considered for routine management of infectious complications and should be reserved for the treatment of severe, potentially life-threatening infections refractory to conventional antimicrobial therapy in prolonged neutropenic patients.

R2070

Severe pulmonary Nocardia otitidiscaviarum infection in a patient with underlying chronic lung disease and long-term corticosteroid therapy, confirmed by 16S rRNA gene sequencing

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Objective: Nocardiosis is a rare opportunistic infection and presents most commonly as pulmonary disease. The species commonly associated with disease are *N. asteroides* sensu stricto type VI, *N. brasiliensis*, *N. farcinica* and *N. nova*. *N. otitidiscaviarum* represents only between 1.3 and 4.8% of clinical *Nocardia* isolates in larger studies from Europe, Japan and the USA. Just a few cases of *N. otitidiscaviarum* infections of the lower respiratory tract were published. We describe the case of a severe pulmonary *N. otitidiscaviarum* infection in an immunocompromised patient with underlying lung disease and review published cases.
Case report: A 75-year-old man, under long-term corticosteroid therapy because of chronic organizing pneumonia since 12 months, was admitted to a regional hospital (day 0) with left-sided pneumonia and empirically treated with ampicillin/sulbactam and ciprofloxacin. A complicating cerebral infarction led to the transfer to the stroke unit/neurological ICU of our tertiary care hospital (day 2). There he needed mechanical

ventilation (day 6) due to respiratory insufficiency. On two occasions, 8 days, respectively 11 days after incubation, gram-positive branching rods were cultured from tracheal aspirate. The strain was identified as *N. otitidiscaviarum* by partial 16S rRNA gene sequencing showing 100% sequence homology with the *N. otitidiscaviarum* type strain DSM 43242. Subsequently, treatment was changed to meropenem (day 25) plus cotrimoxazole (day 27). The patient was further treated on a medical ICU of our institution. Cerebral and pulmonary abscesses were excluded radiologically. Corticosteroids were stopped. Weaning from mechanical ventilation was protracted and complicated by pleural effusion requiring chest tube drainage and reincubation due to CO_2-narcosis (day 61). Meropenem was stopped after 4 weeks but cotrimoxazole continued for long-term treatment. The patient was eventually extubated 73 days after incubation (day 79) and, after altogether 89 ICU days (day 95), was discharged distinctly improved to a rehabilitation facility.

Conclusion: Our patient with severe pulmonary *N. otitidiscaviarum* infection had typical predisposing factors for nocardiosis with underlying lung disease in association with long-term corticosteroid therapy. To our knowledge, so far only six cases with lung infection (1 pyothorax) due to this species were published including two cases in patients without apparent immunosuppression.

R2071

C.R.P. and P.C.T. in septic patients

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Purpose: The purpose of this study was to determine and associate PCT and CRP levels in serum of septic patients that were hospitalized in the intensive care unit for the last 10 months in Hippocration General Hospital.

Methods: During a 10-month interval, 450 serum specimens were examined which corresponded to 92 I.C.U. patients. The PCT value in serum was determined using the immunoluminometric assay method (LIAISON–BRAHMS PCT) taking as normal values 0.10–0.50 ng/ml. The CRP value in serum was determined using the nephelometric method with high sensitivity reagents (Dade–Behring) taking as normal values 0–5 mg/l.

Results: From the 450 samples of 92 patients, 143 (32%) had pathological PCT levels that ranged from 1 to 131 ng/ml and 87% of these septic patients had positive blood cultures. In all 450 serum samples (100%) were found high CRP levels that ranged from 3.0 to over 20.0 mg/l 35 (38%) out of 92 patients with sepsis died.

Conclusions: Our study shows that: (1) The CRP levels remained high in serum measurements of the patients. (2) The PCT values showed some fluctuation (low and high). (3) The patients with high PCT serum levels also had positive blood cultures. (4) There was no connection between PCT and CRP serum levels. (5) High PCT levels remaining in the serum for a long time consist a bad prognostic factor for disease outcome.

R2072

Bacteraemia as a result of bacterial translocation from gastrointestinal tract in patients in the haematological department of a university hospital in Gdansk, Poland

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Objectives: The aim of the study was to estimate the frequency of bacteraemia as a result of bacterial translocation of the microorganisms from gastrointestinal tract.

Materials and methods: We analysed 583 positive blood cultures between 01-01-2004 and 30-09-2004 from 125 patients treated in Haematological Department in Gdansk. The blood samples were incubated in BacT/Alert system (bioMerieux). Strains were identified by classical methods and GNI+ VITEK cards (bioMerieux). Sensitivity was determined by the agar dilution method according to NCCLS guidelines.

Results: We analysed positive blood cultures from the group of 59 pts (36 male, 23 female) where bacteraemia was caused by aerobe intestinal microflora (Gram negative rods, enterococci, candida). Gram negative bacteraemia appeared in 49 cases (38 pts), enterococcal bacteraemia in 38 cases and candidemia in 10 cases (9 pts). In 17 pts, we observed mixed bacteraemia caused by Gram negative rods, enterococci and/or candida. In 16 cases, there were two or more microorganisms cultures from one blood sample. The most often isolated pathogen was *Enterococcus faecium* (79 isolates, 17 pts) and Gram negative rods – *Escherichia coli* (29 isolates, 15 pts) and *Pseudomonas aeruginosa* (20 isolates, 7 pts). The most often isolated yeast was *Candida albicans* (20 isolates, 8 pts). The correlation between microorganisms isolated from blood and stool samples was confirmed in 25 cases caused by Gram negative rods and the lack of correlation was in 23 cases. In all patients with enterococcal bacteraemia we confirmed the correlation between blood and stool samples. The correlation in cases of candidemia was in 8 cases. The homology of microorganisms isolated from blood and stool samples was determinated by resistant phenotype of resistance and biochemical tests GNI+ (VITEK, bioMerieux).

Conclusions: 1. The correlation between the colonization of gastrointestinal tract and microorganisms cultured from blood samples is confirmed in patients of Haematological Department in Gdansk. 2. The most closest correlation was observed in enterococcal bacteraemia and fungaemia. 3. The existence of correlation between bacteraemia and microbial flora of the intestine requires confirmation by other methods (PCR fingerprinting).

R2073

Listeria monocytogenes infection. A clinical and epidemiological report from a Spanish general community-based hospital

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Objective: To describe the frequency, clinical presentation, underlying conditions, and clinical outcome of a series of patients suffering from invasive listeriosis.

Methods: Retrospective review of clinical charts from patients with a positive culture in usually sterile fluids for *Listeria monocytogenes* occurring along a 10-year period (1994–2004) at a hospital that provides specialized care to approximately 390,000 inhabitants.

Results: Sixteen out of seventeen cases occurring along the study period are reported (50% females, 50% males). Mean age: 52.3 years (ranging from 19 to 87); no neonatal cases were found. Underlying conditions: Age over 65 years 6 (37.5%), malignant hematological disease 3 (18.75%), chronic liver disease 2 (12.5%), malignant solid tumor 1, chronic kidney disease 1, and pregnancy 1 (6.25% each one). No patients suffered from HIV-1 infection. Six patients (37.5%) had received prolonged immunosuppressive therapy. Three patients had no known underlying condition, all of them presenting as meningitis. Clinical presentation: Nine patients

presented with bacteraemia or sepsis, one of them as pregnancy listeriosis; five patients presented as meningitis or meningoencephalitis, and two patients as spontaneous bacterial peritonitis. Six cases had in-hospital onset of symptoms. Antimicrobial treatment included either ampicillin or trimethoprim–sulfamethoxazole in all patients. Clinical outcome: Four out of 16 patients died (25%), all of them displaying at least one predisposing condition and three of them older than 70 years.

Comments: Invasive *Listeria monocytogenes* infection is a disease very rarely detected in our hospital. As mortality remains quite high despite correct antimicrobial treatment, clinicians should be alert to rule it out, specially in older patients with predisposing factors.

R2074

Usefulness of procalcitonin and neopterin measurement in the management of exacerbations in chronic obstructive pulmonary diseases

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Objectives: To determine the usefulness of procalcitonin (PCT) and neopterin measurement as inflammatory markers in patients diagnosed of chronic obstructive pulmonary disease (COPD). To establish if differences are observed in PCT and neopterin levels during a clinically stable period, during an acute exacerbation, and during the process until resolution, as well as in COPD patients with pneumonia. The results will be analysed taking into consideration the severity and etiology of the COPD exacerbation.

Methodology: A total of eighty-nine patients diagnosed of COPD were included in the study. Patients were grouped as follows: Group 1: COPD patients in stable period (4); Group 2: COPD patients undergoing an acute exacerbation, and Group 3: COPD patients with clinical, radiographic and microbiological diagnosis of pneumonia (16). In addition, a control group of 10 healthy individuals was included. Comparison between the different groups of patients was conducted by the Mann–Whitney test in order to evaluate the usefulness of the determination of PCT and neopterin. PCT was measured by immunoluminometric assay (Lumitest PCT, Brahms Diagnostica) and neopterin was measured by enzyme immunoassay (Neopterin ELISA, IBL) in serum samples.

Results: The PCT basal levels in the control group patients were lower than 1 ng/ml (median 0.35, 5–95 percentiles 0.258-2-223). The higher values were obtained in COPD patients undergoing pneumonia (median 3.11, 5–95 percentiles 0.158-72-44). The neopterin basal levels in the control group patients were lower than 30 ng/ml (median 0.195, percentiles 0.014–5.334). The higher values of neopterin were also obtained in COPD patients affected of pneumonia. In addition, the PCT levels were higher in COPD exacerbated patients than in stable COPD patients. However, significant differences were only observed when comparing the exacerbated severe COPD patients ($p = 0.018$) to the stable COPD patients.

Conclusion: We can conclude that PCT and neopterin show higher levels in COPD patients presenting pneumonia than in those presenting clinical exacerbation without pneumonia and those in a stable period of the disease. Furthermore, a correlation between PCT and the severity of the COPD is observed.

R2075

Pneumocystis jiroveci (carinii) pneumonia in the era of PCP prophylaxis

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Methods: From 6/1999 to 7/2004 192 BAL have been performed in hematooncological pts. for the reason of pulmonary pathology on chest X-ray or HRCT. 34 pts. had positivity for *P. jiroveci (carinii)* by nested PCR. 4 pts. had false positivity (specificity 91%). 30 cases of PCP (*P. carinii* pneumonia) have been diagnosed. Data from these patients have been reviewed.

Results: Underlaing diseases of these 30 pts. are: NHL – 6 (20%), myeloma – 6 (20%), Hodgkin's lymphoma – 4 (13%), CLL – 4 (13%), acute leukaemia – 2 (7%), aplastic anemia – 1 (3%), CML – 1 (3%), HCL – 1 (3%), MDS – 1 (1%), myelofibrosis – 1 (1%), other – 3 (10%). PCP occured in 5 pts. (17%) in the time of diagnosis, in 12 (40%) during treatment, in 3 (10%) in remission and in 10 pts. (33%) during progression of underlaing disease. 29 pts. (97%) were without PCP prophylaxis and 21 (70%) had steroid treatment. 27 pts. (90%) have been treated with HD co-trimoxazole (3 pts died before start) with 67% treatment response. 15 pts. (50%) needed mechanical ventilation. Overall mortality of these 30 pts with PCP was 50%. The need for mechanical ventilation were higher in pts. with steroids in anamnesis (52% vs. 44%), with >20 mg of steroids/day (67% vs. 20%), with CD4 count $\leq 0.2 \times 10^9$/l or ≤20% (50% vs. 33%) and with underlaing disease in the stage of new diagnosis or during treatment (60%, 58% vs. 33%, 40%).Mortality was higher in pts. with steroids in anamnesis (57% vs. 33%), with >20 mg of steroids/day (53% vs. 40%), with CD4 $\leq 0.2 \times 10^9$/l or ≤20% (56% vs. 33%), with neutrophile count $\leq 1 \times 10^9$/l (60% vs. 45%), pts. on mechanical ventilation (67% vs. 33%) and pts. with underlaing disease in the stage of new diagnosis or progression (80%, 70% vs. 33%, 0%). We did not find higher mortality and higher need for mechanical ventilation in the group of pts. with CD4/CD8 count ≤1. (43% vs. 50%, 14% vs. 55%).

Need for mechanical ventilation and mortality

Condition		n (%)	Mechanical ventilation-n (%)	Death-n (%)
Underlaing disease stage	new diagnosis	5 (17)	3 (60)	4 (80)
	During Treatment	12 (40)	7 (58)	4 (33)
	Remission	3 (1 0)	1 (33)	0 (0)
	Progression	10 (33)	4 (40)	7 (70)
Steroids	Yes	21 (70)	11 (52)	12 (57)
	No	9 (30)	4 (44)	3 (33)
Steroids > 20 mg/day	Yes	15 (75)	10 (67)	8 (53)
	No	5 (25)	1 (20)	2 (40)
CD 4 </= 0.25 × 10e9/l or </= 20%	Yes	18 (67)	9 (50)	10 (56)
	No	9 (30)	3 (33)	3 (33)
CD 4/8 </= 1	Yes	7 (26)	1 (14)	3 (43)
	No	20 (74)	11 (55)	10 (50)
Neu</=1 × 10e9/l	Yes	10 (33)	4 (40)	6 (60)
	No	20 (67)	11 (55)	9 (45)
Mechanical ventilation	Yes	15 (50)	N.A.	10 (67)
	No	15 (50)	NA	5 (33)

Conclusion: Our retrospective analysis demonstrates very low incidence of PCP in the groups of pts with hematological malignancies where PCP prophylaxis is commonly used (e.g. acute leukaemia). By contrast most of pts. with PCP in our institution were pts. with underlaing disease and treatment where PCP prophylaxis isn't routinely used (NHL, Myeloma, Hodgkin's lymphoma, CLL), but steroids were part of treatment protocols (especially >20 mg/day), CD4 count was $\leq 0.2 \times 10^9$/l or ≤20% and underlaing disease was in the stage of new diagnosis or progression. These are pts. who need PCP prophylaxis.

R2076

Peritoneal tuberculosis as a possible complication of imatinib therapy for chronic myelogenous leukaemia

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Background: Imatinib mesylate (STI571 – Glivec) is a selective inhibitor of the Bcr–abl tyrosine kinase, which deregulated expression is involved in the pathogenesis of chronic myeloid leukaemia (CML). We report a case of peritoneal tuberculosis in a patient with global lymphopenia following a four-month treatment with imatinib for a newly diagnosed CML.
Case report: A 37-year-old Swiss male, who was diagnosed with BCR–ABL positive CML in chronic phase, complained after four months of imatinib therapy of abdominal pain, lack of appetite, lost of weight, nausea. Abdominal CT-scan revealed homogenous hepato-splenomegalia, diffuse infiltration of mesenteric fat and ascitis. Ascitis was inflammatory with predominance of lymphocytes and remained sterile on culture. The symptomatology was attributed to an adverse effect of imatinib and the treatment was then interrupted. One month later, he was admitted because of worsening symptoms. An explorative laparoscopy displayed extensive chronic granulomatous peritonitis, and peritoneal tuberculosis was diagnosed by positive *Mycobacterium tuberculosis* PCR of peritoneal biopsies. Chest X-ray was strictly normal. At admission, he presented global lymphopenia (CD4 155 cell/mm^3 and CD8 39 cell/mm^3).
Discussion: This case raises two points: an atypical presentation of tuberculosis in a non-exposed patient and a global lymphopenia in an HIV-negative patient. To the best of our knowledge, this is the first report of an infection suggestive of immune compromise after imatinib treatment. Imatinib is known to induce cytopenia. Moreover, two recent studies have shown that imatinib inhibits the activation and proliferation of T-cells in vitro and delayed-type hypersensitivity in vivo (Cwynarski et al. 2004, Dietz et al. 2004) suggesting that imatinib might affect immunity and could lead to a higher than expected incidence of opportunistic infections. No epidemiological data are yet available to support this hypothesis. Of note, tuberculosis itself induces transient lymphopenia in HIV-seronegative patients and patients with CML are at risk for infection due to a possible dysfunction of the immune response.
Conclusion: Further studies of imatinib effect on immunity and on the incidence of opportunistic infections should be performed. Checking CD4 and CD8 T cell counts in patients during imatinib treatment could be considered.

R2077

Identification of lactic acid bacteria isolated from blood samples and their susceptibility to antibiotics

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Objectives: Since the 1980s members of lactococci, pediococci, leuconostocs and lactobacilli have rarely been indicated as opportunistic pathogens in humans. Generally, these taxa are part of the normal intestinal flora and occur in fermented foods and on plants. Due to their low virulence it is assumed that these bacteria are pathogenic only in immunocompromised hosts. Their sporadic occurrence in human clinical samples is often overlooked and/or they are misidentified.
Methods: A total of 59.845 blood samples were isolated at the Department of Clinical Microbiology (Teaching Hospital, Brno, Czech Republic) during the 2000–2003. Biochemical identification was performed on selected lactic acid bacteria (LAB) by API 50 CH kit and by conventional tests. (GTG) 5-PCR fingerprinting and whole cell protein profile analysis by SDS-PAGE were performed to confirm the biochemical identification results. The MICs for antimicrobial agents (benzylpenicillin, ampicilin, cefotaxim, imipenem, erytromycin, clindamycin, gentamicin, ciprofloxacin, cotrimoxazol, metronidazol, vancomycin and teikoplanin) were determined by using the E-test MIC (Biodisk, Solna, Sweden).
Results: A total of 59.845 investigated blood samples yielded 12.767 (21.3%) infected hemocultures of which 33 (0.25%) contained LAB. They were identified based on phenotypical testing as *Lactobacillus plantarum* (3 strains), *Lactobacillus rhamnosus* (7), *Lactobacillus paracasei* ssp. *paracasei* (2), *Lactobacillus salivarius* ssp. *salivarius* (1), *Lactobacillus fermentum* (1), *Leuconostoc mesenteroides* (9), *Leuconostoc lactis* (5) and *Pediococcus pentosaceus* (1). The (GTG)5-PCR fingerprinting and whole cell protein profile analysis confirmed above identifications and furthermore identified four strains which were biochemically classified at the genus level as *Lactobacillus sakei* ssp. *Carnosus*, *Lactobacillus curvatus*, *Leuconostoc pseudomesenteroides* and *Weissella confusa*. All investigated strains (19) were resistant to vancomycin, teicoplanin and metronidazol and most of them to cotrimoxazol. Susceptibility to cefotaxim, imipenem, gentamicin and ciprofloxacin was variable.
Conclusion: *Lactobacillus rhamnosus* and *Leuconostoc mesenteroides* were the most common LAB species isolated from blood samples. Although isolation of LAB from blood samples is rare, it is of clinical significance and it should be taken into account during treatment decision – especially when they are isolated in pure culture from sterile sites.

R2078

Risk factors for bacterial infections in multiple myeloma treated with vincristine–adriamycin–dexamethasone schedule

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Objective: We evaluated bacterial infections (BIs) in patients with multiple myeloma (MM) treated with vincristine–adriamycin–dexamethasone (VAD) schedule during the last 4 years in our Department.
Design and methods: Sixty-five patients were studied during 290 VAD cycles. VAD was given by continuous intravenous infusion (CII) to hospitalized patients. The characteristics of patients and VAD schedules were retrospectively analysed to detect correlations with the incidence of BI.
Results: By analyzing each VAD cycle, we found that profound hypogammaglobulinemia ($p = 0.05$) and post-treatment neutropenia ($p = 0.09$) were associated with a higher risk of infection, while renal function impairment was significantly correlated with BI risk at multivariate ($p < 0.001$) analysis. We evaluated only the first 3 months of therapy, characterized by a significantly higher incidence of BI than the later period ($p < 0.0001$).Continuous schedule ($p = 0.07$), and profound hypogammaglobulinemia ($p = 0.01$) were associated with a tendency to a higher risk of infection. The high probability of CII-related infection was demonstrated to depend on the frequent development of nosocomial infections.
Conclusion: Patients with profound hypogammaglobulinemia who receive VAD as first line treatment are at a major risk of BI up to the completion of the third month of therapy. These patients require antibacterial prophylaxis with intravenous immunoglobulins which could be appropriate and effective.

R2079

Catheter-related bacteraemia due to *Mycobacterium chelonae* complex (M. Fuerth)

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Objectives: M. Fuerth was described in 2002 as a member of the rapid growing *Mycobacterium chelonae* complex. Its clinical significance and optimal therapeutical regimens have still remained unclear.
Methods: Here we report on a case of a 39 years old woman with catheter related disseminated infection due to M. Fuerth following bone marrow transplantation due to aplastic anemia.
Results: M. Fuerth could be cultivated in April 2004 from two blood cultures and in high numbers from a central venous catheter. Therapy was started using imipenem and clarithromycin. During the following 4 months, no signs of infection could be observed, but the patient developed disseminated purple coloured infiltrations of her the skin, which were initially regarded as cutaneous GvHD. In September 2004, a skin biopsy revealed large amounts of mycobacteria, which could also be subsequently identified as M. Fuerth. According to susceptibility testing results, a therapy with linezolid (Zyvoxid™) was started. During the next three weeks, the lesions improved and were sterile upon follow-up biopsy and culture. Ultimately, the patient succumbed due to chronic transplant-associated complications.
Conclusion: As other members of the rapid growing mycobacteria, M. Fuerth is able to cause catheter-related and disseminated infections in severely immunocompromised patients. Linezolid, which has in vitro activity against many mycobacteria was effective led to resulting in rapid improvement of the mycobacterial skin infiltrations and sterilization of blood cultures and skin biopsies.

R2080

Massive pericardial effusion due to Salmonella enteritidis in a patient with rheumatoid arthritis

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Objectives: Although pericardial involvement is frequent in patients with rheumatoid arthritis (RA), it is rarely symptomatic and the course is mostly benign and self-limiting; also, septic pericarditis is extremely uncommon. In this case report, we describe a patient with RA who developed massive pericardial effusion due to Salmonella.
Methods: The patient, a 48-year old man who suffered RA for two years, was on low-dose corticosteroid and methotrexate treatment for the last four months. He presented with palpitations and retrosternal pain. On physical examination he was not in cardiopulmonary distress. Erythrocyte sedimentation rate was 80 mm/h and the leukocyte count was 11300/mm^3. An echocardigraphic examination revealed minimal pericardial fluid collection which was considered to be due to RA and the dose of corticosteroid was increased. However, the amount of fluid increased and periods of fever (38 °C) were observed. A pericardiocentesis was performed and 600 mls of purulent fluid was drained. The pericardial fluid cultivated to BACTEC system were passaged to Endo and Blood agar plates. The identification and susceptibility tests were done with VITEK automated systems (bioMerieux, VITEK, Inc. USA) and conventional microbiological methods. The isolate was serotyped by using Salmonella O Antiserum Group 04, Group 09 and Salmonella antiserum poly a–z.
Results: The strain was identified as *Salmonella enteritidis* and found susceptible to all 15 antimicrobials (ampicillin, ampicillin/sulbactam, cefazoline, cefepime, cefotaxime, cefotetan, ciprofloxacin, ofloxacin, imipenem, meropenem, gentamycin, tobramycin, amikacin and trimethoprim/sulfametaxazole) studied. The blood culture remained sterile. The patient was given ciprofloxain p.o. 2 × 500 mg/day for two weeks. After the completion of the treatment the pericardial fluid had disappeared with remarkable clinical improvement.
Conclusion: Although pericardial involvement is frequent in RA, these patients are predisposed to various infections because of immunosuppressive drug treatment and infectious etiology should be considered in the presence of atypical features such as increasing fluid during steroid treatment and fever.

Immunology, host defenses, immunotherapy

R2081

Antibody titre to hepatitis B vaccine after 5 years of vaccination in Iranian children

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Background: The first dose of hepatitis B vaccine in first year of life induces immunity about 85–95% and level of antibady(Ab) decreases subsequently. The need to assece the boster dose of Hepatitis B vaccine is the main purpose of this study.
Materials and methods: 4160 children aged 6 years from three province of IRAN whom were vaccinated at birth, 1.5 and 9 months by recombinant hepatitis B vaccine were selected randomly and a questionnaire were filled for weight at birth, growth condition, chronic diseases and any medication and tested for antibody titre to Hepatitis B surface antigen. The level of Ab under 10 MIU/ML were assumed negative.
Result: 2609(65%) of subjects were boy and 1481(35%) girls. From 4160 children, 1248(30%) were negative for hepatitis B Ab. 1017(39%) of boys and 473(32%) of girls were seronegative(non-immune).
Conclusions: Our study suggests that high percentage (30%) of vaccinated children are non-immune after 5 years and need the revaccination.

R2082

Serotypes of Salmonella isolated strains in northern Greece and antimicrobial resistance rates

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Objectives: *Salmonella enterica* is one of the main causes of diarrhoea world wide. In this study, we present the serotypes of *Salmonella* isolates from diarrheal patients over 2 years in our hospital in Northern Greece and determine the antimicrobial resistance rates.
Methods: Over a 2-year period a total of 648 stool specimens from diarrheal patients were cultured. Identification of the isolated strains was made by conventional microbiological methods while determination of their antibiotic susceptibility against ampicillin (AMP), ampicillin/sulbactam (SAM), tetracycline (Te), chloramphenicol (Cl), ciprofloxacin (Cip), ceftriaxone (CRO) and trim-

ethoprim–sulfamethoxazole (SXT) was performed using Auto Scan 4 of Dade Boehring. The disc diffusion method of Kirby Bauer was used when necessary according to the NCCLS recommendations. Serotyping was performed with monospecific antisera by the slide agglutination method.
Results: A total of 53 *Salmonella* strains were isolated, 11 of them (20.8%) derived from adults and the majority 42(79.2%) from children. There is also a seasonable distribution of *Salmonella* cases with a peak in the summer months. The most prevalent serotype was *S. enteritidis* 9:g,m (75.5%), followed by *S. typhimurium* 4,5:i (18.8%), *S. goldcoast* 6.8:r:1,w(1.9%), *S. blockley* 6.8:k:1,s (1.9%) and *S. thompson* 6,7,14:k:1,s(1.9%). 49 strains (92.4%) were susceptible to all antibiotics tested while three strains (5.7%) 2 *S. typhimurium* and 1 *S. goldcoast* were resistant to AMP, SAM, CL, Te and one strain of *S. enteritidis* (1.9%) was resistant to Te. Medical case histories revealed two interesting incidences of family infection. In one, *S. enteritidis* was isolated from five members of the same family who had eaten infected meat. In the second case, *S. typhimurium* was isolated from two infants after close contact with their grandfather, who was found to be a chtonic carrier of the same strain.
Conclusions: *Salmonella enteritidis* predominates over the other serotypes. Children are more susceptible than adults to salmonellosis. Although in our region the resistance rates are low in comparison with other countries the rational use of antibiotics and continuous surveillance are essential in order to prevent the dissemination of resistant Salmonella strains.

R2083

Acute morphine treatment reduce innate immunity against systematic infection of HSV-1 in mice

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Objective: The opioids including morphine are believed to be responsible for many systemic effects in drug abusers. Involving immune system the opioids increased susceptibility to infections. The effect of acute administration of morphine on Systemic HSV-1 infection was evaluated in this study.
Method: HSV-1 was propagated and titered on BK cell line. Three groups of BALB/c mice were received 50 mg/kg, 75 mg/kg or PBS (positive control) subcutaneously, 24 h after ip injection of 2×10^7 TCID50 HSV-1. Under the same conditions a group of mice just received 75 mg/kg morphine as negative control. The survival rate was followed for 14 days. The cytotoxic activity of NK cells using MTT assay.
Results: Survival analysis demonstrated that comparing to control group administration of 75 mg morphine significantly reduces innate immunity to hsv-1 infection. The NK cell activity has been reduced in morphine-treated group in comparison with saline-treated one.
Conclusion: The results showed that adverse effects of Morphine on innate immunity can be taken as a result in enhanced rate of mortality in HSV-1 infected mice.

R2084

Effect of the function of polymorphonuclear leukocytes and interleukin-1 beta on wound healing in patients with diabetic foot infection

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Objectives: Disorders of polymorphonuclear leukocytes (PMNL) phagocytoses and intracellular killing activities play vital role in pathogenesis of diabetic foot infections. High blood levels of interleukin-1 beta (IL-1 beta), which is a proinflammatary cytokine, are detected at the initial phase of inflammations. In this study, we aimed to investigate the changes in the phagocytic and intracellular killing activities of PMNL and blood levels of IL-1 beta during the treatment of diabetic foot infections (DFI).
Methods: Thirty-eight patients with DFI [(24 male, 14 female, mean age 66.15 ± 9.8(37–84)] were enrolled in this prospective study between March 2003 and June 2004. Venous blood samples (20 ml) were obtained in the pretreatment period and at 2nd and 4th weeks of the treatment period. Polymorphonuclear leukocytes (1×107 cells/ml) were separated from venous blood with EDTA by Ficoll–Hypaque gradient centrifugation. These PMNL were incubated with *Candida albicans* blastospores and number of ingested organisms per 100 PMNL were calculated. Wound care, rational antibacterial therapy and hiperbaric oxygen therapy constituted the treatment protocol. The IL-1 beta levels were detected in the three period by ELISA (Quantikine HS, R&D Systems, Inc., US). Statistical analysis was performed by Mann–Whitney U–test and $p < 0.05$ was admitted to be meaningful.
Results: Twenty-three cases responded to the therapy completely. Amputation was performed to eight of 15 cases who did not respond to the therapy. A statistically significant increase in phagocytic activities of PMNL were detected only in responder group, whereas no significant change in intracellular killing activities of PMNL due to treatment were detected in none of the groups. A statistically significant decrease in the blood levels of IL-1 beta was detected in responder group at the 2nd week. In non-responder group, IL-1 beta levels increased slightly but it was not statistically significant. At the 4th weeks of the treatment, the IL-1 beta levels decreased slightly in both groups but it was not statistically significant. On the other hand, there was significantly change in IL-1 beta level between responder and non-responder groups in 2nd weeks of the treatment (1.97 ± 3.96 vs 4.91 ± 6.88, $p = 0.025$).
Conclusion: The present study confirmed that increased phagocytic activity of PMNL and decreased blood levels of IL-1 beta correlate well with good clinical prognosis in the treatment of DFI.

R2085

Chlamydia pneumoniae and risk factors of acute myocardial infarction

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Objectives: Acute myocardial infarction (AMI) is still the most important cause of death. Recently, it is believed that the microorganisms that causes chronic or persistant infections may play a role in the development of AMI (besides the classical risk factors). In this study, seropositivity of *Chlamydia pneumoniae* in patients suffering from acute myocardial infarction is searched and correlation of its presence with other classical risk factors is questioned.
Methods: Between March and May 2004 a hundred patients (mean age 62.7) who were diagnosed as AMI considering typical chest pain, Electrocardiography findings, serum cardiac enzyme values and 50 healthy control (mean age 59.8) were included in this study. Inquiry was made by asking classical risk factors to both groups. *C. pneumoniae* IgG antibodies were searched in serum samples by microimmunofluorescence assay (Euroimmun, Germany) and the titres over 1/100 were accepted significant.
Results: *C. pneumoniae* IgG antibodies were positive in 67% of patient and 40% in the control group ($p < 0.05$). The relation between the positivity of *C. pneumoniae* IgG antibody and the classical risk factors (sex, smoking, high total cholesterol level) were found significant ($p < 0.05$).

Conclusions: We believe that *C. pneumoniae*'s property of developing chronic infection triggers processing of other AMI risk factors although it is not a direct risk factor for AMI.

R2086

Pre- and post-operative changes in complement C3, complement C4 levels in acute appendicitis

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Objectives: Intraabdominal infection activates alternate pathway of complement system. Complement C3 is an important component of alternative pathway whereas complement C4 is a component of classical pathway of complement activation. C4 and C3 deficiencies are associated with pyogenic infections such as acute appendicitis. Our study is aimed to evaluate the preoperative and postoperative changes in C3 and C4 levels in acute appendicitis.
Patients and Methods: Our study involved 33 patients who underwent appendicectomy for acute appendicitis. Serum C3 and C4 levels were measured immediately before operation and postoperatively in one hour. All serum samples were tested by using nephelometric method (Dade-Behring, Germany). Results were analysed statistically (SPSS 9.0 Package program for Windows) with Chi-square test and $p < 0.05$ values were accepted as significant.
Results: C3 levels were found low in 22(67%) patients and normal in 11(33%) patients preoperatively whereas low in 30(91%) patients and normal in 3(9%) patients postoperatively ($p = 0.016$). C4 levels were found low in 9(27%) patients and normal in 24(72%) patients preoperatively. However in 12(36%) patients have low C4 levels and in 21(64%) patients have normal C4 levels postoperatively.
Conclusion: Postoperative C3 levels were found significantly low compared with the preoperative C3 levels in the patients underwent appendicectomy. C4 levels have no significant difference between preoperative and postoperative measurements.

R2087

CD4 and CD8 levels in Crimean-Congo haemorrhagic fever, and relation to the outcome

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Objective: Crimean–Congo haemorrhagic fever (CCHF) is a tick-borne infectious disease with severe hemorrhagia. There is not any study on immunology of the disease and T cell response in acute stage of CCHF. This study was aimed to investigate CD4, CD8 levels and CD4/CD8 ratio in CCHF.
Methods: Serum samples of 21 suspected and confirmed patients were collected at admission (early stage of disease), during hospitalization (acute stage of disease) and at discharge from hospital (recovery periods of disease). CD4–CD8 values were determined by flow cytometry.
Results: CD4 and CD8 values were normal in most of the patients, CD4 values were slightly increased in only 2 patients, and CD8 values increased in 1 patient and decreased in 1 patient. There is no significant difference between CD4 and CD8 values and CD4/CD8 ratios of the patients at early stage of disease, at acute stage of disease, at recovery periods of disease and healthy control ($p > 0.05$). CD4/CD8 ratios were usually normal, but reversed in 4 patients (19.0%) at early and acute stage of disease, and spontaneously returned to normal levels at recovery periods. To investigate effect of CD4/CD8 ratio on clinical outcome of disease, features of patients with reversed CD4/CD8 ratio and patients with normal CD4/CD8 ratio were compared. There were no difference respect to bleeding, ecchymosis, mortality, platelet and leukocyte counts, aspartate amino transferase (AST), creatine phosphokinase (CK), lactate dehydrogenase (LDH) and partial thromboplastine time (PTT). Mean hospitalization time of patients with reversed CD4/CD8 ratio were longer than others (12.2 vs. 7.1 days, $P = 0.01$).
Conclusion: CD4–CD8 values and CD4/CD8 ratios in CCHF were usually normal. The data demonstrate that during the acute phase of CCHF CD4/CD8 ratio may reverse. To determined role of CD4 and CD8 in CCHF further studied are need.

R2088

Changes in serum cytokines profile and nitric oxide levels in cystic echinococcosis patients at preoperative and postoperative period

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Objectives: Cytokines and nitric oxide play an important role in the human immunological response. The aim of study to determine their role in immune response in patients infected with *Echinococcus granulosus.*
Methods: Thirty-two patients with cystic echinocosis (CE) were studied and all underwent surgery. Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin IL-1beta, receptor of soluble IL-2 (sIL-2R), IL-6, IL-8, nitrate/nitrate, and C-reactive protein (CRP) were evaluated before surgery, on first day, and three months post operation. Data were compared with those obtained from 30 healthy volunteers.
Results: IL-6 was elevated in vast majority of CE patients (96.9%). IL-8 was increased in 12/32 (37.5%). Slight increases of sIL-2R, and TNF-alpha levels were found in limited number of them particularly those showing cysts in the central area of the liver (5/32, 6/32). IL-1 beta level was not elevated in any patient. CRP and nitrate /nitrate levels were also increased. A positive correlation between CRP and IL-6 ($r = 0.78$, $p < 0.001$) was found confirming the link between inflammation due to CE and activation of monocytes. All patients were completely recovered and studied parameters levels were declined to normal levels except one patient in whom recurrent disease occurred at interval of 2 years from the first operation.
Conclusion: These results suggest that there are different immunoregulatory events and cytokines response during CE and may be in part related to slight monocytosis in CE patients. IL-6, NO and CRP were involved in the host immune response against *E. granulosus* and may be useful markers in diagnosis, monitoring CE management and evaluation surgical stress.

R2089

Detection of Fc receptors released by streptococcal isolates of anginosus group

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Objectives: Oral streptococci, especially those belonging to the anginosus group, are the main facultatively anaerobic microorganisms involved in oral infections. The aim of the study was to detect soluble Fc receptors (FcR) secreted by some streptococcal strains of anginosus group isolated (either as single bacteria or in association with other microorganisms) in pus samples collected from patients with different pyogenic oral and maxillofacial infections.

Methods: The strains were identified at species level using the Rapid ID 32 STREP system (Bio-Merieux, France). Cell-free supernatant samples obtained from the culture of each isolate were blotted onto nitrocellulose membrane (Bio-Rad Laboratories) using a multifiltration apparatus. Horseradish peroxidase – rabbit anti-peroxidase complexes (PAP) (DAKO-Immunoglobulins a/s, Dakopatts, Denmark) were used to react with the bound FcR and afterwards, with the second antibody (goat anti-rabbit antibodies conjugated with horseradish peroxidase). In addition, 4-chloro-1-naphthol (Sigma Immuno Chemicals) was used to detect horseradish peroxidase in order to visualize the immune complexes.
Results: Forty isolates were identified as *S. anginosus*, while only one and three isolates were found to belong to *S. intermedius* and *S. constellatus* species, respectively. Positive FcR reactions (grey-blue dots) were obtained in all test samples.
Conclusion: *Streptococci* of anginosus group involved in pyogenic infections secrete small amounts of FcR which can be detected by this sensitive dot-immunobinding assay.

Pathogenesis, animal models including experimental treatment

R2090

Role of innate immunity in the pathogenesis of meningitis

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Objective: Microglial cells behave as cells of innate immunity in the brain. In order to study the pathogenesis of meningitis blood monocytes were triggered by cerebrospinal fluid (CSF) and by sera from patients with bacterial and abacterial meningitis
Methods: Cerebrospinal fluid and blood serum were collected upon admission from nine patients with bacterial meningitis and twenty-four patients with abacterial meningitis. Mononuclear cells were isolated from healthy individuals after density gradient centrifugation of heparinized whole blood over Ficoll-Hypaque. They were then washed three times with Phospate-Buffered Saline (pH: 7.2) and suspended in flasks. After incubation for one hour at 37°C under 5% CO_2, non-adherent cells were removed and monocytes were re-suspended in 12-well plates in RPMI with 10% FBS and 2 mM glutamine at a density of 1×10^5 cells/well. After 30 minutes of incubation at 37°C in 5% CO_2 100 μl of sampled CSF and 100 μl of sampled serum of each patient were added into different wells of each of the volunteers. Cells were subsequently incubated for 18 hours at 37°C in 5% CO_2. TNF-α, interleukin-1β (IL-1β), IL-10, IL-12, IL-6 and interferon-γ (IFN-γ) were measured in culture supernatants with an enzyme immunoassay; procalcitonin (PCT) by an assay based on immunochemoluminescence.
Results: Mean values of TNF-α and IL1β (pg/10000 cells) produced after triggering with CSF and serum from patients with bacterial meningitis were 126.35 and 51.55 pg/10000 cells, respectively and 82.86 and 66.54 pg/10000 cells from patients with abacterial meningitis, respectively. Respective mean values of IL-1β were 44.63 and 9.68 pg/10000 cells and 18.59 and 19.48 pg/10000 cells; of IL-6 119.92 and 65.53 pg/10000 cells and 66.49 and 37.83 pg/10000 cells; of IL-10 43.53 and 34.07 pg/10000 cells and 20.04 and 6.13 pg/10000cells; of IL12 84.36 and 31.76 pg/10000 cells and 24.31 and 22.51 pg/10000 cells; of IFN-β 65.08 and 17.04 pg/10000 cells and 75.03 and 18.17 pg/10000 cells; and of PCT 1.49 and 0.73 ng/10000 cells and 0.64 and 0.39 ng/10000 cells.
Conclusion: Triggering of innate immunity elicits cytokine responses that are higher in bacterial than abacterial meningitis; IL-1β, IL-6, IL-10 and PCT are important mediators in bacterial meningitis and IL-6 and IFN-γ in abacterial meningitis. Further research is mandatory to fully elucidate the clinical relevance of these findings.

R2091

Clarithromycin as an effective immunomodulator when administered late in experimental sepsis by pan-resistant *Klebsiella pneumoniae*

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Objectives: There is literal evidence that clarithromycin possesses an immunomodulatory role in experimental sepsis (Giamarellos-Bourboulis et al. AAC 2004). The present study was designed to evaluate its activity when administered 24 hours after bacterial challenge in experimental sepsis by pan-resistant *K. pnumoniae*.
Methods: Sepsis was induced in 40 New Zealand rabbits after ligation of the right pelvo–ureteral junction and inoculation of the test isolate in the renal pelvis. Animals were then divided into four groups: A, controls; B, clarithromycin; C, amikacin; and D, both agents. Therapy in groups B, C and D was administered 24 hours after bacterial challenge; two consecutive intravenous of 80 mg/kg of clarithromycin and one dose of 10 mg/kg of amikacin. Three days after bacterial challenge, animals were sacrificed and tissue samples were cut for quantitative culture and pathology. A semiquantitative histology climax was applied based on the addition of findings of acute and chronic inflammation, each scored from 0 to 3 depending on their severity.
Results: (In parenthesis comparisons with group A). Mean ± SD of pathology scores of liver of groups A, B, C and D were 2.57 ± 1.13, 1.55 ± 0.53 (p: 0.009), 2.22 ± 0.44 and 1.67 ± 0.70 (p: 0.018), respectively. Respective values of spleen were 3.43 ± 0.53, 3.44 ± 0.53, 3.22 ± 0.44 and 3.33 ± 0.71. Respective values of the right kidney were 5.43 ± 0.97, 3.78 ± 1.48 (p: 0.032), 3.78 ± 1.64 (p: 0.032) and 3.11 ± 2.36 (p: 0.004). Respective values of the right lung were 1.42 ± 0.53, 1.89 ± 0.33, 2.00 ± 0.50 and 1.55 ± 0.53. No differences were recorded in tissue bacterial counts between groups of treatment.
Conclusions: Administration of clarithromycin attenuated organ inflammation in experimental sepsis by pan-resistant *K. pneumoniae* without affecting tissue bacterial load. Results are favorable for a considerable immunomodulatory effect of clarithromycin.

R2092

Is there any association between fungal colonisation of gastric mucosa and gastric ulcer healing? A model of fungal colonisation in healthy adult rats

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Objectives: The aim of the study was: 1/to evaluate the influence of isolated from patients with gastric ulcers/GU/and chronic gastropathy/CG/*Candida* strains, on gastric secretion and gastric ulcer healing in rats in conditions of Aspirin/ASA/ oral administration. 2/to evaluate the influence of probiotics on GU healing.
Methods: We investigated 60 Wistar rats equipped with gastric fistula, with GU induced by acetic acid/ulcer area 28 mm^2/. Animals were divided into 3 groups. I: rats inoculation for 25 days with *C. albicans*: 105 CFU/ml/i.g. was preceded by 5 days supply of proteinase 0.2 U/kg i.g., promoting fungal colonization and aspirin/ASA/: 40 mg/kg/i.g. II: Rats received *Candida* ASA and proteinase as group I, besides *Lactobacillus acidophilus* 106 CFU/ml i.g. III: rats with GU-induced, given vehicle/saline/and proteinase. Animals were sacrificed after 4, 15, 25 days upon induction. The ulcer area was measured with planimetry, the gastric blood flow/GBF/was determined H_2 gas clearance. Gastric biopsy was taken for quantitative and qualitative mycological investigation and for the histopathology. Plasma levels of IL-1β, TNF-α, gastrin were measured. RT-PCR expression of IL-β, TNF-α was evaluated in gastric tissue.
Results: In rats inoculated with fungi, given ASA, gastric output was reduced in over 40% and GBF was decreased ,in comparison with rats given vehicle/group III/. The rats from group III showed progressive decrease in the area of GU by 13%, 45% and 95% at 4, 15 and 25 day, respectively. In contrast , the ulcers were present till 25 day in all rats inoculated with *Candida* . In rats given probiotic, decrease of GU area was observed more rapidly, in comparison with group I. In *Candida* colonized rats, upregulation of TNF-α, IL-β mRNA and the rise of plasmagastrin, TNF-α, IL-β, IF-γ levels was recorded.
Conclusions: 1. Fungal colonization could be achieved in rats by antisecretory or ASA treatment. 2. Fungal infection markedly reduces gastric secretion, delays gastric ulcer healing, probably due to fail of GBF at ulcer area and maintaining the mucosal inflammation, involving expression and release of IL-β, TNF-α. 3. Administration of probiotic reduces the delay of GU healing caused by *Candida* inoculation.

R2093

Serum antibody profile against *Helicobacter pylori* VACa and CAGa genes in Turkish patients with duodenal ulcer

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Objective: To evaluate the impact of major virulance factors of *Helicobacter pylori* (Hp) on the development of duodenal ulcer (DU) in Turkish, dyspeptic patients.
Methods: Prospective study from a tertiary referral hospital. Patients who were referred to our endoscopy unit due to dyspepsia between June 2003 and March 2004 and diagnosed to have DU or normal endoscopic findings [non-ulcer dyspeptic (NUD) control group] were included. Exclusion criteria were as follows: previous eradication treatment for Hp, the use of non-steroidal antiinflammatory drugs, the use of antisecretory drugs and antibiotics in the previous two weeks and of bismuth compounds in the previous one month. Four biopsies from the antrum and three from the body were taken in order to assess the current Hp status by histology, rapid urease test and culture. Blood samples were taken and patients who were seropositive for anti Hp IgG were further analysed by westernblotting against cagA and vacA. Age and sex adjusted multiple logistic regression analysis was used to determine the impact of cagA and vacA genes.
Results: 62 seropositive patients with NUD (36.9 ± 12.3) (93% Hp-positive) and 63 seropositive patients with DU (43.14 ± 16.27) (97% Hp-positive) were eligible for the final analysis. All patients' sera were tested for antibodies of class G and A against cagA and vacA genes. 9 of 62 (15%) NUD and 30 of 63 (48%) DU patients were positive for anti-cagA IgA. Age and sex adjusted multiple logistic regression analysis only disclosed the presence of anti-cagA Ig A as a risk factor for the development of DU pointing out to a recent infection with a highly virulant strain of Hp.
Conclusion: Recent infection with HP strains having cagA gene, seems to be a promoting factor for Hp development of DU in Turkish population.

R2094

The anti-candidal activity of *Pseudomonas aeruginosa* strains isolated from clinical specimens

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Objective: To investigate in vivo and in vitro the anti-candidal activity of *Pseudomonas aeruginosa* strains against *Candida* species.
Methods: Forty-four *P. aeruginosa* strains isolated from various specimens of intensive care patients in the study. *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C krusei* ATCC 6258 and a clinical isolate of *C. tropicalis* were used as *Candida* strains and anti-candidal activity of the strains were examined by using Kerr's method.
Results: The total inhibition rates obtained by using blood agar for *C. albicans, C. parapsilosis*, *C. krusei* and *C. tropicalis* were 41%, 34%, 34% and 25%, respectively. These rates were 45%, 39%, 48% and 25%, respectively when Sabouroud dextrose agar used. In mice model of concomitant subcutaneous infection with *Candida* species and *P. aeruginosa*, no yeast was recovered from skin cultures despite 100% detection of *P. aeruginosa*.
Conclusion: *P. aeruginosa* strains isolated from intensive care patients showed anti-candidal activity against these *Candida* species and it can be important for the treatment of the patients.

Molecular bacteriology (incl. diagnostics)

R2095

Real-time PCR identification of *Bacillus anthracis* strains containing PAG and CAPR genes using Real-time

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Objectives: The M. Aikimbayev Kazakh Scientific Center for Quarantine and Zoonotic Diseases monitors anthrax throughout Kazakhstan. Recent opportunities allowed the initiation of molecular biology testing for bacterial agents. Through sponsored collaboration between the Kazakhstan Institute and the Armed Forces Institute of Pathology real time PCR assays were developed and optimized. This collaborative effort introduced Real-Time PCR as a new technology to the Kazakhstan Public Health System for their potential implementation.
Methods: Primers sets selected and designed using Integrated Diagnostics Inc. Web–based Primers Selection Program for protective antigen and capsule genes. These primer sets were optimized on an Idaho Technologies Inc. Ruggedized Advanced Pathogen Identifiication Device (RAPID) using Syber Green as the reporter dye.
Results: Limit of Detection (LOD) studies showed a sensitivity to the 1 fg level (approximately 2 copies of genomic DNA). Testing multiple replicates of DNA preparation at the 10 fg concentration confirmed the reliability of the assay to consistently detect DNA at this concentration. Specificity testing was done using a panel of *B. anthracis* isolates and other *Bacillus* species including multiple isolates of *B. cereus* and *B. thruingiensis*. Both the PAG and CAPR primer sets were detected at the 10 fg concentration and melting curves confirmed their presence. Agarose gels also confirmed the presence of an amplicon of the correct size in reaction containing *B. anthracis* DNA but not are near neighbor PCR reactions. Specificity panel testing showed no cross-amplification with non-*B. anthracis* DNA; all wild type *B. anthracis* DNA were positive for pag and cap and expected results were observed from lab-modified *B. anthracis* strains (pX01-) or (pX02-) DNA.
Conclusion: These results coupled with the high speed runtime (<1 hour) and small foot-print of the RAPID thermal cycler show strong potential for adaptation of this technology by the Kazakhstan Public Health System. The research described in this publication was made possible in part by support provided by the U.S. Defense Threat Reduction Agency (DTRA) under the project «KB0-1950-AL-03» and administered by U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF).

R2096

Determination of *Helicobacter pylori* cagA gene prevalence in Iranian GUD and PUD patients

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Introduction: *Helicobacter pylori* are commonly associated with gastritis but only sometimes it causes clinically significant infectious diseases such as gastric and peptic ulcer diseases. The development of disease depends on the virulence of the infecting *H. pylori* strain, the susceptibility of the host, and environmental co-factor. The cytotoxin associated protein encoded by cagA gene is an important virulence factor that is produced by some *H. pylori* strains, and has been used as virulence marker in some populations. The aim of study was to determine the prevalence of cagA gene as a virulence factor on *H. pylori* strain types in Iranian GUD and PUD patients.
Material and Methods: In this study, biopsy specimens were obtained from antrum of 180 patients, who were admitted to the ward of internal medicine, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran. A positive *H. pylori* strain was identified by cultured isolates, biochemical tests standard methods, and the molecular typing was used by PCR technique to detect cagA gene.
Result: Among 129 biopsy specimens who were taken during the study period, 105 (81.33%) patients had GUD and 24 (18.6%) patients had PUD, in which 63 (59%) and 19 (80%) patients were infected by *H. pylori* infection, respectively. The cagA gene was detected in 37 (59%) of GUD and 19 (100%) of PUD patients whom were positive for *H. pylori*.
Conclusion: There was significant difference in the prevalence of the cagA gene between GUD and PUD patients ($p < 0.001$). Moreover, the presence of the cagA gene in *H. pylori* may be used as an indicator marker for increased risk of PUD.

R2097

Epidemiologic analysis and antibiotic resistance of *Shigella sonnei* isolated from patients in Seoul

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Shigella sonnei is a major cause for diarrheal disease in developed as well as in developing countries. Epidemiologic studies of this organism have been limited by the lack of a simple and effective method for comparing strains. The study of epidemiologic markers is important in an attempt to trace the sources of infection. We used PFGE, ERIC-PCR to study the genetic relatedness of 78 isolates of *S. sonnei* collected in Seoul. For quantitative illustration of the relationships within a large body of isolates, computer generated dendrogram were used to determine the number of isolates in pulsotypes at Dice coefficients of similarity of 80%. From 2000 to 2003, 78 isolates of *Shigella sonnei* were isolated from patients in Seoul. By the PFGE experiment, Over 80% band similarity has divided 6 Group(A ~ F) band patterns. *Xba*I digestion produced about 40 fragments and their sizes ranged from 32.4 to 582 kb. Isolated from the same outbreak showed identical PFGE patterns, Most of the different outbreak strains and sporadic strains showed identical or different PFGE patterns. The ERIC-PCR technique successfully typed all isolates examined and produced bands in the 300 to 2,000 bp size range. The susceptible strains to Chloramphenicol, Amikacin and Cefoxitin was the most prevalent 100%, respectively. In case of the multiple antibiotic resistant pattern, the most prevalent pattern was NA-SM-SXT-TE, followed by AM-NA-SM-SXT-TIC-TE. During an outbreak of infection, the aim of bacterial typing is to provide laboratory evidence that epidemiologically related isolates are also genetically related. Isolated from the same outbreak showed identical PFGE patterns, Most of the different outbreak strains and sporadic strains showed identical or different PFGE patterns. We found that PFGE and ERIC-PCR have the highest discriminatory power for differentiation of strains of *S. sonnei*. ERIC-PCR fingerprinting is particularly useful because of its simplicity and represents a less time consuming procedure. We conclude that it is possible for a typical clinical laboratory to analyze a large amount of PFGE information on *Shigella* isolates obtained under controlled conditions. Such data analysis should enhance surveillance capabilities and give indications of further work to various aspects of bacterial pathogenicity of the species.

R2098

Inactivation of mraZ and mraW genes of dcw cluster in *Salmonella enterica serovar Typhimurium* SL1344

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The versatility and adaptability of bacterial pathogens as well as complexity and variety of antimicrobial resistance mechanisms even against last generation antibiotics actually in use, are causing a serious health problem in developing countries with re-emerging of infectious diseases considered totally eradicated. Our working group is trying to discover a new antibacterial target highly specificic, selective and essential for survival of our pathogenic bacterial study model: *Salmonella enterica serovar Typhimuruium* SL1344 (STM). It presents as an important virulence factor its capacity to replicate intracellularly in epithelial cells, been necessary to develop a very complex molecular mechanism to improve penetration, intracellular survival and replication in the host cell. Focusing bacterial division process and specifically the mraZ and mraW genes, located at the beginning of the division cell wall cluster, we are trying to determine the potential character of this genes like a useful target in design of therapeutic strategies that will allow to limit infection and avoid the acquisition of antimicrobial resistance mechanisms. Since the pathogenicity of STM is given by its capacity to penetrate to non-phagocitic host cells, we have designed a deletion mutant of STM for the mraZ and mraW genes implied in the process of cellular division. This mraZW – mutant was obtained by using a very simple PCR and homologous recombination technique. We first used a set of 60 nt-primers to amplify the chloramphenicol resistant gene flanked by FRT regions (FLP Recognition Target) followed by homologous regions of genes adjacent to mraZ and mraW genes. We then used the PCR product to transform competent STM cells containing the low number copies and temperature sensitive pKD46 vector, which express the red recombinase of phage lambda under the control of arabinose inducible promoter. Afterwards, we selected the chloramphenicol resistant mutant. Finally, this antibiotic resistance gene was eliminated using the pCP20 plasmid that shows ampicillin and chloramphenicol resistance, temperature-sensitive replication and thermal induction of a recombinase FLP synthesis. We have subjected this mutant to several tests like the in vitro intracellular infection test using non-phagocitics cell-lines: HeLa, NIH/3T3 and NRK. In parallel, an 'in vivo' test using a mouse model in order to determine the kinetic value of intracellular proliferation has been carried out.

R2099

Identification of the pathogens caused the outbreak of *Streptococcus suis* toxic shock syndrome and meningocephalitis of swine and human

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Objectives: To reveal the relationship between the pathogens of toxic shock syndrome and meningocephalitis outbreak of pigs and human in 1998 in East China.
Methods: Epidemiological survey, isolation and identification of pathogenic bacteria were performed.
Results: Characters of the disease were quick onset, serious symptoms, short course and high mortality. The main clinical manifestations of the disease were high fever, sometimes with nausea, vomiting and diarrhoea, then might developed to myositis, fascitis, DIC, multiple organ failure, shock, and usually died in 2–3 days. Among 25 patients, 16 manifested clinically as streptococcal toxic shock syndromes (STSS) and 9 streptococcal meningocephalitis syndrome, with mortality 81.25% and 11.11%, respectively. 16 bacterium strains isolated from blood and cerebrospinal fluid specimens from the diseased pigs and patients were identified as *Streptococcus suis* based on biochemical reactions and 16S rRNA gene sequence analysis. The isolates from both pig and human also showed indistinguishable antibiotic and pulse-field gel electrophoresis patterns.
Conclusion: The pathogen isolated from the blood of patients and pigs were identified as the swine *streptococci*. It suggests that the outbreak of *Streptococcus suis* sepsis spreads from pigs to humans.

R2100

Real-time PCR screening of women with term-ruptured membranes for carriage of group B *streptococcus*

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Objectives: To evaluate a Light Cycler PCR assay for the detection of group B *streptococcus* (GBS) carriage in women with term-ruptured membranes in comparison with conventional culture.
Methods: Low vaginal swabs were obtained from 143 pregnant women who presented with term rupture of membranes. After incubation for 1 h in brain–heart infusion broth, DNA was extracted with the InstaGene matrix (BioRad), and real-time PCR for amplification and detection of the cfb target was performed with the LightCycler (Roche). Samples were processed in parallel by conventional culture on HSA and ISLAM plates, with confirmation of GBS isolates by latex agglutination.
Results: Twenty (14.0%) samples were positive for GBS by conventional culture, whereas the PCR assay detected GBS carriage in only 10(7.0%) cases, although two PCR-negative samples were positive on repeat testing. The PCR false-negative results did not always correlate with low numbers of target cells, thereby indicating a problem with extraction and/or inhibition rather than sensitivity per se. The negative and positive predictive values of the PCR assay were 92.5% and 100%, respectively, but the overall sensitivity of the assay was only 50%.
Conclusions: Although the real-time PCR assay generated a result within 3 h of receipt of a sample, the current method highlighted the need to further optimise DNA extraction and purification protocols for use in the clinical setting. Further studies will continue to address the optimisation of the sample processing and DNA extraction steps.

R2101

Use of PCR in the diagnosis of sexually transmitted infections in Lithuania

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Today's medicine takes advantage of the latest achievements in natural sciences and successfully employs new techniques to the diagnostics and treatment of diseases. Recently molecular-genetic science has been applied in the field of diagnostics of sexually transmitted diseases (STD) in addition to the conventional culture-based and serological methods: the technology of in vitro specific DNA-amplification or Polymerase Chain Reaction (PCR). The molecular methods have many advantages: high sensitivity, high specificity, option for standardization and

genotyping. The samples collected for molecular diagnostics tolerate transportation, testing procedures are rapid and these techniques can be performed in less than 24 hours. It is very important for patient and physician. In the molecular diagnostic laboratory Biomedical research centre (Lithuania) PCR technique has been applied for the direct detection and identification of sexually transmitted pathogens (*C. trachomatis*, *U. urealiticum*, *M. hominis*, *M. genitalium*, *N. gonorrhoea*, HPV and 16/18 genotype, HSV-I/II, *T. vaginalis*, *G. vaginalis*, etc.) in different clinical samples (cervical, urethral and vaginal swabs).

Objectives: To describe the epidemiology of prevalent sexually transmitted pathogens in different clinical samples using molecular genetic methods.

Materials and methods: In this analysis were included data collected from 1 January 2003 to 1 January 2004. During this time men and women with ailments or prophylactic referred to Biomedical research centre and 11713 PCR tests for genital pathogens were done in laboratory. Our PCR tests for detection microbial pathogens consisted of the following stages: DNA purification from different clinical samples; DNA amplification with specific primers and PCR product visualisation by agarose gel electrophoresis.

Results: We detected 15.3%(1797/11713) tests positive for sexually transmitted pathogens. The prevalence of *C. trachomatis* was 15.9%(416/2616), *U. urealiticum* – 29%(509/1758), *M. hominis* – 9.8%(132/1349), *M. genitalium* – 6.5% (72/1117), *N. gonorrhoea* – 2.4%(21/879), *G. vaginalis* – 27%(314/1164), *T. vaginalis* – 5.3%(57/1075), HPV – 25.6%(228/892), HPV – 16/18 – 33.8%(22/65), HSV – 3.3%(26/798).

Conclusions: It was detected that *U. urealiticumi* was the most common pathogen in the country population (29%), while *C. trachomatis* only 15.9%. The identification of genital infections by PCR is specific and rapid test in laboratory diagnostic.

Diagnostic methods (other than molecular)

R2102

Development of a new p24 antigenaemia enzyme linked immunosorbent assay (ELISA) for detection of human immunodeficiency virus

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Human immunodeficiency virus (HIV) is a member of the Retroviridae family. The HIV genome resembles that of other retroviruses with the typical gag, pol, and env gene organization. HIV establishes a permanent infection once the proviral DNA integrates into the host cell genome. HIV has been shown to infect a variety of cells, including peripheral lymphocytes, macrophages and other CD4+ cells. HIV is associated with immune system dysfunctions and, is responsible for opportunistic infections involved in AIDS. Transmission of HIV through blood transfusion and diagnosis of infection in hospitals and public health settings continues to be a worldwide concern. Polyclonal, mono-specific antibody against HIV recombinant p24 antigen, the capsid protein region, has been developed in rabbit and purified with gel filteration and affinity chromatography techniques. The purified antibody has been conjugated with the horseradish peroxidase enzyme and a capture enzyme linked immunosorbent assay (ELISA) has been developed for detection of p24 Antigenemia. HIV-negative human sera failed to react with the developed assay, thereby showing the specificity of the HIV antigenaemia assay. Seventy-five human positive serum samples as well as human negative serum samples were tested for the presence of p24 antigen by the developed and a reference assay (Abbot Lab., USA). There was a 100% concordance when the samples tested with both assays. The results of this study showed that the developed p24 antigenaemia assay could be used for detection of HIV infection in human early before appearance of any detectable antibodies against the virus. This assay appears to be highly sensitive and specific to HIV. The test also has several advantages, including a relatively short test time and, most importantly, an adequate and reproducible supply of antibody to be used. Further research is needed to better evaluate the developed assay reactivity in a larger number of HIV positive sera.

R2103

The comparison of Western blot test with FTA-ABS test as confirmatory tests in syphilis cases: a cross-sectional study in Turkish population

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Objectives: We aimed to compare and detect the serological confirmatory performances of Western blot (WB) and FTA-ABS tests as confirmatory tests in early (ES) and latent syphilis (LS) cases from different medical clinics of Istanbul with cases that showing biological false positiveness and cases with infected with other spirochetes (leptospirosis, borreliyosis) and healthy blood donors (HBD).

Methods: Forty-nine cases with syphilis (29 ES, 20 LS), 60 control individuals (20 cases with biological false positiveness, 20 cases infected with spirochetes and 20 HBD) were included cross-sectionally, in this study. The presence of reactive antibodies against to the specific and natural antigens of *T. pallidum* were detected with commercial Euroimmun Anti-treponema pallidum Western blot (WB) and FTA-ABS test kits.

Results: 29 cases with ES and 20 cases with LS were found positive in WB/IgM and WB/IgG tests, respectively but 26 cases with ES and 19 cases with LS were found positive in FTA-ABS/IgM and FTA-ABS/IgG tests, respectively. No WB positivity was found in any of control cases. While the per cent agreement of WB/IgM and FTA-ABS IgM tests in the diagnosis of ES cases was 90%, the per cent agreement of WB/IgG and FTA-ABS/IgG in the diagnosis of LS cases was 95%. The confirmatory performances (sensitivity, specificity, positive predictive value, negative predictive value and accuracy value) for WB/IgM test in the diagnosis of ES cases were detected 100% for all of them and the same parameters for FTA-ABS/IgM'nin were detected 90%, 100%, 100%, 90% and 94%, respectively. The same parameters for WB/IgG test in the diagnosis of LS cases were detected 100% for all of them and the same parameters for FTA-ABS IgG were detected 95%, 100%, 100%, 95% and 97.5%, respectively.

Conclusions: As conclusion, an ideal confirmation test for every periods of syphilis must be optimal, objective, fast, specific and away from subjective results which show variation depending

on the experience of microbiologist who check the samples on florescence microscope like FTA-ABS test. Routinely used treponemal and non-treponemal screening and diagnostic tests can show diagnostic efficacy insufficiency in different periods of syphilis depending on the different reasons. We suggest that WB test is useful than FTA-ABS test which is already and commonly used as a confirmatory test in the serological diagnosis of both ES and LS.

R2104

Procalcitonin and the diagnosis of early syphilis

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Objectives: The aim of this study was to determine procalcitonin (PCT) and C-reactive protein (CRP) levels in early syphilis (ES) cases and evaluate these indicators in diagnosis of syphilis.
Methods: Twenty-nine patients with ES, 30 patients with bacteraemia and 30 healthy blood donors (control group) were included in this study. PCT levels in blood samples were measured by monoclonal immunoluminometric method.
Results: The mean PCT levels (minimum–maximum) were found 0.80 (0.55–1.680) ng/ml in ES group, 7.5 (0.68–35.4) ng/ml in bacteraemia group, and 39 (0.15–1.25) ng/ml in control group, The PCT levels in the ES cases were statistically higher than those observed in healthy blood donors ($p < 0.01$). The area under the ROC (receiver operating characteristic) curve using PCT to predict early syphilis was 0.920 (95% CI: 0.831–1.008). The sensitivity, specificity, positive predictive value and negative predictive value for PCT were 100%, 90%, 90%, and 100%, respectively at 0.5 ng/ml, which was an optimal concentration. Consequently, PCT levels were found slightly higher than the cut-off value (mean: 0.80 ng/ml) in ES.
Conclusions: In conclusion, this research is the first preliminary study for determination of PCT levels in ES cases in spite of few number of case-control cases. Our results suggested that PCT is a useful and additional marker at 0.5 ng/ml optimal serum concentration according to the ROC curve when conventional laboratory test results were false positive and false negative, Nevertheless, we believe that new, extended serial case-control studies are needed to further support our results.

R2105

The microagglutination test as a complement to ELISA examination in serological diagnosis of *Legionella* infections

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In Poland, laboratory diagnosis of Legionella infections generally is based on serological investigation. Bacteria *L. pneumophila* sg 1 are more frequent cause of severe pneumonia, less frequent are *L. pneumophila* other serogroups or other Legionella species. Examination of serum sample using ELISA IgA, IgM and IgG assays is the method of choice in our laboratory for detection *L. pneumophila* sg 1. For *L. pneumophila* non-1 or other Legionella species infection the microagglutination test has been used.In our study we determined the significant titre of serum antibody to some *L. pneumophila* non-1 serogroups and other Legionella species on the base of results of examinations of serum samples collected from blood donors. The titre of antibody was determined using 14 antigens prepared in-house from the reference strains *L. pneumophila* and others Legionella. Statistical analysis of obtained results allowed to determine cut-off titre for all used antigens. The value of cut-off was 64 for *L. pneumophila* sg 1, 2, 4, 5, *L. longbachae*, cut-off = 128 for *L. pneumophila* sg 3, 7, 8, *L. micdadei*, *L. jordanis*, and cut-off = 256 for *L. pneumophila* sg 6, 12, *L. bozemanii*, *L. anisa*. Using those cut-off points we are able to eliminate not significant titre due to some cross-reactivity of *L. pneumophila* serogroups.Determination of specific serum IgA, IgM and IgG antibody levels to *L. pneumophila* sg 1 by commercial ELISA is more sensitive, standarised, informative and faster than microagglutination test. However, from our point of view the microagglutination test should be used as complement test to ELISA, especially for diagnosis of infection due to *L. pneumophila* non-1 serogroups or other Legionella species. In 16% of all serum samples tested this year the significant titre of antibody specific to one of serogroup of *L. pneumophila* non-1 was found using MAT. In those sera increased but not significant level of IgM antibodies to *L. pneumophila* sg 1 (in range 0.32–0.75) or IgA antibodies (0.38–0.53) was determined by ELISA. It is probably connected with cross-reactivity of some antigenic structures of *L. pneumophila* strains. Moreover, two cases of legionellosis due to *L. micdadei* were diagnosed. In those cases the antibiotic had been changed and the patients recovered. Because of unspecific symptoms and rather high mortality rate in legionnaires' disease the microbiological diagnosis of Legionella infection should be done using as many supplementary techniques as possible.

R2106

Comparison of different laboratory methods for detection of methicillin-resistant *Staphylococcus aureus*

M. Rahbar, M. Yaghoobi, A. Fatahi *(Tehran, IR)*

Objective: The aim of this study was to compare conventional phenotypic methods for detection of methicillin resistant *Staphylococcus aureus* in laboratory practice.
Methods: This study was conducted on a selection of 96 clinical isolates of *Staphylococcus aureus* in Milad hospital of Tehran. In our study four different laboratory methods including disk diffusion, oxacillin screen agar, E-test and latex agglutination compared for detection of MRSA. The disk diffusion method performed with oxacillin disks (1 μg) and interpreted according to National Committee for Clinical Standards (NCCLS) guidelines. Screening for MRSA also performed by using Muller–Hinton agar plate containing 6 μg/ml oxacillin and supplemented with 4% NaCl as recommended by NCCLS.A commercial latex agglutination test, the MaslatexTM, was assessed for the detection of penicillin binding protein 2a (PBP2a), the mecA gene product. Minimum inhibitory concentration (MIC) were measured for all isolates by E-test (AB Biodisk, Solna, Sweden) were according to the manufacture's guidelines.
Results: Of 96 isolates of *S. sureus* by disk diffusion method 52 had inhibitory zone in the resistance range (10 mm), 9 in the intermediate range (11–12 mm) and 36 in susceptible range (13 mm). By E-test method of 96 isolates 51 isolates were MRSA and 45 isolates susceptible to methicillin. Oxacillin screen agar showed only two false positive MRSA. The sensitivity and specificity oxacillin screen agar methods was 96% and 95% respectively. The MRSA-screen latex agglutination showed 54 (three false positive) MRSA isolates and 42 isolate were susceptible to methicillin. The sensitivity and specificity for this method were 94% and 93% respectively.
Conclusion: The oxacillin screen agar test is reliable alternative for detection of MRSA in clinical laboratory where molecular method are not available and finally MRSA latex agglutination kit offer an interesting new approach to early detection of MRSA.

R2107

Evaluation of external proficiency testing results among 532 microbiology laboratories of Tehran 2004

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Objective: The aim of this study was to evaluate external proficiency testing in microbiology laboratories located in Tehran and suburbs for identification of three unknown bacteria.
Methods: The 14th survey of external programme for 2004 distributed to 532 laboratories in Tehran. The selected microorganisms were: *Shigella flexneri*, *Sreptococcus agalactiae* and *Stenotrophomonas maltophilia*. *S. flexneri* were sent to all 532, *S. agalactia* to 395, and *S. maltophilia* to 137 microbiology laboratories. We asked all laboratories for identification each unknown organism, performance of susceptibility testing for *S. flexneri* and return their answer to reference laboratory within 2 weeks receipt of samples. Scoring of results determined according to WHO external surveys criteria.
Results: Of 532 laboratories we received answer from 428 (80.5%) laboratories. Of 428 laboratories 205 (38.5%) produced correct answer for identification of S. flexneri and received 3 score of points. and 31(5.8%) laboratories produced incorrect answer for identification of this organism. The remaining 192 (36.1%) laboratories received 1–2 score of points (mean score 2.2, SD ± 0.91). Of 137 labs only 11(8%) labs produced correct answer for identification of *S. maltophilia* and 66 (48.2%) labs received zero score of points. The remaining labs 23 (16.8%) received 1–2.5 score of points (mean score 81, SD ± 1.13). Of 395 labs 146 (37%) identified *S. agalactiae* correctly and obtained 3 score of points and 32 (8.15) were not able to identify this organism and therefore received 0 score of points The reaming laboratories 137(36%) obtained 1–2.5 score of points (mean sore 2, SD ± 1.1). The results of susceptibility testing were satisfied and the majority of laboratories produced correct answer for susceptibility testing of *S. flexneri* (mean score 4.52). It is assumed that the result of external quality control in Tehran microbiology laboratories was not acceptable.
Conclusion: Our study reveals that unfortunately proficiency testing in microbiology laboratories of Tehran is poor .The poor results were due to: defective reagents, l culture media inappropriate settlement of internal quality control programme and of unqualified technologists.

R2108

Sensitivity and specificity of laboratory based serological tests for detection of *Helicobacter pylori* infection compared to histopathological test

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Objectives: *Helicobacter pylori* (HP) role in duodenal ulcer, gastric ulcer, gastritis, intestinal metaplasia, maltoma and adenocarcinoma of stomach is established. Then detection of HP by non-invasive methods such as serological test (ELISA) is important. In order to determine of sensitivity and specificity of ELISA in detection of HP infection comparing to histopathology (Gold standard) this study was done.
Methods: In an analytic study 232 patients with gastrointestinal signs were studied. Endocopy was done and 2–3 biopsies were taken from antrum. Specimens were processed by shandon tissue processor (citadel) and prepared sections were stained by Hematoxylin–Eosin and Giemsa. Slides were studied by two pathologist and one microbiologist. Also 5 cc blood was taken to determine anti HP (IgG) by ELISA method. Patients' data including age, sex, clinical signs, histological diagnosis, existence of HP in biopsies and results of ELISA tests were recorded and analysed by statistical tests.
Results: Of 232 patients 130 (56.1%) were females and 102 (43.9%) males. They were in age group 10–80 years. The most common clinical sign of infected patients was epigastric pain and the least were hiccup and vomiting. The most common pathologic findings were chronic active gastritis with ulcer or intestinal metaplasia and then chronic active gastritis alone. In the base of histopathology 222 (95.7%) patients had HP infection, by ELISA method 197 patients were IgG positive. ELISA sensitivity was 85%. Of 10 patients who were negative in histology in 2 patients IgG was negative. Specificity of ELISA was 20%. ELISA method showed positive predictive value 95.9% and negative predictive value 5.7%.
Conclusions: This study showed that serologic test (ELISA method) IgG is a good test for screening but no for diagnosis. We recommend ELISA method be used for screening and then diagnostic tests such as histology or microbiology be used for definite diagnosis. There is no relation between H.P infection age, sex and clinical signs.

R2109

Toxin A negative/toxin B positive *Clostridium difficile* strains isolated from patients with diarrhoea in a tertiary hospital in Athens

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Objectives: During the last five years, toxin A negative/toxin B positive (A–B+) *Clostridium difficile* (Cd) strains were isolated in Asia, Canada and Europe. No A–B+ Cd strains have been isolated from patients in Greece so far. The aim of this study is to present five cases of diarrhoea caused by A–B+ strains in our hospital, over a two year period.
Methods: Over the last two years, we prospectively examined 913 stool samples obtained from patients with diarrhoea for the presence of Cd and its toxins A&B. Culture for Cd was performed on cycloserine–cefoxin blood agar and the strains were identified by conventional methods. Toxin A was detected from stools by an ELISA assay (Vidas, bioMérieux) and a chromatographic assay (Color Pac, BD). Toxigenicity of the isolated strains was examined using Color Pac test for toxin A and Premier toxins A&B test (Meridian, USA) for toxins A&B. Cd strains negative by Color Pac and positive by Premier test were considered A–B+. The MIC's of the A–B+ isolates to antibiotics were determined using E-test (AB Biodisk, Sweden).
Results: Cd was isolated in 70/913 (8%) stool samples. Toxin A was detected in 79/913 (8.6%) samples. Five of 70 (7%) Cd strains were A–B+, two (3%) were A–B– and 63 (90%) were A+B+. The A–B+ strains were isolated from five patients (age range 50–85 years), hospitalized in different wards of the hospital during 2003 (4 patients) and 2004 (1 patient). All patients had more than one risk factors to develop Cd associated diarrhoea such as antibiotic therapy (3), malignancies (2), chemotherapy (2), advanced age (4). All five A–B+ strains were resistant to clindamycin, three were resistant to penicillin, but all were sensitive to vancomycin and metronidazole.
Conclusions: This is the first report of isolation A–B+ Cd strains from hospitalised Greek patients with diarrhoea. The strains were resistant to clindamycin and all the patients had high risk factors to develop Cd associated diarrhoea.Laboratories should apply diagnostic assays to detect both toxins A&B, especially in patients with risk factors to develop Cd intestinal infections.

R2110

Accuracy of cefoxitin disk diffusion for the detection of oxacillin resistance in *Staphylococcus lugdunensis* and *Staphylococcus saprophyticus*

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Objectives: The NCCLS interpretive criteria for oxacillin against coagulase-negative staphylococci (resistant: MIC > 0.25 mg/l or <18 mm) may overcall resistance for *Staphylococcus lugdunensis* and *Staphylococcus saprophyticus*. Recent reports indicate that cefoxitin is better than oxacillin in detection of oxacillin-resistance in staphylococci. In this study we compared the cefoxitin disk with four other methods for the detection of oxacillin resistance in *S. lugdunensis* and *S. saprophyticus*.
Methods: A total of 64 clinical isolates of *S. lugdunensis* and 78 of *S. saprophyticus* recovered from patients at our institution were studied. The isolates were identified using the MicroScan system (Dade Behring. Sacramento, CA) and confirmed by additional tests. All *S. lugdunensis* were mecA negative. Among *S. saprophyticus*, 5 were mecA positive, and 73 mecA negative. Following standardised NCCLS methods, oxacillin susceptibility was determined using: disk diffusion with oxacillin 1 mcg and cefoxitin 30 mcg disks, oxacillin microdilution using the MicroScan system, and detection of PBP2a by the latex agglutination test (OXOID. Hampshire. UK). The detection of the mecA gene by PCR was used as the 'gold standard' assay.
Results: Sensitivity and specificity of cefoxitin disk for the detection of oxacillin resistance in *S. saprophyticus* were 100% and 81%, respectively. Specificity against *S. lugdunensis* was 96%. All mecA positive isolates were cefoxitin-resistant (<25 mm), however 17 mecA negative *S. saprophyticus* (22%) and 2 mecA negative *S. lugdunensis* were cefoxitin-resistant. The specificities for oxacillin disk diffusion, oxacillin microdilution, and PBP2a detection against *S. lugdunensis* were 61%, 23%, and 100%, respectively. Against *S. saprophyticus*, the sensitivities and specificities for oxacillin disk, oxacillin microdilution, and PBP2a were: 100% and 5%; 100% and 19%; 60% and 98%, respectively.
Conclusion: Cefoxitin disk diffusion is more specific than oxacillin disk and oxacillin microdilution for the detection of oxacillin resistance in *S. lugdunensis* and *S. saprophyticus*. The cefoxitin disk is an accurate surrogate test for *S. lugdunensis*, but is less specific in the case of *S. saprophyticus*.

R2111

Identification of genital mycoplasmas: comparison of two methods

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Objectives: Mycoplasmas represent a unique group of microorganisms. Mycoplasma hominis (Mh) and ureaplasma urealyticum (Uu) are common genital inhabitants that have been associated with a variety of clinical conditions in pregnancy, infertility and pelvic inflammatory disease. The aim of the study was to compare two methods for the isolation of the organisms from the lower genital tract.
Methods: We studied 97 vaginal secretions from an equal number of women attending our hospital. All samples were cultured for aerobic and anaerobic bacteria. For the identification of both Mh and Uu two methods were used.One was the commercial kit Mycoplasma IST 2 (BioMerieux, France) and the other was an 'in house' method which is used for years in our laboratory. For the identification of Mh we prepared DNA-PPLO agar, while Uu was identified by its ability to metabolize urea using T-broth, a preparation containing 10% urea broth.
Results: Out of the 97 samples, with the commercial kit we isolated 26 positive samples (26.8%) for Uu and 6 positive (6.2%) for Mh. On the other hand, our method resulted in 28 (28.9%) positive samples for Uu and 4 (4.1%) positive for Mh. The commercial kit offers the advantage of performing the antibiotic susceptibility testing. The most sensitive antibiotics for mycoplasmas were tetracycline (96.2%) and deoxycycline (92.2%). To our surprise, quinolones (Ofloxacin and Ciprofloxacin) showed decreased susceptibility rates (26.9% and 3.8%, respectively), while the intermediate sensitivity rates were very high (69.3% and 73.1%, respectively). In 42.3% of the cases Uu was the only pathogen isolated. Uu coexisted with bacterial vaginosis in 38.4% of cases, while with other pathogens like Gram-negative rods, Gram-positive cocci and yeasts in 11.5%, 3.9% and 3.9%, respectively. Concurrent infections by both Uu and Mh were in all cases accompanied by bacterial vaginosis.
Conclusions: The two methods proved to be similar in sensitivity and specificity. The commercial kit has the advantage of the antibiotic susceptibility testing. Mycoplasmas were moderately sensitive to quinolones probably because of the irrational use over the last years. Both Uu and Mh correlated positively with bacteria involved in the specific clinical entity bacterial vaginosis.

R2112

Detection of cytolethal distending toxin for diagnosis of *Campylobacter jejuni* gastroenteritis

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Objective: *Campylobacter* spp. are recognized as a major cause of acute bacterial enteritis in the developed countries. Several potential virulence factors have been proposed as important factors in the pathogenesis of *Campylobacter jejuni* e.g. motility, adherence, invasion and toxin production. The only toxin of *C. jejuni* characterized at the molecular level is the cytolethal distending toxin (CDT); cdt genes have also been found in various other Gram negative bacteria. The aim of this study was to examine whether the level of CDT expressed had any impact on the clinical outcome in patients infected with *C jejuni*.
Methods: The levels of CDT expressed by 30 clinical *C. jejuni* isolates, from patients with campylobacteriosis, were determined using a HeLa cell assay. The presence of the three CDT coding genes, cdtA, cdtB and cdtC was tested in these isolates by PCR. The presence and titres of serum neutralizing antibodies to CDT in the corresponding patient serum was also measured. Clinical data were obtained from these patients using a standardised questionnaire.
Results: PCR examination of the cdt genes revealed that 29 of the strains had the wild type sequence; the remaining strain had major deletions in the CDT genes. Three of the 30 *C. jejuni* strains had undetectable levels of CDT, 20 strains had low CDT titres while 7 had high titres. CDT neutralizing antibodies were found in all patient sera. The levels of anti-CDT antibodies did not correlate with the levels of IgG, IgM or IgA against whole cell heat-stable *C. jejuni* antigens.
Conclusion: The results showed a trend in low CDT titre increasing the risk for patients to become febrile. Neither CDT expression nor the presence of CDT neutralizing antibodies in patient serum correlated to clinical outcome. Thus, this study indicated that CDT cannot be used for diagnostic purposes.

R2113

Usefulness of chromogenic agar Candida ID2 for differentiating *Candida dubliniensis* from *Candida albicans*

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Objective: To test the hypothesis that *Candida dubliniensis* and *Candida albicans* isolates can be differentiated on the basis of colony color in the chromogenic media Candida ID2 (bioMérieux, France) and CHROMagar Candida (CHROMagar, France).
Methods: One hundred *C. dubliniensis* and 100 *C. albicans* isolates were tested on Candida ID2 and CHROMagar Candida agar plates. Isolates had been previously identified by conventional mycological methods, as the germ tube induction test in serum, microscopical morphology and chlamydospore formation in corn meal agar with Tween 80, and carbon source assimilation with the commercial kit API ID 32C (bioMérieux). C. dubliniensis identification was confirmed by PCR with specific primers. Prior to testing, each strain was subcultured for 24–48 h at 36 ± 1°C on Sabouraud glucose agar to ensure viability. Plates were incubated at 36 ± 1°C and read for visual colony color independently by three investigators after 24 and 48 h of incubation.
Results: All yeast isolates grew well on both agars after 24 h of incubation, although growth on CHROMagar Candida was slower and colonies smaller. After 48h of incubation, all *C. dubliniensis* isolates produced blue turquoise colonies on Candida ID2, whereas 91 *C. albicans* isolates grew as blue cobalt colonies and 9 as blue turquoise colonies (Chi square, $p < 0.01$). However, on CHROMagar Candida 13 of *C. albicans* isolates evaluated gave a lighter shade of green and 12 gave dark green color. In contrast, 62 *C. dubliniensis* isolates gave dark green color.Therefore, the sensitivity and the specificity for differentiating between *C. albicans* and *C. dubliniensis* on Candida ID2 chromogenic medium were 100% and 91%, respectively.
Conclusion: Candida ID2 agar provides a simple laboratory tool for the differentiation of *C. dubliniensis* from *C. albicans* in clinical microbiology laboratories.This work was financed in part by the project IE019 ETORTEK-2002 (Diamolfun subproject) from the Departamento de Industria, Comercio y Turismo del Gobierno Vasco-Eusko Jaurlaritza.

R2114

Electroencephalographic findings in patients with clinical features of meninges excitation

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This study presents 80 patients who had been treated at Clinic for Infectious diseases during the last two years, and who had exhibited the signs of meninges excitation, with or without neurological disorders, but with normal cytological and biochemical findings in cerebrospinal fluid.The most common diagnoses were virosis, bronchopneumonia, pharyngitis, vertiginous syndrome, urinal infections, etc. The majority of the patients were 10–25 years old. Among them, 5% reported previous convulsions, end even 14% informed us of earlier head trauma. Pathological electroencephalographic (EEG) findings were observed in 50% of the male and 63% of the female patients. The EEG disorders occurred mostly between the 5th and the 15th day of the disease. Among the patients with pathological EEG findings, 94% were conscious, 3% somnolent, and 3% soporal. According to the location of EEG changes, the temporal region dominated – 27%, and the frontal region was the rarest – 8%. Most of them had unspecific EEG disorders, a few exhibited irritative changes, steeper waves or specific changes. In our opinion, earlier convulsions and head traumas in these patients explain way they reacted with meningeal or neurological symptoms to other infection.

R2115

Analysis of consecutive blood cultures performed in one year by an infectious team: were three haemocultures still necessary?

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Introduction: Blood cultures for the purpose of isolating bloodborne pathogens have routinely included the use of media for detection of both aerobic and anaerobic organisms. Although methods for the detection of anaerobic bacteria from blood cultures have improved, the per cent recovery of anaerobes isolated from blood cultures has declined. This study analyses all blood culture isolates in view of clinical condition for one year.
Materials: During the year 2002 the infectious disease team (IDT), who help for the diagnosis and treatment of all infected patients in Saint-Joseph hospital, analysed all the positive bottles. The IDT determined the clinical significance of all blood positive culture. Contamination was determined if one bottle out of two or three sets (2 bottles) collected on the same day grew with coagulase negative staphylococci, corynebacterium species, bacillus species and if there is a habitual pathogen with no clinical signification and clinical improving without treatment. A beginning of antibiotherapy in case of false positive positive bacteraemia was reported.
Results: For the year 2002, 8094 sets were utilised. 931 were positives. 377 (40 %) bottles were considered as contamination with 207 (22 %) aerobic and 170 (18%) anaerobic, concerning 274 patients. 31 contaminations induced antibiotic therapy. 554 bottles were considered as a true bacteraemia for 487 patients. 48 (%) bacteriemic patients have positive anaerobic bottle only. Bacteria present only in anaerobic bottle were anaerobes (16 cases), *Staphylococcus aureus* (5 cases), *Escherichia coli* (14 cases), others enterobacteriacae (8 cases), 2 *Streptococcus pneumoniae* and others streptococci (5 cases). Infections concerned in these cases were intra-abdominal (27 cases), soft-tissue infections (3 cases), pyelonephritis (11 cases) and others (7 cases).35 of 48 bacteriemic patients in only anaerobic bottle were positives since the first set, 12 since the second set with 8 patients treated with active antibiotherapy and one patient in the fourth set.
Conclusion: In view of these results, we think that only 2 sets of bottles are necessary for blood stream infection diagnostic.

R2116

The application of Avidity Index testing to identify recent HIV infection

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Valuable epidemiological data can be obtained by determining the incidence if new HIV infection. Currently the only FDA approved system to determine how recent a HIV-1 infection occurred is STARHS – Serologic Test Algorithm for Recent HIV Seroconversion. This presentation describes the application of avidity index (AI) testing to determine how recent HIV-1 infection was acquired. The protocol involved the modification of Bioelisa HIV-1+2 (rec) (BIOKIT), by the introduction of a

dissociation step, which resulted in the disruption of the binding between antibody and antigen. The assay protocol was developed and evaluated using well characterised samples collected from HIV infected individuals using a range of serological and molecular assays. An AI of 60% was identified as a threshold below which HIV infection probably occurred within the previous 90 days. Avidity index has the advantage of determining the relative timing of HIV infection using only one sample, which is often the case when performing unlinked epidemiological investigation. Data will be presented showing the application of the assay to the investigation of newly identified HIV infection.

R2117

Comparison of 2 *Chlamydia trachomatis* IgG ELISAs with the MIF test

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Introduction: In the fertility work-up *C. trachomatis* antibody testing is also used. It is a less invasive procedure than laparascopy in the detection of tubal pathology as a result of a C. thachomatis infection. This study was performed because ELISA's are less prone to subjectivity and are easier to automate as compared to the microimmunofluorescence test (MIF).
Objectives: To compare 2 ELISA kits (*Chlamydia trachomatis*-IgG/IgA-pELISA medac, MEDAC and Vir-ELISA anti-Chlamydia-IgG/IgM, Viro-immun) with the current gold standard MIF technique (FOCUS) which is our routine technique.
Methods: 72 serum samples from individual patients were analysed for IgG antibodies with the 3 methods described above. All tests were performed according to the manufacturers instructions. The Medac ELISA is coated with synthetic *C. trachomatis*-specific peptide, whereas the Viro-immun ELISA is coated with purified, homogeneous, inactive antigen.
Results: In the MIF test we have found 32 IgG positive and 40 IgG negative serum samples. In the Medac ELISA we have found 28 positives and 44 negatives of which 4 were positive in MIF (sensitivity 88.8%/specificity 100%). In the Viro-immun ELISA there were 36 positives of which 5 were negative in MIF, 1 equivocal and 35 negatives of which 1 was positive in MIF (sensitivity 96.9%/specificity 88.8%).
Conclusion: Both ELISA's performed well as compared to the MIF test and further prospective study to evaluate the diagnostic accuracy of the ELISA test will be done.

R2118

Procalcitonin and fever as markers of bacteraemia in patients with sepsis

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Objective: To assess the clinical informative value of Procalcitonin (PCT) plasma concentration and fever as markers of bacteraemia in patients with sepsis.
Material and methods: All adult patients admitted between Jan 2002 and Aug 2004 who fulfilled the criteria for sepsis/severe sepsis according to the American College of Chest Physicians/Society of Critical Care Medicine. Fever and plasma PCT with a semi-quantitative method were measured and two sets of blood cultures were taken at admittance. Patients without proven bacterial sepsis were excluded. We compared results in patients with or without bacteraemia using *t* test, chi-square test and descriptive statistics.
Results: 117 patients with bacterial sepsis/severe sepsis were included, 73 (62%) with bacteraemia: 24 with Gram-positive bacteraemia and 49 with Gram-negative bacteraemia. There were no statistically significant differences between either bacteremic and nonbacteremic ($p = 0.46$) or between Gram-negative and Gram-positive ($p = 0.37$) bacteremic patients, regarding fever. At cut-off value of 2 ng/ml, PCT discriminate between bacteremic and non-bacteremic septic patients ($p = 0.0002$) with a positive predictive value of 80% and a sensitivity of 78% for bacteraemia. There are no significant differences for PCT values between Gram positive and Gram-negative bacteremic patients but at cut-off of 10 ng/ml for PCT the positive predictive value was 73% for Gram-negative bacteraemia.
Conclusion: Fever does not have a discriminatory power for bacteraemia in septic patients and the use of PCT assessment could help physicians limit the number of blood cultures to be processed.

R2119

Urogenital mycoplasma diagnosis, identification and sensitivity testing to 7 antibiotics

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U. urealyticum (U.u) and M. hominis (M.h) are species in the family Mycoplasmataceae, which is the smallest bacteria replicating in culture medium and they do not possess peptidoglycan cell walls. These tiny microorganisms can be found commensal in lower genitourinary tracts of sexually active men and women. Moreover, they cause many disorder such as non-gonococcal urithritis, postpartum fever, infertility, and pelvic inflammatory disease. 15 human species have been found to date all belonging to the mollicutae class. They differ from other bacteria in their lack of a cell wall and hence a natural resistance to α-lactams, as well as by the presence of a membrane rich in sterol obtained through their adhesion to eukaryotic cells.
Objective: The aim of present study was to evaluate the occurrence of U.u and M.h in non-gonococcal urethritis and to determine the bacterial resistance to seven antibiotics in order to determine the most suitable treatment strategy.
Methods: A total of 83 patients were enrolled into the study. Urethral samples were taken with a dacron swab placed into urethra 2–3 cm in males, and vaginal samples were taken from the endocervical region in women. Mycofast EvolutioN 3 detects urogenital mycoplasma growth in a liquid milieu. During growth, U.u and M.h metabolise urea and arginine respectively resulting in a colour change of the medium, which contains phenol red indicator, from yellow-orange to red. This colour change is due to liberation of ammonia resulting in an alkaline pH of the medium.
Results: Of the 83 patients in the study, 8 (9.6%) were men and 75 (90.4%) were women with signs of urethral discharge and/or urethral discomfort and lower urinary tracts symptoms. Mycoplasma Mycofast EvolutioN 3 (International Microbio, France) was used for the isolation of U.u and M.h. U.u was isolated from 40 (48.1%) patients. 30 (36.1%) of them had only U.u, and the rest had mixed pathogen organisms 9 (10.8%) U.u and M.h. While 1 (1.2%) patient had M.h only. 7 antibiotics (doxycycline, pristinamycin, roxithromycin, azithromycin, josamycin, azithromycin, ciprofloxacin and ofloxacin) were used in this system. In the evaluation of antibiotic susceptibility, higher resistance was obtained against ciprofloxacin and oflaxin in U.u.
Conclusion: Our results indicated that deoxycicline or pristinamycin should be the first choice when empirical treatment is

necessary. This commercial kit is good for the isolation of U.u and M.h.

R2120

Comparison of the antimicrobial agents on the MicroScan Synergies plus Neg Combo Type 2 Panel with an NCCLS reference panel

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Objectives: The MicroScan Synergies plus Neg Combo Type 2 Panel combines early MIC results with the capacity to hold and read panels incrementally up to 18 h. The accuracy of this panel in detecting antimicrobial resistance was evaluated by comparing results from this panel format to results obtained with an NCCLS Reference Panel.
Methods: Fresh and stock gram-negative isolates were tested. The Synergies plus Neg Combo Type 2 panel contains the antimicrobials ampicillin (Am), ceftazidime (Caz), and gentamicin (Gm) in MIC format (>3 dilutions), and amikacin (Ak), ampicillin/sulbactam (A/S), ceftriaxone (Cax), cefuroxime (Crm), ciprofloxacin (Cp), gatifloxacin (Gat), imipenem (Imp), levofloxacin (Lvx), meropenem (Mer), piperacillin/tazobactam (P/T), tobramycin (To), and trimethoprim/sulfamethoxazole (T/S) in breakpoint (BP) format (less than or equal to 3 dilutions). The number of organisms evaluated for each antimicrobial ranged from 329 to 761, and varied depending on the indications for use with that antimicrobial.
Results: The essential agreement between Am, Caz and Gm and the reference method ranged from 98.0% for Am to 99.1% for Gm. Absolute categorical agreement (CA) for these three antimicrobials was 95.2% for Am, 96.0% for Caz and 96.3% for Gm; major error (MJ) rates ranged from 0.6% for Am to 0% for Gm. Very major (VMJ) error rates ranged from 0.8% for Am to 0% for Caz and Gm. For the BP format antimicrobials, CA ranged from 88.1% for A/S to 99.1 for T/S. MJ rates for BP ranged from 1.8% for Crm to 0% for Ak, Gat, Lvx, Mer and To. VMJ error rates for BP ranged from 8.3% for P/T (4/48 resistant organisms, 1-dilution errors) to 0% for A/S, Cax, Cp, Gat, Imp, Mer and T/S.
Conclusion: This study showed that the antimicrobials on the MicroScan Synergies plus Neg Combo Type 2 panel provide appropriate performance in the detection of Gram-negative bacterial resistance in comparison with an NCCLS broth microdilution reference panel.

R2122

Evaluation of BACTEC LX blood culture system

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Objective: Evaluate the performance of the new fully automated BACTECTM LX Blood Culture diagnostic system. This system utilizes a finely resolved beam of infrared light through the culture headspace to obtain an infrared absorption spectrum. This infrared absorption spectrum provides headspace composition information. The growth of microorganisms is determined algorithmically by either kinetic analysis of the headspace or by net headspace composition. This evaluation includes data on instrumentation and reagents that are currently in clinical evaluation.
Methods: The study includes ATCC strains of Enteric bacteria, Staphylococci, Streptococci, Enterococci, Obligately-oxidative Gram negative bacteria. BACTEC Aerobic Plus and BACTEC Anaerobic Plus media were both included in the study. Suspensions of cells from a fresh culture on solid media were used as inocula. The target inoculum per bottle was 10 CFU. The test included banked human blood to determine the effect of blood volume on the system. The BACTEC LX system incubates at 35 °C, agitates the cultures and tests for CO_2 accumulation on a 20-minute cycle. The system was compared to the BACTEC 9240.
Results: The average time to detection for the Aerobic Plus medium for all bacteria was 18 (±8) h for the BACTEC 9240 system and 18 (±8) h for the BACTEC LX. The average time to detection for the Anaerobic Plus medium for all bacteria was 25 (±14) h for the BACTEC 9240 system and 26 (±13) h for the BACTEC LX.
Conclusions: The data demonstrates that under typical workflow conditions in the laboratory the BACTECTM LX Blood Culture system is equivalent to the BACTEC 9240 system. This test was performed on the BACTECTM LX Blood Culture system in the configuration to be evaluated clinically at multiple sites in a controlled comparison study. The BACTECTM LX Blood Culture system utilizes technology that provides data from the direct measurement of carbon dioxide. This type of system allows quantification of carbon dioxide (true delayed vial entry detection). The system is less susceptible to secondary noise effects since the system is a direct measure of carbon dioxide and not a secondary measurement like sensor technology.

R2123

Evaluation of the ROBOBACT system for automatic processing of vaginal cultures for screening of *Streptococcus agalactiae* in pregnant women

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Context: Group B *streptococcus* (GBS) is the most common cause of early-onset neonatal sepsis in developed countries, and determination of the GBS colonization status in pregnant patients near term is essential for the provision of prophylactic measures to prevent early-onset disease.
Objectives: To determine if GBS recovery rates of a semiautomatic method, Robobact (RB, Rotec), is similar to selective SB Broth (SBB, Biomedics).
Patients and methods: 294 pregnant women were evaluated for GBS colonization by combined lower vaginal-anorectal swabs (LVRS). Mean age was 30.3 years old (range 17–42; standard deviation 1.56). Swabs were inoculated to both culture broths as preenrichment media. Subculture in Blood Agar was done manually in SBB culture 18 h later, and by an automatic system with RB 6 h later. Investigation of GBS was done with agglutination test (BioMerieux).
Results: LVRS were positive in 18.7% and 17.4% of pregnant women with SBB and RB methods respectively ($p = 0.75$). With

combination of both methods 19.7% were positive (3 cases only detected with RB and seven only with SBB).
Conclusions: These results indicate that ROBOBACT system (ROTEC) is a reliable method that can be used to screen for maternal GBS colonization with similar results than SB Broth. (Biomedics) in only 24 h.

R2124

Fever of unknown origin and ferritin level: three cases of adult-onset Still's disease

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Background: Fever of unknown origin (FUO) is a clinical picture which has both infectious and noninfectious causes. Neoplasm and connective tissue diseases are most common noninfectious etiological categories. Among connective tissue diseases, Adult-onset Still's disease (ASD) accounts for approximately one quarter of cases. Clinical and laboratory findings are nonspecific in ASD and serum ferritin levels were markedly elevated.
Purpose: In this study, we presented three cases of FUO diagnosed as ASD with different clinical and laboratory findings whereas ferritin levels markedly elevated in all patients.
Cases: Cases presented here were 23, 25 and 20 years old male patients. Clinical and laboratory findings were summarized in the table.

Conclusion: As shown in the table clinical and laboratory findings of all patients were different and nonspecific. Long lasting fever, arthralgia with elevated levels of CRP, ESR, and ferritin were common findings. Most published studies focused on the potential usefulness of serum ferritin assay for diagnosing ASD. In FUO cases, after exclusion of infections and malignancies, diagnosis of ASD was supported with the elevation of serum ferritin levels strongly.

Clinical and laboratory findings	Case 1	Case 2	Case 3
Fever	+	+	+
Rash	–	+	+
Arthritis	+	–	+
Arthralgia	+	+	+
Sore throat	+	+	+
Wasting	+	+	+
Conjunctivitis	+	–	+
Lymphadenopathy	+	–	–
Hepatomegaly	+	+	+
Splenomegaly	+	+	–
WBC (mm^3)	32500	20800	21100
ESR (mm/hours)	153	63	87
CRP (mg/L)	297	330	267
AST (U/L)	38	174	178
ALT (U/L)	100	398	140
RF	–	–	–
ANA	–	–	–
Ferritin (ng/L)	7361	7500	616

Mycobacterial infections (incl. diagnostics)

R2125

Mycobacterial skin infections: pathologic section results

I. Fedotova *(Kharkov, UKR)*

Objective: Detection of acid fast bacilli (AFB) in chronic granulomatous inflammation is an important clue for mycobacterial infection.
Methods: We reviewed of 77 pathologic sections of mycobacterial skin infections to study histopathologic features and the correlation with the presence of AFB in the section was performed.
Results: All cases showed granulomatous inflammations that can be categorized into 4 types: mixed cell, suppurative, tuberculoid and palisading granuloma. AFB was found in 15 sections (19.5%). 44.15% compared to the AFB negative group. Mixed cell granuloma was the most common histologic feature, but suppurative granuloma was the most common histological feature (47.12%) in which AFB could be found, which was statistically significantly different from other types of granuloma. Tuberculoid granuloma was more common in the AFB negative group (10.53%) compared to the AFB positive group (9.37%) but the difference was not statistically significant. In cases that AFB could not be found, the inflammation tended to be located in the upper half of the dermis.
Conclusion: AFB can be more frequently detected in suppurative granuloma that might be located in any portion of the dermis.

R2126

Multi-drug resistance *Mycobacterium tuberculosis* in a centre of Tunisia

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The multi-drug resistance (MDR) of *Mycobacterium tuberculosis* represents currently the biggest problem concerning tuberculosis on the individual and collective scale. Through 800 stumps of *Mycobacterium tuberculosis* isolated in the laboratory of microbiology of Farhat Hached university hospital in Sousse – Tunisia during a period from January 1990 to August 2004, we have represented the main features of this problem. The 800 stumps of *Mycobacterium tuberculosis* have been isolated for more then 90% from sputum. The study of the sensitivity to the antituberculosis drugs has been achieved by method of proportion. The global resistance to isoniazid (INH) is 5%, to rifampin (RMP) is 2.5%, to ethambutol (ETB) is 2.5%, to pyrazinamide (PZN) is 2.8% and to streptomycin (STM) is 2.5%. The multidrug resistance interested 11 strains/patients.

- 5 patients present a secondary resistance causing by a bad therapeutic observance.
- 2 patients present a secondary resistance due to a bad digestive absorption.
- 5 patients present a primary resistance.

Therapeutic assays after bacteriological diagnosis were limited. The prognosis was fatal for 5 patients whereas 6 continue to live with a multi-drug resistance tuberculosis. In view of the gravity

of this regional, national and world problem, some intense preventive measures must be applied rigorously.

R2127

Determination of *Mycobacterium tuberculosis* drug resistance in Mazandaran province, Iran – 2002

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Objectives: Tuberculosis (TB) is one of the most important infectious disease and the main cause of mortality in the developing countries (4). Drug resistant leads to failure in treatment which is followed by unaffordable expenses (6). Administrators of Iran to plan necessary educational programmes, and as soon as possible eradicate tuberculosis in Iran.
Methods: Sputums from the individuals referring to health centre suspected of having TB were collected in sterile containers, decontaminated by PETROPH method and concentrated, supernatant was removed and collected in container having disinfectant then sediment was inoculated onto the Lowenstein–Jensen medium and incubated at 37 (C for 4 weeks. Smears were prepared from the collected samples, Zeihl-Neelsen staining was performed and looked for the presence of mycobacterium for 10 min growth on the medium 4 weeks after the inoculation it was transferred to the partment of mycobacteriology Culture media were observed for the growth of *mycobacterium tuberculosis* (TB). The results were recorded. At Masih daneshvari TB centre in Tehran, to be confirmed for the growth of TB and performing antibiogram.
Results: In this study, on 45 TB growth cultures, drug sensitivity was done for 4 essential drugs that are Izoniazid (INH), refampin (Ref), ethambotol (Eth) and stereptomycin (ST) hich are used against TBCases resistant to Izoniazid and 37 sensitive as result 26.66% resistant. For rifampin 37 cases sensitive and 8 cases resistant, that is 26.66% resistant. For Ethambitol 5 cases resistant and 40 cases sensitive, as a result 16.66% resistant. In case of streptomycin 17 cases resistant and 28 sensitive, with 37.77% resistant. Also two simultaneous resistant to rifampin and streptomycin was observed, and one case with simultaneous resistant to Izoniazid, rifampin and streptomycin was noticed.
Conclusion: The results of this study indicate that, resistant to INH, Rif, Eth, and ST is 26.66%, 16.66% and 37.77% respectively, which reveals an increasing pattern of TB drug resistant. Hence, increase in educational training of the physicians and paramedical personals for the proper short course treatment and implementation of directly observed treatment short course (Dots) strategy considered as prior.

R2128

Haemorrheological alterations in Nigerian pulmonary tuberculosis patients

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Objective: Pulmonary tuberculosis (PTB) is a major infectious disease with very high incidence in developing countries and this is expected to rise with the incidence of HIV infection. Despite the known consequences of PTB on blood flow, rheologic properties have not been viewed with any serious research attention. This study was designed therefore, to investigate the possible haemorheological effects of the infection.
Methods: Haemorheological parameters were studied in 40 (17 males and 23 females) and 10 (5 males and 5 females) newly diagnosed PTB patients but confirmed to be HIV negative. Their ages ranged between 25 and 45 years. Also, 50 apparently healthy controls with age and sex matched were compared. Haematocrit (HCT), plasma viscosity (PV), erythrocyte sedimentation rate (ESR) and plasma fibrinogen concentration (PFC) were estimated with standard methodologies while student t-test was used to compare the data.
Results: Patients (treated and untreated) show statistically significant increase in PFC, PV and ESR ($P<0.01$ respectively) while there was a statistically significant decrease in the HCT ($P<0.01$). However, treated patients show an improved rheological values than the untreated ones (i.e. reduced ESR and PV with an increased PCV, $P<0.01$ respectively). There was no established male and female differences amongst the patients.
Conclusion: Hyperfibrinogenaemia with hyperviscosity are possible consequences of PTB infection and coupled with reduced PCV and increased ESR indicates chronic signals of altered haemorheology which may predispose the patients to increased cardiovascular risks. The inclusion of haemorheological parameters in the monitoring of patients on treatment is hereby emphasized.

R2129

Challenges of *M. tuberculosis* drug resistance in Russia

E.V. Sevastyanova, V.I. Golyshevskaya *(Moscow, RUS)*

Despite some stabilization and even decrease in TB incidence, epidemic situation for TB in Russia remains difficult. Aggravation of the epidemic situation for TB is primarily caused by proliferation of multi-drug resistance among new cases and prevalence of HIV-infection. The major condition of TB control both among civilians and prisoners is bacteriology verification and imperative drug resistance determination, especially MDR. In 2003 MDR was 8.0% among new cases (newly-detected patients) in Russia; primary MDR among prisoners reached 17.8%. In different Russian regions frequency of drug resistant cases significantly ranged. This was mainly caused by non-standardized drug resistance determination methods, also by disadvantages or impediments, such as lack of substances for drug resistance determination, lack of equipment, lack of reagents and lack of professional staff, no regular external quality control, other. Recently a number of Russian laboratories have implemented rapid methods of drug resistance determination along with traditional absolute concentrations. Only quality and degree of standardization of such studies, as a major condition, will assure reliability and repeatability of results.

R2130

Serum total adenosine deaminase activity in active pulmonary tuberculosis in comparison to other infectious diseases in Iran

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Objective: In order to have an acceptable rapid test helping the clinicians in the diagnosis of active pulmonary tuberculosis, we evaluated the importance of elevated serum adenosine deaminase in active pulmonary tuberculosis versus other infectious diseases.
Methods: We measured serum total adenosine deaminase level in 3 groups: 1. Cases of active pulmonary tuberculosis who were confirmed by positive sputum smears for acid fast bacilli in association with compatible clinical and radiologic findings.

2. Cases of other infectious diseases including Brucellosis, Endocarditis, Salmonellosis, meningitis confirmed by clinical findings and related laboratory tests.
3. Healthy controls.Serum adenosine deaminase levels were measured before treatment was started. Data analysis was performed by Chi square, ANOVA and LSD. The significance level was evaluated for P value of less than 0.05.
Results: We evaluated 51 (21 females and 30 males aged 47.7 ± 19 years) cases of active pulmonary tuberculosis, 11 (6 females and 5 males aged 44.7 ± 21 years) cases of other infectious diseases and 50 (14 females and 36 males aged 48.4 ± 11 years) cases of healthy individuals. Mean serum total adenosine deaminase level in pulmonary tuberculosis (42.4 ± 21.5 IU/ml) and other infectious diseases (38.3 ± 23.4 IU/ml) was meaningfully more than controls (26.6 ± 8.2 IU/ml, $p < 0.0001$ and $p < 0.03$ respectively), but the difference in pulmonary tuberculosis and other infectious diseases was not statistically significant. There was no significant difference in age and gender between the above groups.
Conclusion: We conclude that serum total adenosine deaminase increases in infectious diseases but it cannot differentiate pulmonary tuberculosis from other infectious diseases. This project has been supported by Infectious Diseases and Tropical Medicine Research Centre of Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

R2131

Tuberculosis and diabetes: risks and realities

I.C. Bygbjerg *(Copenhagen, DK)*

The vicious combination of tuberculosis (TB) and HIV is now well-established, though not well-controlled. More unnoticed is the meeting between TB and diabetes, predominantly type 2 (DM2,) particularly in low-income countries in fast transition. In India, e.g., about 10% of adults have DM2 and another 10% pre-stages (impaired glucose-tolerance), while 1% may have active TB and 30% have are at risk of (re-)activating TB. In Mexico, the risk for reactivating TB in patients with DM2 was increased 4–6 times, which is comparable with increased risk for TB among HIV-infected. The World Health Organisation preview that by year 2025 about 350 mio. may have DM2, more than 70% in developing countries. It remains to be seen if TB may double or quadruple in the wake of the DM2 epidemic, as has been the case with TB in HIV. The mechanism(s) behind the increased risk for re-activation of TB in DM is poorly understood. Present knowledge (and lack thereof) will be highlighted and suggestions given for future research and possible measures to control this emerging health problem.

R2132

Meningo-encephalo-mielo-polyradiculonevritis with *Mycobacterium tuberculosis*: case report

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Objectives: Tuberculosis still represents a public health problem in developing and underdeveloped countries. We report a case of central nervous system tuberculosis.
Methods: A 17 year-old female was admitted in our hospital for fever, headache, loss of weight and progressive disability to walk. She presented: fever, later cervical lymphadenopathy and the objective neurological signs were: coma, nuchal rigidity, Kernig signs were positive, lack of abdominal cutaneous reflexes and osteotendinous reflexes, paraplegia, convergent strabismus. We performed: CT brain scan, routine hematological and chemical tests, chest X-ray, lumbar puncture, CSF culture, blood culture.
Results: CT brain scan revealed cerebral edema. Routine hematological and chemical tests showed leukocytosis (17100/mm^3). Chest X-ray was normal. Blood culture was negative. Lumbar puncture revealed opalescent CSF with 122 WBC/mm^3 (lymphocytes), increase in protein level (200 mg%), decrease in glucose level (10 mg%); culture was positive for *Mycobacterium tuberculosis*. She received antituberculosis chemotherapy with isoniazid, rifampin, pyrazinamid, and streptomycin for 41 days. After 30 days of treatment the CSF parameters returned into normal values and the clinical evolution was very good.
Conclusions: Our case shows that *Mycobacterium tuberculosis* infection can affect the entirely central nervous system in absence of systemic infection.

R2133

Tuberculous meningitis: a 5-year review

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Objectives: To assess the present epidemiology, clinical presentation and outcome of patients with tuberculous meningitis (TBM).
Methods: A retrospective study was conducted including 118 cases of tuberculous meningitis hospitalized in the Department of Infectious Diseases Iasi between January 1999 and January 2004.
Results: Among the 118 patients with tuberculous meningitis children (5 months–15 years) accounted 29% and elderly (>65 years) – 6% of the cases. Clinical condition at the time of admission was: coma in 13.5% of the cases, other consciousness abnormalities – 50%, cranial-nerve palsies – 18.6%, hemi/monoparesis – 8% of the cases. A history of contact with tuberculosis or prior tuberculosis was documented in 36% and 21% of the cases, respectively. In 60% of the patients antituberculous therapy was initiated in the first day after admission, 23% of patients received adequate treatment in the second day and 12% were treated after 48 hours. In 52% of the cases other location of tuberculosis was revealed: pulmonary TB (42%), lymph nodes (8%), bones and joint (5%), etc. A positive CSF culture result *M. tuberculosis* was available in 17% of the patients and 12% of patients were smear positive. The CSF albumin level was very high (>3 g/l) in 18% of the patients and glucose level was markedly low (<0.3 g/l) in 47% of the cases. Complications occurred in 49% of the patients: hydrocephalus (15%), tuberculoma (4.2%), hemi/monoparesis (12%), and cranial nerve palsies (18.6%). Ninety-five patients recovered (80%), 10 patients (8%) required neurosurgery procedures for complications of TBM and 13 patients (11%) died.
Conclusions: The prognosis was generally poor for patients with CSF glucose level at the time of admission and for those presenting late, with stage 2 and 3 disease even when treatment was begun on the day of admission.

R2134

Evaluation of molecular assays for detection of *Mycobacterium tuberculosis* multi drug resistant strains and genotyping this isolates by spoligotyping

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Objectives: The increasing incidence of tuberculosis and multidrug resistance (MDR) among *Mycobacterium tuberculosis* strains remains one of the major public health problems in Poland. The geographical position of Lublin means that potentially MDR

strains from former Soviet Union countries are more likely to be present. Rapid detection of MDR can optimize the efficacy of antituberculosis therapy and control their transmission. The aim of the study was to compare current methods of drug susceptibility testing with two molecular assays.
Methods: The Inno-Lipa Rif. TB was applied to detect mutations in rpoB gene conferring rifampicin resistance and a non-commercial low-density macroarray (MDRTB screening array) detecting mutations in rpoB and kat G, inh A genes responsible for rifampicin and isoniazid resistance, respectively. Differentiation of *M. tuberculosis* complex species was confirmed using biochemical analyses, niacin accumulation test and spoligotyping based on polymorphism of the chromosomal direct repeats (DR) locus, which contains a variable number of short DR interspersed with non-repetitive spacers.
Results: 18 MDR strains cultured from patients with pulmonary and extrapulmonary tuberculosis in the laboratory of Public Hospital were subjected to susceptibility testing for rifampicin and isoniazid by the resistance ratio method on L–J medium. All these strains were analysed by the Inno-Lipa Rif, MDRTB screening array and spoligotyping. Phenotypic testing established that 17 of 18 isolates were rifampicin and isoniazid resistant using the resistance ratio method – 'the gold standard' to compare with. By the Lipa assay 17 of 18 strains (94.4%) were scored as rifampicin resistant. The MDRTB screening array had the same results for both rifampicin and isoniazid resistance – 15 of 18 strains (83.3%); 2 of the 18 isolates demonstrated unusual pattern of hybridisation in the MDRTB screening array. Among the 18 isolates identified as *M. tuberculosis* by conventional methods, 17 strains (94.4%) were confirmed as M. tuberculosis whereas 1 strain was identified (5.6%) as *M. bovis* using spoligotyping. The *M. bovis* strain had the absence of the spacers 39 to 43 in DR region, characteristic of all *M. bovis* strains.
Conclusion: Our study shows that rapid commercial and non-commercial molecular methods may be useful tools to detect MDR strains and to distinguish *M. tuberculosis* and *M. bovis* which is often difficult by traditional methods.

R2135

Comparison of BacT/ALERT and Löwenstein–Jensen media and rapid differentiation of *Mycobacterium tuberculosis* complex from other mycobacteria using para-nitrobenzoic acid from clinical specimens at a chest disease hospital in Istanbul, Turkey

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Objectives: To evaluate the performance of BacT/ALERT 3D (bioMerieux, France) for isolating of mycobacteria in comparison with the Löwenstein–Jensen medium. Additionally, a series of studies were conducted to detect the sensitivity of *p*-nitrobenzoic acid test for differentiation of *Mycobacterium tuberculosis* complex with other mycobacteria at Social Security Institute Sureyyapasa Chest Disease Hospital, Istanbul.
Methods: During our investigation, a series of different samples such as sputum, bronchial aspiration, pleural fluid, bronchoalveolar lavage, gastric lavage, urine, periton fluid and lenfadenopathy drenage recovered from patients in the three major services, were treated with *N*-acetyl-L-cysteine-3% NaOH decontamination and concentration procedure. Each sample was then cultivated in BacT/Alert 3D MP bottle and Löwenstein–Jensen medium in 37 °C. Acid-fast staining was employed for smear preparation. Time and presence of growth on BacT/Alert 3D MP bottle was monitored and recorded. Additionally, we have evaluated efficiency of BacT/Alert MP medium in rapid differentiation of mycobacteria by adding PNB.
Results: Among the smear-positive and smear-negative specimens, more Mycobacteria grew on BacT/ALERT MP then L–J media. In smear-positive samples, BacT/ALERT 3D system showed a few days earlier then in smear negative samples. Contamination ratios for BacT/Alert 3D and L–J were 3 and 4%, respectively. The total mean time of detection of BacT/ALERT MP bottles were approximately 19 days shorter than Löwenstein–Jensen media. It has been determined that all bottles with *Mycobacterium tuberculosis* complex showed no growth or late growth compared to bottles containing *p*-nitrobenzoic acid. But other mycobacteria bottles showed same type of growth as the bottles containing *p*-nitrobenzoic acid. Mean time of *p*-nitrobenzoic acid test for other mycobacteria and *Mycobacterium tuberculosis* complex have not exceed more than 5 days.
Conclusions: It has been showed that the performing of *p*-nitrobenzoic acid test in BacT/ALERT MP bottles is sensitive and rapid. Thus total testing time of mycobacteria at the system provides the ability to meet the 21 days target for detection and identification of *M. tuberculosis* complex as recommended by the Centers for Disease Control and Prevention, Atlanta, GA, USA.

R2136

Early diagnosis of lung tuberculosis by automatic system probetec ETTH (Becton-Dickinson)

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Introduction: To study the sensibility and specificity of the strand displacement amplification (SDA) technique compared with baciloscopies (BC) by automatic system Probatec ETTH (Becton–Dickinson) in patients with high potentiality of lung tuberculosis (LTB).
Method: A total of 195 samples from respiratory origin (sputums, bronchial aspirates, broncoalveolar lavages, induced sputums) from patients with high clinical likelihood of LTB, from the General Internal Medicine and Neumology departments of our centre. All the samples underwent (1) a decontamination process; (2) were homogeneizated and concentrated by the method of *N*-acetil-cisteine-NaOH; (3) BC was made in all the samples; (4) culture in liquid medium (BactAlert 3D, BioMerieux) and solid (Löwenstein–Jensen, Becton–Dickinson); (5) *M. tuberculosis* complex (MTC) DNA was tested in all the samples by SDA technique and by the automatic system Probatec ETTH (Becton–Dickinson) following the trader instructions.
Results: A total of 61 (31.3%) samples were positives for MTC DNA, of these samples 49 (80.3%) were BC positives and 12 (19.7%) were negatives. In those 3 samples (1.5%) with BC positive and MTC DNA negative, atypical mycobacteria were presented in 2 cases and in the other one a culture contamination eluded its identification. MTC DNA positive and BC negative were in 12 samples (6.1%), in these MTC only could be demonstrated in 4 (33.3%), in the others the half were from patients with LTB therapy started.
Conclusions: SDA method is quickly, efficient and easy to the early diagnosis of LTB (4–6 h) overall in cases with BC positives. We have achieved a sensibility of 94.2% compared with BC and a specificity of 91.6%. We consider that this technique cannot be used by LTB therapy monitorization.

R2137

Drug resistance pattern of *Mycobacterium tuberculosis* in a technical university medical faculty in Turkey, 2000–2004

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Multidrug-resistant tuberculosis, a disease caused by *Mycobacterium tuberculosis* strains that are resistant at least to rifampin and isoniazid, entails extended treatment, expensive and toxic regimens, and higher rates of treatment failure and death. To document the presence of multidrug-resistant tuberculosis (MDR-TB) strains in patients from Turkey on Black Sea region between January 2000 and October 2004. We retrospectively analysed the outcomes in 249 patients treated and untreated , and tests of primer and seconder resistance to isoniazid, rifampicin, streptomycin, ethambutol and multi drug resistance on their specimens were performed using the Bactec TB-460 system. The most commonly encountered culture-positive specimens were sputum (67%) and BAL fluids (14.4%). The drug resistance pattern of *M. tuberculosis* among these isolates was as follows: 249 strains were resistant to 22.9% isoniazid, 14.8% rifampin, 20.9% ethambutol, 7.6% streptomycin, and 13.2% isoniasid and rifampin drugs.

R2138

Cerebellar abscess and syringomyelia due to isoniazid resistant *Mycobacterium tuberculosis*

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Objectives: Tuberculous brain abscess (TBA) is a rare clinical entity, even in endemic areas. These lesions may occur during the treatment of tuberculous meningitis. Drug resistances were not well defined in Central Nervous System tuberculosis.
Case report: A 19 years-old, immunocompetent, young man was admitted with diplopia, nausea, vomiting and a change in mental status. The patient had a history of tuberculous meningitis diagnosed before six months in another hospital and given anti-tuberculosis treatment with isoniazid (INH), rifampicin (RF), pyrazinamide (PRZ) and ethambutol (ETB) for two months and followed by two drugs treatment with INH and RF for four months. Computed tomography (CT) scan of the brain showed a non-communicating hydrocephalus. The patient was operated for hydrocephalus and placed a ventriculo-peritoneal shunt. Two mounts later, the patient was hospitalized again for fever, dysfagia and left hemiparesis. Cranial CT scan was within normal; however magnetic resonance imaging (MRI) examination revealed irregular multiloculer peripheral contrast enhancement lesion with hypointense necrotic centres within the left cerebellar hemisphere extended to the upper posterior cervical subarachnoid space through left medullacerebellar fissure. The abscess was drained by surgical operation. Acid resistant bacillus (ARB) was demonstrated in the abscess material by Zeihl-Neelsen stain and polymerase chain reaction (PCR). Isoniazid resistant *Mycobacterium tuberculosis* was grew on Lowenstein–Jensen culture medium. One month after the operation quadryparezi was developed. Cervical MRI was revealed cervico-thoracic syringomyelitic cavity. The treatment was given with four drugs (INH, RF, PRZ, and ETB) for 10 months and it was continued with three drugs until 24 months.
Conclusions: The patients with tuberculous meningitis should be observed closely for complications like brain abscess and all isolates of M. tuberculosis must be tested for drug resistance.

Fungal infections

R2139

Pneumocystis jiroveci in patients with pulmonary infections

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Objectives: In patients with acute or chronic respiratory problems mainly tuberculosis (TB) suspected cases, there is a concern that other opportunistic infections apart from TB, such as *Pneumocystis jiroveci*, may be missed owing to lack of diagnostic facilities. The aim of this study was to investigate the extent of *P. jiroveci* pneumonia in patients with respiratory tract infections especially in TB patients registered for smear-negative.
Methods: Three hundred specimens, included bronchoalveolar lavage, and sputum collected from patients having different pulmonary infections were tested by nested PCR for *P. jiroveci* and TB.
Results: *P. jiroveci* was detected in 15 (5%) patients, 7 (46.7%) were TB negative by both acid fast-smear and PCR, the other 8 (53.3%) had dual infections with TB. These patients included old patients, alcoholic, heavy smokers, and post primary TB patients. Results suggest that these might be risk factors for Pneumocystis pneumonia (PCP). Patients with PCP had somehow different clinical presentation and radiographic appearances from patients with TB. The PCP infections might be overlooked due to concurrent infections with TB, which makes the diagnosis difficult.
Conclusions: The use of sensitive diagnostic methods for the diagnosis of PCP in patients with respiratory tract infections who are not responsive to treatment is recommended.

R2140

Susceptibility testing of pathogenic fungi with fluconazole and tea (*Camellia sinensis*) on *Candida albicans* PTCC-5027

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Tea (*Camellia sinensis*) has recently been shown to inhibit growth of a range of bacteria and microfungi. In my study *Candida albicans* standard strain PTCC-5027 were tested for in-vitro susceptibility to fluconazole and tea containing whole extract of Lahijan fresh tea leaves and black tea, Chinese green tea and tea polyphenols and caffeine. A rapid reversed phase HPLC method for the determination of polyphenols, using a binary gradient system, was developed. At the present research, which is aiming at determining the minimum inhibitory concentration of tea extract and tea polyphenols and caffeine at concentration (6.26–200 mg/ml) on (500, 1000, 2000 cells *Candida albicans* PTCC-5027) at two period 24 and 48 h. The macrodilution broth test technique was performed to do this. Fluconazole was used for comparing in this study, and we found resistance of that strain with fluconazole and MICs > 64 μg/ml. The 100% minimum inhibitory concentrations (MFC) calculated in our study are evidence of antifungal activity of polyphenols against fluconazole-resistance *Candida albicans* PTCC-5027. The obtained results confirmed that caffeine in rather than polyphenols produced better treatment outcomes. The obtained MFC for polyphenols in comparison to the whole extract of tea has better inhibitory effect at 48 h on *Candida albicans* PTCC-5027.

R2141

Aspergillosis of the maxilla sinus in patients from maxillofacial surgery clinic in Cracow, Poland, between 1998 and 2004

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Objectives: The aim of the paper was to analyze the occurrence of the aspergillosis of maxilla sinus among patients hospitalized in Maxillofacial Surgery Clinic connected with the estimation of the results of laboratory-clinical diagnostics applied and together with therapeutical procedure.
Methods: The subject of the analysis was the group of 398 patients with chronic inflammation of the maxilla sinus hospitalized in the clinic between 1998 and 2004. The diagnosis of aspergillosis was performed on the basis of interview, object, subject studies, radiological studies, mid-operation estimation of the state of mucous membrane and inside of sinus, and on the basis histological and mycological examinations. Aspirates taken during operation from maxilla sinus were subject to mycological investigation. Diagnostics included: direct microscopic examination, culture on Sabouraud agar, microculture and estimation of MIC for itraconazole and voriconazole using E-tests.
Results: Aspergillosis of maxilla sinus was diagnosed in 12 patients (5 males, 7 females) in age from 19 to 74, respectively in years: 2000: 2, 2001: 1, 2002: 2, 2003: 5 and 2004: 2 cases. In mycological examinations in 5 patients the presence of septate mycelium was proved in direct preparations and in one case the presence of *Aspergillus* conidiophores was also stated. In 3 persons positive result from preparation was proved by culture. Moulds from genus *Aspergillus* was identified. In 2 patients isolates were recognized as *Aspergillus niveus* and *A. candidus*. In histological examination in 9 patients the presence of foreign body was also stated in lumen of sinus and numerous septate mycelium was also identified in preparations. All patients were treated with combined surgical and antifungal therapy using itraconazole and in one patient ambisone.
Conclusions: Applied therapeutical procedure caused significant improvement of local and general condition of patients, which was confirmed by the results of mycological investigations and by the interview which took place along time after surgical operation. In one patient invasive form of aspergillosis was observed together with the expanding inflammation process to eye-socket and to lower part of front skull.

R2142

Adverse events and outcomes in patients with fungal infections treated with amphotericin B deoxycholate

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Objectives: Amphotericin B deoxycholate (AmBd) has been a standard therapy for most systemic mycoses. It is associated with significant adverse events (AEs) including nephrotoxicity (NT). Objectives were to describe AmBd therapy, AmBd related AEs, and clinical outcomes.
Methods: Retrospective chart review was undertaken in an academic centre in Geneva, Switzerland, on patients with IFI receiving AmBd as initial therapy. Data abstracted included: AmBd mean daily doses (MDD), AEs, clinical/microbiologic response to antifungal treatment (AFT), and mortality. NT: 50% increase in baseline serum creatinine (SCr) or if baseline SCr > upper normal limit, an absolute increase of 1 mg/dL. Success was defined as complete or partial clinical response to the AFT. Wilcoxon rank-sum test was used for continuous variables and the Mantel–Haenzel chi-square test for proportions.
Results: Twenty nine patients were abstracted from 1990 to 2000 (62% male, 28% solid tumor, 24% AIDS, 14% leukaemia; 94% Candidiasis). Average MDD: 0.57 ± 0.26 mg/kg. Mean duration of treatment: 18 ± 16 days. Nine patients (35%) were switched to alternative AFT (35% azoles; 3% lipid formulation of amphotericin B). Eighteen patients (62%) experienced AmBd related AEs. Eight patients (33%) experienced NT. Solid tumor rate was higher in NT patients (50% vs. 13%, $p = 0.05$). Nephrotoxic medications distribution was not different in NT and non-NT patients. NT patients showed higher MDD than non-NT patients (0.72 vs. 0.48 mg/kg; $p = 0.04$). Non-significant numeric differences were observed between NT and non-NT patients for success rate (25% vs. 56%), hospital length of stay (HLOS) (33 vs. 23 days), and mortality rate (63% vs. 50%).
Conclusion: Patients with IFI, treated with AmBd had high AEs and mortality rates. Descriptive analyses suggest how AmBd related NT could have negative impact on clinical outcomes, and HLOS. This adds to the body of knowledge and highlights need for less toxic, more effective AFT.

R2143

Candida albicans endocarditis in a human immunodeficiency virus infected patient successfully treated with caspofungin and vegetectomy: a case report

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History: A 22-year-old male i.v. drug user acquired acute HIV infection in September 2003. At that time he was admitted to a hospital outside Croatia and treated for mixed bacterial (*Streptococcus sanguis*) and fungal (*Candida albicans*) sepsis with tricuspid valve endocarditis. His hospitalization was complicated by pulmonary embolism, femoral thrombosis and bilateral pneumonia that required bilateral thoracic drainage. His *Candida albicans* infection was treated with liposomal amphotericin B for 3 weeks followed by oral fluconazole. He was discharged in December 2003 in a stable condition and given fluconazole (400 mg/day) and methadone maintenance therapy.
Case presentation: The patient was admitted to our hospital in March 2004 because two blood cultures taken in the outpatient setting grew *Candida albicans* despite continuous fluconazole therapy. Echocardiography was performed and a vegetation 2 cm in diameter was found on the tricuspid valve, and there was also a thrombotic mass 0.5 cm in diameter in the right ventricle. A 2-week course of liposomal amphotericin B with intravenous fluconazole (400 mg/day) was given, followed by oral fluconazole (400 mg/day). The patient became afebrile, however, a blood culture taken in May 2004 grew *Candida albicans* again. On June 2nd surgery was performed with excision of the vegetations on the tricuspid valve and in the right ventricle. One the day prior to surgery treatment with intravenous caspofungin (70 mg on day 1 followed by 50 mg/day) was started. *Candida albicans* grew from the cultured vegetations, it was susceptible to fluconazole and amphotericin B. Intravenous caspofungin was given for 4 weeks followed by oral fluconazole (400 mg/day). In the following 4 months no recrudescence of endocarditis was observed. Antiretroviral treatment was started in April 2004 with a combination of stavudine, lamivudine and lopinavir/ritonavir. It was given throughout the course of illness and was well tolerated.
Conclusion: Initial caspofungin treatment followed by fluconazole maintenance therapy, in addition to vegetectomy, was successful in the treatment of *Candida albicans* right-sided endocarditis in our HIV infected patient.

R2144

Survey of the prevalence and identification of dermatophytosis in Northern Iran, 2003–2004

J. Hashemi, A. Nasrollahi Omran *(Tehran, Tonekabon, IR)*

Objective: For 15 months in the period from June 2003 to September 2004, we experimented 400 patient suspected to dermatophytosis refer to clinics medical mycology to have itching and irritation.
Materials and methods: Skin samples were taken by scraping from patients and collected. Diagnosis was confirmed by direct microscopy carried out with a 20% KOH preparation and culture were performed in Petri dishes on mycobiotic agar and dermatophyte medium (DTM), and dermatophyte diagnosis tests for taxanomic identification of species. Thus after to complete questionnaire, we analysis prevalence of dermatophytosis.
Results: Our results showed that: Totally, that 210 cases of them suffered from dermatophytosis, that disease confirmed by microscopic examination and culture positive. %70 (147 case) of this patients suffered from disease were male and rest were female 30% (63 case). Age groups between 10 and 50 were effect by this disease, with a lightly higher incidence in the age group was 20–30 (57%). Seventy per cent (147 case) of patients living in the cities rest 30% (63 case) were villager. Majority of the patients were driver 30% (63 case), rest were student 20% (42 case), officer 20% (42 case), collar worker 20% (42 case) and 10% (21 case) farmer. The frequency of clinical types according to the anatomic site involvement of dermatophytosis was groin, thus tinea cruris 50% (105) was the predominant clinical form of all ring worm infection see, rest were 20% (42 case) of cases were tinea manum, 17.6% (37case) tinea pedis, 4.76% (10 case) tinea capitis. 4.76% (10 case) tinea corporis, 1.9% (4 case) tinea unguium. Ninety five per cent (2 case) tinea barbae. Eighty per cent of tinea capitis was ectothrix rest were 5% endothrix and 15% tinea favosa. *Epidermophyton floccosum* was most common etiological agent 28.5% (60 case) rest encountered dermatophytosis including: *T. rubrum* 23.8% (50 case); *T. violaceum* 7.14% (15 case); *T. mentagrophytes* 14.28% (30 case); *T. verrcosum* 4.76% (10 case); *T. schoenleinii* 11.9% (25 case); *T. sonsudens* 4.76% (10 case); *M. canis* 476% (10 case). We found that anthropophilic infections 85.72% (180 case) appeared to be six time frequent than zoophilic ones 14.28% (30 case)
Conclusion: Statistical evaluation of obtained data indicated the decreased trends towards frequency of tinea capititis and the growing trends toward tinea cruris. This study highlights a common problem in many areas of the northern Iran and suggests that further measures regarding public health and specially personal hygiene must be undertaken in order to reduced the risk of dermatophytosis.

R2145

Colonisation of oral cavity by Candida species

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Objectives: The aims of the study were to assess the colonization of oral cavity in otherwise healthy individuals with oral symptoms (dry mouth and burning mouth syndrome) by Candida species and to determine their susceptibility to antimycotic drug nystatin. Nystatin is frequently used for topical prophylaxis or treatment of oral candidiasis.
Methods: This study examined the oral yeast colonization in 104 participants. Samples were collected with tongue swabs and cultured on Sabouraud's dextrose agar at 37 °C for 48 h. All isolates were identified by germ tube production, chlamydospore development on cornmeal agar with 0.5% Tween 80 (Difco, Detroit, USA) and ID 32 Candida identification kit (bioMerieux, Marcy-l'Etoile, France). The susceptibility profile was evaluated by disk diffusion method for nystatin (NeoSensitabs, Taalstrup, Denmark) on Mueller–Hinton agar.
Results: At the time of sampling, 30 (28, 84%) individuals were positive on colonization by Candida species. 27 (90%) of isolates corresponded to *Candida albicans* while two isolates (6.6%) were identified as C. kefyr (*C. pseudotropicalis*) and one (3, 3%) as *C. glabrata*. All the isolates were susceptible to nystatin.
Conclusion: *Candida albicans* was the main Candida species that colonized oral cavities while non-*C. albicans* species were rarely detected. Since all isolates were susceptible, nystatin can be safely used for prophylaxis of oral candidiasis in patients with oral symptoms such as dry mouth or burning mouth syndrome.

R2146

Confirmation of *C. dubliniensis* from other *Candida* spp.

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Objectives: *C. dubliniensis* (CD) is often identified as *C. albicans* (CA) specially in routine mycological practice because two species shares characteristics, they are 'germ-tube' positive and produce chlamydospores. The aim of this study was to distinguish CD from other *Candida* spp. in immunocompetent and immunodeficient patients clinical specimens.
Methods: All *Candida* spp. isolates were tested using algorithm-tolerance at 45 °C, osmotic resistance in broth containing 6.5% of NaCl and phenotypic features on CHROM agar and Pal agar. For quality control we used *C. albicans* ATCC9028 and *C. dubliniensis* CBS7988.
Results: One hundred and twenty three strains of *Candida* spp. isolated from genitourinary tract of immunocompetent patients were tested for CD and was no such strain confirm. In 93 *Candida* spp. from oral cavity in haematological patients and 10 swabs from HIV positive persons we confirm only one strain as CD. CD growth on Sabouraud-agar at 37 °C and do not at 45 °C does not growth in broth with 6.5 % NaCl, on Pal agar colonies have characteristic pseudo-hyphal roots (with chlamydospores under 400×). On CHROMagar CD have dark blue-green colour distinguishable from light-blue CA colonies.
Conclusions: Using algorythm ('germtubes') and chlamydospore formation together with two methods: growth at 45 °C and cultivation on Pal agar present easy methods for distinguish *C. dubliniensis* from *C. albicans* and other *Candida* spp. in clinical laboratory specimens.

R2147

Candidosis due to *Candida guilliermondii*: an analysis of eleven cases

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Objectives: In the attempt to separate colonization from infection, we reviewed the clinical records of patients who had *Candida guilliermondii* identified on clinical specimens.
Methods: Retrospective study of all positive cultures for *C. guilliermondii* obtained during October 1997 to August 2003 at Santa Casa Complexo Hospitalar, Brazil. The records of the Mycology Laboratory were reviewed attempting to identify positive cultures to *C. guilliermondii*. Clinical records of these patients were also reviewed. Candidemia was diagnosed if a peripheral positive blood culture was temporally related to signs and symptoms. Histopathologic or cytopathologic examination showing *C. guilliermondii* from specimens of needle

aspiration or biopsy, excluding mucous membranes, were considered evidence of invasive infection, as well as positive culture result on sample obtained by sterile procedure from normally sterile site.
Results: Eleven patients were included in this study. Specimens corresponded to blood (n = 5), ascitis fluid (n = 2), pericardial fluid (n = 1), urine (n = 1), colon biopsy (n = 1), and esophagus mucosa (n = 1). Patients were mainly male (54%), and age ranged from 19 days to 73 years. Six patients were immunosuppressed (3 transplant recipient, 2 AIDS, and 1 cancer), and 4 had been exposed to invasive procedure (2 peritoneal dialysis, and 2 abdominal surgery). For the 5 patients with candidemia, there was a favorable response with amphotericin B treatment. Only one case, an immunocompetent patient, received no antifungal therapy. Two cases were presented of *C. guilliermondii* peritonitis, associated to dialysis catheter; both had favorable evolution after antifungal therapy and catheter removal.
Conclusions: In this series of 11 patients, we demonstrated that, although *C. guilliermondii* can be associated with invasive disease, its presence may represent colonization or even infection with low pathogenicity, mainly in immunocompetent patients. There was a frequent association between *C. guilliermondii* and catheter use (intravenous, peritoneal or urinary), which is common to Candida species infections. Aseptic technique should be rigorously performed during the process of blood sampling, to avoid further mistakes in the interpretation of the isolation of *C. guilliermondii* from clinical specimens.

R2148

Importance of the coexistence of Candida fungi and bacteria *Helicobacter pylori*

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Objectives: Except of well known role of *Helicobacter pylori* (Hp) in the pathogenesis of ulcer disease (UD) and chronic gastritis (CG), the role of the presence of Candida (*C.*) fungi in the stomach is now evaluated. The problem of the coexistence of Candida and Helicobacter pylori in the gastric mucosa and their influence on ulcer healing and the course of chronic gastritis has not been explained. The aim of study was to (1) evaluate the frequency of *C.* in patients with gastric ulcer (GU), duodenal ulcer (DU) and CG; (2) estimate the frequency of Hp in GU, DU and CG; (3) evaluate the frequency of coexistence of *C.* and Hp in above patients; (4) identify the *C.* species, isolated from patients with GU, DU and CG; (6) evaluate the influence of Hp eradication on the frequency of the presence of *C.* in the stomach.
Methods: We examined the group of 63 patients, including14 patients after Hp eradication therapy, aged from 18 to 72 years, with dyspeptic and ulcer symptoms. Clinical investigation included: clinical history, gastroscopy with biopsies for urease test, histopathology, mycology and evaluation of the Hp presence. Also brush smear from changed gastric mucosa and aspirate of gastric juice were taken for mycological investigation. Bacteriological investigation included: (a) Hp culture in the incubator with CO_2 flow, (b) identification of Hp species by evaluation of urease, catalase and oxydase activity. Mycological investigation of examined material was evaluated on the basis of qualitative and quantitative investigation. In the identification of isolated fungi: Albicans ID2, ID 32C ATB System (bio Merieux) was used.
Results: (1) Presence of *C.* in the stomach was found in 47% and 57% patients before and after Hp eradication therapy respectively. (2) We did not find *C.* and Hp in 53% and 29% in not eradicated and eradicated patients respectively. (3) Coexistence of *C.* and Hp in the stomach was found in 10% of cases. (4) *C. albicans* was the most frequently isolated species.
Conclusions: (1) *C.* and Hp coexistence was found in 10% of cases. (2) Hp eradication therapy containing proton pump inhibitor and antibiotics may increase the frequency of fungal colonization of stomach mucosa.

R2149

Molecular characterisation of GTP-binding protein gene in dermatophyte pathogen *Trichophyton rubrum*

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Trichophyton rubrum (T. rubrum) is an anthropophilic dermatophyte. Infections caused by the fungus are mostly confined to the keratinized epithelial layer. *T. rubrum* is the most common cause agents of dermatophytosis in human skin and nail tissue. Some properties of *T. rubrum* have been investigated in molecular level, however, no information is available regarding the GTP-Binding protein in this dermatophyte. GTP-Binding proteins regulate a variety of processes including sensual perception, protein synthesis, various transport processes, and cell differentiation. In the present study, we try to characterize the GTP-Binding protein gene in this dermatophyte pathogen. *T. rubrum* was obtained from patients with dermatophytosis and cultured in appropriate conditions. Nucleic acids (DNA and RNA) were isolated from obtained mycelial mass by standard methods. Pairs of 21 nt primers were designed from highly conserved regions of the GTP-binding protein genes in other eukaryotic cells. Mentioned primers were utilized in PCR by using isolated genomic DNA as well as cDNA template of *T. rubrum*. Predicted molecules have been amplified and were sequenced. By the time 350 nucleotides of this gene are sequenced. The characterization of this PCR fragments which revealed significant homology with other GTP-binding protein in GenBank (NCBI, NIH, USA) is still under investigation.

R2150

Molecular characterisation of vacuolar-ATPase gene in dermatophyte pathogen *Trichophyton rubrum*

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Trichophyton rubrum (*T. rubrum*) is one of the dermatophyte fungi which invade the skin of human. Several properties of this fungus have been investigated so far. However a few studies were carried out in the field of molecular biology of this fungus. In the present study we tried to identify the vacuolar ATPase proteins (V-ATPase) in this fungus. Pairs of 21 nt primers were designed from highly conserved regions of the same gene in other fungi. Mentioned primers were utilized in PCR by using isolated genomic DNA as well as cDNA template of *T. rubrum* and the PCR fragments were then sequenced. By the time, 568 nucleotides have been sequenced from this new gene which encodes a polypeptide with 119 amino acids. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for both DNA and its deduced amino acid sequence revealed significant homology with V-ATPase genes and proteins from other eukaryotic cells. The molecular characterization of this gene as well as definition of its possible role in the physiological function of *T. rubrum* are still under investigation. The new gene has been submitted to the international GenBank (NCBI, NIH, USA).

R2151

Aspergillus arthritis in an immunocompetent patient

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Case: *Aspergillus* is an opportunistic pathogen that causes severe clinical diseases in immunosuppressive patients. It is widely distributed in nature and rarely causes disease in immunocompetent patients. A 69 year-old immunocompetent female patient was admitted to our hospital one and a half month after implantation of bilateral knee prothesis. She had no other underlying disease. Her complaints were fever, cough, hemoptysis, pleuritic chest pain and dyspnea on the 5th week after operation. She was hospitalized with the suspicion of pulmoner thromboembolism and anticoagulant therapy was started. The purulan drainage from both knees was noticed during the follow up. Polymorphonuclear leukocytes and Gram-positive cocci were seen in the microscopic examination of the joint fluid. Teicoplanin treatment was begun. She improved, fever resolved but necrotic areas appeared during teicoplanin treatment. The pathological examination of the necrotic material demonstrated fungal hyphae and the culture grew *Aspergillus niger*. Amphotericin B was started along with debridman and the patient improved dramatically. After 42 days of amphotericin B therapy, both knees and the patient were well without prothesis replacement. She was still in good health three months after discharge.
Conclusion: Prosthetic joint infections are troublesome complications of orthopedic surgery. Usually Gram-positive microorganisms cause such infections but in descending order Gram-negative bacilli or *Candida* spp. may be the causative agents. Aspergillus arthritis is a rare clinical entity especially in immunocompetent patients. Other interesting side of this case is the rescument of the prothesis with medical therapy and debridman only.

R2152

Fungaemia by yeasts in a Portuguese university hospital: epidemiology and susceptibility profile over a 2-year period

C. Pina-Vaz, A. Gonçalves-Rodrigues *(Porto, P)*

Fungaemia is reported as the 4th cause of septicemia in the international literature, with inherent serious morbidity and significant mortality. No local epidemiological data on this problem are yet available.
Objectives: To define the prevalence of Fungaemia due to yeasts, respective risk factors, reservoirs, distribution by species and its susceptibility antifungals pattern.
Methods: All the yeasts isolated from blood cultures during 24 months (2002–2004) at an university hospital of Porto were collected. Data like demographical aspects, hospital department, presence of central venous catheter or other indwelling medical devices, concomitant diseases, as well as antimicrobial therapy were collected. Additionally, concomitant isolation of yeasts from distinct body sites was also registered. The nosocomial nature of such infections was investigated. Following biochemical characterization of isolates, performed by Vitek system (BioMérieux), the susceptibility pattern to antifungals like amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin, was determined according to NCCLS protocol M27-A2.
Results: A total of 160 blood yeasts strains were identified, representing 0.3% of all positive blood cultures; 31% from Internal Medicine, 24% from Surgical Departments, 18% from Hematology/Oncology and 17% from Infectious Diseases Department. *C. albicans* and *C. parapsilosis* were isolated in similar percentage, 33.5%; 12.8% were *C. tropicalis*; 7.7% *C. glabrata*; 6.9% *Cryptococcus neoformans*; 2.5% *C. guillermondii* and 1.25% *C. lusitanea*. All the strains were susceptible to amphotericin B except C. lusitanea; 20% of isolates were resistant (R) to fluconazole, 7% being susceptible dose-dependent (S-DD); 26% were R to voriconazole (MIC > 1 μg/ml); 22% R to itraconazole while 30% S-DD; 41% were R to caspofungin (MIC > 1 μg/ml).
Conclusion: Fungaemia due to yeasts represents is an important concern among us, being non-albicans species of Candida prevalent comparing with other international studies. Due to this fact, resistance to antifungals was found in a significant extent.

R2153

C. krusei showed promoted resistance to oxidative stress

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The increase of invasive infections by non-albicans species of *Candida* is of great concern as such isolates are more resistant to antifungals. *C. krusei* is a paradigma example, whose pathogenicity is not yet full understood, being often negative for mechanisms like germ tube formation or production of enzymes like coagulase (1). Recent studies have implicated a role for adaptative responses to oxidants in pathogenesis. The reactive oxygen species (ROS) are produced mainly by phagocytic cells of the host immune system, its microbial effect leading to the destruction of potential pathogens.
Objectives: To study the response of *C. krusei* to some oxidative stress agents.
Methods: Blastoconidea of 10 clinical strains of *C. krusei* and 10 strains of *C. albicans* were stained with 50 μg/ml of MitoTracker Red CM-H2XRos (Molecular probes) during 15 min and exposed to oxidant stress agents: 0.4 mM of H_2O_2, 0.5 mM of menadione and 3 μM of plumbagine, all from Sigma, during different periods of time (15, 30 and 60 min). After treatment the cells were analysed in a flow cytometer (Beckman Coulter XL-MCL). MitoTracker Red CM-H2XRos does not emit fluorescence until entering an active respiratory cell, where is oxidized by agents of ROS. Strains affected by those agents, ROS, will show an increase in intensity of fluorescence.
Results: All *C. albicans* showed an increase of the intensity of fluorescence after being exposed to oxidative stress agents, which increased with the time of incubation. Conversely, minor variations of the intensity of fluorescence were seen in all *C. krusei* strains tested.
Conclusions: *C. krusei* are relatively protected to agents of ROS compared to *C. albicans* strains. Further work is need to clarify its importance and role regarding pathogenicity
(1) Rodrigues AG et al. J. Clin. Microbiol. 41: 5792–5793, 2003.

R2154

Species distribution and antimycotic susceptibility of *Candida* strains isolated from pulmonary tuberculosis patients

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Objectives: To determine species of *Candida* fungi inducing respiratory tract infections in pulmonary tuberculosis (TB) patients and analyze their resistance to antimycotics.
Methods: Culture (inoculation onto Sabouraud medium with chloramphenicol) and microscopy of bronchoalveolar lavage,

sputum (we estimated quantitative concentration of *Candida* spp. in specimens), samples from lung cavities and pleural cavity (aspirates, biopsies), lung tissue samples (resection); identification of isolated strains (morphology on corn-meal agar and CandiSelect medium, 'Auxacolor' test-system, Bio-Rad); antimycotic resistance determination (using microdilution method validated with NCCLS M27-A reference method – 'Fungitest' test-system, Bio-Rad).

Results: During 2002–2004 we studied 502 patients with different forms of pulmonary TB. Inoculation of clinical samples revealed strains of *Candida* spp. in 163 patients (32.5%). In 89.2% from this group culture results were confirmed by positive microscopy of clinical samples. We detected 8 species of *Candida*. Detection rate of *C. albicans* strains was 64%, *C. glabrata* – 11%, *C. krusei* – 9%, *C. tropicalis* – 8%, *C. parapsilosis* – 4%, *C. kefyr* – 2%, *C. guilliermondii* – 1%, *C. lusitaniae* – 1%. Susceptibility to antimycotics (fluconazole, itraconazole, amphotericin B, ketoconazole, miconazole, 5-fluorocytosine) was tested in 328 *Candida* strains (211 strains of *C. albicans*, 41 – *C. glabrata*, 27 – *C. tropicalis*, 24 – *C. krusei*, 16 – *C. parapsilosis*, 5 – *C. kefyr*, 3 – *C. guilliermondii*, 1 – *C. lusitaniae*). Resistance to fluconazole was detected in 5% of all studied strains (in 2% strains of *C. albicans*, 15% – *C. glabrata*, 21 % – *C. krusei* and 4% – C. tropicalis). Resistance to itraconazole was in 7% strains (in 6% strains of *C. albicans*, 17% – *C. glabrata* and 15% – *C. tropicalis*). We also found strains resistant to miconazole (1% strains), ketoconazole (3% strains) and 5-fluorocytosine (2% strains). Strain of *C. lusitaniae* was resistant to amphotericin B.

Conclusions: Pulmonary TB patients administered long-term adequate course of chemotherapy (4–7 antituberculosis drugs) compose a risk group for candidosis. Considering the experimental data on Candida clinical strains resistance, we deem that before administration of specific treatment for pulmonary candidosis with fluconazole and itraconazole it is advisable to identify the strain and determine its susceptibility to antimycotics.

R2155

Candida species isolated from blood and other sterile fluids: distribution and susceptibility

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Objective: During the last decades yeast infections have emerged as important causes of nosocomial infections. As *Candida* infections are associated with significant morbidity and mortality, monitoring of *Candida* species distribution among significant isolates and their resistance is required.

Material and methods: All consecutive strains of *Candida* spp. isolated from blood and other normally sterile fluids cultures at the General Hospital of Athens '*G. Gennimatas*' during a 5.5-year period (1999–2004) were examined. Isolates were speciated by the Vitek II system (BioMerieux, France). Susceptibility testing to amphotericin B, fluconazole, flucytosine, itraconazole and voriconazole was performed by the E-test (AB Biodisk, Sweden); result interpretation was according to NCCLS.

Results: A total of 81 *Candida* spp. strains were isolated from a respective number of patients hospitalized in Surgery (n = 27, 33.5%), Internal Medicine (n = 39, 48%) or ICU (n = 15, 18.5%); 18 persons (22%) had received fluconazole prophylaxis previously. Sixty five (80%) strains originated from blood and 16 (20%) from other sterile fluid cultures. Nine *Candida* species were identified. *C. albicans* represented the most common (n = 32, 39.5%) followed by *C. parapsilosis* (n = 19, 23.5%), *C. glabrata* (n = 14, 17.3%) and C. tropicalis (n = 8, 9.9%). However, C. *albicans* isolation rates decreased steadily from 1999 to 2004 (from 58.3% to 22.2%), while *C. glabrata* isolation rates increased (from 8.3% to 33.3%). All strains were susceptible to amphotericin B (MIC, <1 mg/l) and a single *C. krusei* strain (1.2%) was flucytosine resistant (MIC, ^{3}32 mg/l). Resistance to fluconazole (MIC, ^{3}64 mg/l) and itraconazole (MIC, ^{3}1mg/l) was observed in 15 (18.5%) and 24 (29.6%) strains, respectively. *C. glabrata* and *C. krusei* were the species more often resistant to the azoles (9 out of 14 and 2 out of 2 strains, 64.3% and 100%, respectively), while *C. albicans* was the least resistant (1 out of 32 strains, 3.1%). Resistance to voriconazole (MIC, >1 mg/l) was detected among 6 (7.4%) isolates, 3 strains of *C. glabrata* and 2 strains of *C. parapsilosis*.

Conclusion: Similarly to data from other countries, our results from Greece show increasing isolation rates of species other than *C. albicans* in blood and other sterile fluid cultures. Increased frequency of *C. glabrata* strains resistant to conventional azoles was detected, but voriconazole demonstrated good in vitro activity against *Candida* species.

R2156

Epidemiology and in vitro antifungal susceptibilities of *Blastoschizomyces capitatus*: a 14-year overview

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Background: *Blastoschizomyces capitatus* (BC) is an uncommon but frequently fatal cause of invasive infections in immunocompromised patients, particularly those with hematological malignancies. Data on its antifungal agent susceptibilities are limited. We evaluated the epidemiology and antifungal susceptibilities of *Blastoschizomyces capitatus* in our hospital during a 14-year period.

Methods: From 1990 to 2003, 80 isolates of *Blastoschizomyces capitatus* (44 patients) were recovered. The in vitro activities of amphotericin B (AMB), flucytosine (FC), fluconazole (FZ), itraconazole (IZ), ketoconazole (KZ), voriconazole (VZ) and anidulafungin (AL) were determined by the microdilution method (NCCLS M27-A2).

Results: The ratio of isolates to patients with *B. capitatus* during the study period was: 1990–1996 (5/5), 1997–2003 (75/39). Among the clinical isolates of BC, 23 were collected from deep sites (9 blood, 7 lower respiratory tract, 5 sterile fluid, 1 biopsy and 1 abscess) and 67 were recovered from mucosal sites with no systemic involvement. The overall MIC90 (mg/l) of AMB, FC, FZ, IZ, KZ, VZ and AL against 45 available BC were: 0.5, 1, 32, 1, 1, 0.5 and 8. There were no differences in susceptibility between isolates from deep and mucosal sites.

Conclusions: A clear increase in the rate of isolation of *B. capitatus* occurred during the second period of the study in our institution. Although there are no established breakpoints to define antifungal susceptibility to BC, voriconazole and amphotericin B appeared to be the most active antifungal agents. Our data shows the reduced susceptibility of BC to flucytosine, fluconazole, itraconazole, ketoconazole and anidulafungin.

R2157

Evaluation of Candiselect®4 a chromogenic medium for yeasts differentiation

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Objectives: Candiselect®4 (BioRad) is a chromogenic medium recommended by manufacturer for selective isolation of yeasts, direct identification of *Candida albicans* and presumptive

identification of *C. glabrata*, *C. tropicalis* and *C. krusei*. The identification is based on the species specific color and colony morphology, namely: pink-purple for *C. albicans*, dark turquoise and smooth for *C. tropicalis*, dark turquoise and rough for *C. krusei* and pale turquoise for *C. glabrata*. In this study we evaluated the performance of Candiselect® 4 for the identification of clinical yeasts isolates.

Methods: A total number of 204 strains included 57 C. albicans, 43 C. glabrata, 18 C. krusei, 13 C. tropicalis, 23 C. parapsilosis, 7 C. inconspicua, 6 C. kefyr, 6 C. guilliermondi, 4 C. dubliniensis, 2 C. rugosa, 1 C. norvegensis, 1 C. lusitaniae, 1 C. lipolytica, 19 S. cerevisiae, 2 C. neoformans, and 1 P. anomala. 13 strains were obtained from BCCM IHEM Collection and 191 were a clinical isolates collected in Department of Microbiology Medical University of Wroclaw and identified previously using standard methods (e.g. ID-32C tests, BioMérieux) and on the base of rDNA restriction fragment length polymorphism. As a quality control six typical Candida strains from ATCC and 34 bacterial isolates were used. The yeasts cultures on Candiselect® 4 medium were incubated at 37 (C for 4 days. The colony morphology and pigmentation were read visually after every 24 h.

Results: A pink-purple colonies were found in *C. albicans* and *C. dubliniensis* only. After 48 h a color of *C. dubliniensis* colonies became purple-bluish and differ from *C. albicans*, which were purple-violet with a pink halo around. The color and colony morphology of all strains of *C. tropicalis*, *C. krusei* and *C. glabrata* were in agreement with manufacturer description. Among the remain investigated *Candida* and non-*Candida* species the colorless or white colonies were very seldom and different shades of blue and green were observed. There was a problem with discrimination between *C. glabrata* and some *C. inconspicua*, *C. parapsilosis*, *C. kefyr*, and *C. norvegensis*. The sensitivity and specificity values for *C. albicans* were 98% and 87%, for *C. tropicalis* 100% and 98.4%, for *C. krusei* 100% and 100%, and for *C. glabrata* 100% and 90%.

Conclusion: Candiselect® 4 could be regarded as a useful medium for presumptive identification of most commonly isolated *Candida* species, including fluconazole resistant.

R2158

In vitro activity of amphotericin B against Aleuriconidia derived from *Aspergillus terreus*

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Aspergillus terreus is an uncommon but emerging fungal pathogen, which causes lethal infections often refractory to amphotericin B (AMB). It has been suggested that aleuriconidia might be the source of AMB resistance. This study used aleuriconidia as inoculum and compared the minimal inhibitory concentration (MIC) of AMB and voriconazole (VRZ) to those obtained for conidia of 31 *A. terreus* strains using a broth microdilution method. In addition, we examined the in vitro susceptibility of hyphae derived from aleuriconidia and conidia of A. terreus. The MIC ranges of AMB for conidia and aleuriconidia were 1.25–5 μg/ml and MIC90 was 2.5 and 5 μg/ml, respectively. The MIC ranges of VRZ for conidia and aleuriconidia were 0.25–1 and 1–2 μg/ml, and MIC90 was 1 and 2 μg/ml, respectively. The MIC ranges of AMB for hyphae of conidia and aleuriconidia were 1.25–5 μg/ml, and MIC90 was 2.5 μg/ml; the MIC ranges of VRZ were 1–2 μg/ml, and MIC90 was 2 μg/ml. In conclusion, our data confirm that AMB MICs from *A. terreus* aleuriconidia do not differ dramatically in comparison to MICs obtained from conidia. VRZ was significantly more active against aleuriconidia and showed consistently lower MICs.

R2159

Clinical evaluation of Aspergillus-PCR for detection of invasive aspergillosis in immunosuppressed patients

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We evaluated the value of Aspergillus PCR as a tool for diagnosing invasive aspergillosis in patients at risk. Aspergillosis was assessed according to European Organization for Research and Treatment of Cancer/Mycosis Study Group definitions. Nine and 17 patients with proven and probable aspergillosis were evaluated. Whole blood samples prior (n=101) and during antifungal treatment (n=267), and tissue specimens (n=9) and/or bronchoalveolar lavage fluids (n=17) were investigated. In patients with proven infections the sensitivities of PCR of lung samples were 100%, of blood samples prior treatment were 66%, during treatment 55%. Clearance of fungal DNA from blood was associated with resolution of clinical symptoms in 2 of 4 patients with proven infection. Consecutive positive PCR results for Aspergillus are associated with fatal outcome as 2 of 5 patients died. In patients with probable infections the sensitivities of PCR of lung fluids were 85%, of blood samples prior treatment were 57%, during treatment 42%. The benefits of PCR diagnosing and screening of whole blood is limited if sampling takes place once treatment has started. The performance of Aspergillus PCR from tissue samples should be recommended in addition to microscopic examination and culture technique for sensitive detection of fungal infection.

R2160

Identification of dermatophytes using partially digested ITS2-PCR

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Objectives: The routine identification of dermatophytes is a time-consuming process, depending on morphology. The forms of the spores are very informative, but this is mostly pending on the expertise of the researcher. The objective is to design a fast, easy and robust identification technique which allows the identification to species level from cultured dermatophytes.

Methods: Internally transcribed spacer region 2 (ITS2)-PCR followed by fragment analysis (De Baere et al., 2001) has been shown to be a fast and reliable identification technique for yeasts. The used primers are universal for all fungi, so also molds and dermatophytes. The dermatophytes are however consisting of a variety of species of which some are highly related and ITS2-length is identical. Therefore we added a partial restriction digestion to increase the discriminatory power.

Results: In total 29 species (each represented by 1–5 strains) were tested. For 16 of those species a unique fingerprint was obtained, which was different from all other species and which was constant for all members of that species, except for 3 species for which minor intraspecific differences were obtained. For other species we found different fingerprints, some of them unique, some also found for other species, which leads only for some members to a precise identification up to species level. Finally, a number of species had identical fingerprints so that final identification could not be obtained.

Conclusion: Partially digested ITS2 fragment lengths analysis enables the identification of dermatophytes up to species level (for most of the species) or to a cluster of 2–4 species (which are sometimes highly related by other methods as well). Observed discrepancies might in part be explained but not yet resolved taxonomy and may indicate the existence of not yet described

species or the need for reclassification of strains. The technique can be seen as a fast identification technique for most of the routinely encountered dermatophytes. It worked especially well for the genera Arthroderma, Epidermophyton, Microsporon while Trichophyton species were more difficult to identify.

R2161

Successful use of posaconazole in a paediatric case of necrotising fasciitis caused by *Rhizopus*

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Objectives: Victims of severe trauma are susceptible to opportunistic fungal infections such as zygomycosis, a rare but life-threatening infection commonly caused by *Rhizopus* species. We describe the case of a child with necrotizing fasciitis caused by *Rhizopus* who was successfully treated with posaconazole (POS), an investigational triazole antifungal agent with in vitro activity against Zygomycetes.
Case Report: A 12-year-old girl sustained bilateral haemorrhagic contusions of the temporal brain lobes, fractures of the skull and left tibia, and deglovement of the left leg after being hit by a truck. Drug-induced coma was initiated to control resistant intracranial hypertension. Initial treatment of the leg injury included almost daily removal of necrotic skin and muscle tissue and hyperbaric oxygen therapy. Thirteen days after the accident, *Rhizopus* spp and Trichosporon oviodes were cultured from multiple wound swabs. Subsequently, therapy with liposomal amphotericin B (LAMB) at 200 mg/d was initiated. Three days later, several areas of necrotic black ulcerations suggestive of invasive zygomycosis were observed, and the patient showed signs of sepsis. To avoid leg amputation, all suspected sources of infection were excised, daily hyperbaric oxygen treatment was given, and POS oral suspension (800 mg/d in divided doses) was added to the LAMB therapy. POS was given via nasogastric tube for the first 28 days and orally thereafter. After 2 days, most signs of sepsis disappeared, though the patient continued to experience fevers for the next 2 weeks. LAMB was discontinued after 9 days because drug-associated toxicity was anticipated. Necrotizing fasciitis responded gradually to antifungal treatment. Two weeks after initiation of POS, *Rhizopus* was no longer detected histologically or by culture of superficial tissue or muscle specimens. After 6 weeks, cultures were negative for Trichosporon. POS was continued for 3 months. After 8 months, neither local nor systemic fungal infection was observed. The patient is continuing physiotherapy and can walk. Despite signs of frontal-lobe syndrome, her neurologic status is improving.
Conclusion: POS demonstrated activity against *Rhizopus* and appears to have prevented limb amputation and systemic spread of infection. It is noteworthy that POS, as an oral suspension, was successfully administered to an unconscious patient. The successful outcome of this case further supports the activity of POS against zygomycosis.

R2162

Subtyping and susceptibility testing of *Microsporum canis* isolates from preadolescent tinea capitis to four oral antifungal agents by broth microdilution and E-test

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Tinea capitis, a dermatophyte infection involving the scalp hair shaft, is primarily affecting preadolescent children. Across 19 European countries a survey conducted by the European Confederation of Medical Mycology (ECMM) revealed that the commonest agent of tinea capitis remains *Microsporum canis* (Hay et al. J. Eur. Acad. Dermatol. Venereol. 2001;15: 229–33).
Objectives: To identify the *M. canis* subtypes prevailing among isolates from preadolescent tinea capitis and determine their susceptibility to the newer antifungals that are currently shown to be effective, safe and of shorter treatment duration than oral griseofulvin.
Methods: Twenty-six isolates were delineated by PCR fingerprinting with a decameric minisatellite oligonucleotide sequence. Broth microdilution (BMD) susceptibility testing against terbinafine (Novartis, Basel, Switzerland), itraconazole (Janssen Pharmaceutical NV, Beerse, Belgium), fluconazole and voriconazole (Pfizer Inc., Sandwich, Kent, UK), was performed according to the NCCLS M38 A guidelines. E-test (AB Biodisk, Solna, Sweden) was performed supplementing the RPMI 1640 medium with zinc and thiamine to support *M. canis* growth and facilitate 48–72 h minimum inhibitory concentration (MIC) reading.
Results: PCR fingerprinting disclosed the same discrete *M. canis* subtypes. The BMD range of MIC50 (mg/l) of terbinafine was 0.03-equal or greater than 0.5, itraconazole 0.03-equal or greater than 0.25, fluconazole 0.25-equal or greater than 64, voriconazole 0.03–0.5. The overall agreement between BM and E-test was 85–90%.
Conclusions: The proposed RPMI-1640 solid medium can adequately support growth of *M. canis* for recording 48–72 h concordant BMD and E-test MICs. Regular conventional and molecular epidemiological surveillance and testing for antifungal susceptibility to the newer oral antifungal agents is an essential component in the management of tinea capitis.

R2163

Enzymatic profile of *Cryptococcus neoformans* isolates from patients with AIDS and lymphoproliferative diseases

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Objectives: *Cryptococcus neoformans* is an important pathogen in patients with impaired cell-mediated immunity and is the most common cause of fungal meningoencephalitis. *C. neoformans* is a facultative intracellular pathogen with several well-defined virulence factors that include the production of a polysaccharide capsule, melanin and growth at 37 °C. Identification and characterization of enzymes produced by *C. neoformans* are important because of their potential role in virulence, induction of host immune response and for epidemiological typing.
Methods: Thirty-nine strains were included in the study, 29 obtained from patients with AIDS and 10 from patients with lymphoprolipherative diseases. The enzymatic profiles of *C. neoformans* isolates were obtained by using the API-ZYM system (bioMérieux, France) as a whole-cell assay with an inoculum of 106 cells/ml. The phospholipase activity was tested using the egg-yolk plate method. The ratio of colony diameter to the diameter of the colony plus the pericolonial precipitation zone on agar (Pz) correlated with phospolipase activity.
Results: All tested strains were identified as grubii or neoformans variety by standard methods. The API-ZYM system is a screening test for the activity of 19 enzymes. Eleven of the 19 enzymes were found to be positive and no significant difference between two groups of isolates was observed. The activity of butyrate esterase was detected in 38 strains and activities of caprylate esterase lipase, leucine arilamidase and acid phosphatase were registered in 37 isolates. The activities of following enzymes were negative: lipase, cystine arilamidase, trypsin, chymotrypsin, alpha-galactosidase, *N*-acetyl-beta-glucosaminidase,

alpha-mannosidase and alpha-fucosidase. Seventeen enzymatic patterns were identified according to the profiles obtained by API-ZYM system. Only one strain, isolated from an AIDS patient, was detected as phospholipase negative. There was no significant difference in phospholipase production between isolates obtained from patients with AIDS and lymphoproliferative diseases (mean Pz, 0.52 vs. 0.55; $p>0.05$).
Conclusion: We found no difference in the enzymatic activity between strains infecting two different groups of immunocompromised patients. Each enzymatic activity has its own pathway, which could be separately related to *C. neoformans* virulence, and it was recently demonstrated that extracellular phospholipase activity is a virulence factor for this fungus.

R2164

The epidemiology and antifungal sensitivity results of nosocomial infections caused by *Candida* species

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Objective: To determine epidemiologic and antifungal sensitivity patterns of nosocomial Candida infections in Çukurova University Research Hospital where antibiotic consumption and nosocomial infection rate are high.
Materials and methods: Between January and June 2004, 160 patients with nosocomial candida infections according to CDC criteria were involved. Identification of candida isolates antifungal susceptibility tests by ID 32C (bioMerioux, France) and by ATB Fungus 2 (bioMerioux, France) respectively.
Results: In patients with candidiasis, the site of infection and their frequency were determined as bloodstream, urinary tract and other sites, 42 (26.3%), 104 patients (65.0%), and 14 patients (8.7%) respectively. Risk factors statistically significant for bloodstream infection were determined as total parental nutrition, central venous catheter and prior antibiotic use ($p < 0.05$). Of all patients 77.5% and 76.2% of patients with candidemia were hospitalized in intensive care units. *C. albicans* (50.6%) the most prominent Candida species was followed by *C. glabrata* (16.3%), *C. tropicalis* (16.3%), *C. parapsilosis* (10.0%), *C. krusei* (3.1%), *C. lusitaniae* (1.2%) and *C. kefyr*, *C. sake*, and *C. pulcherrima*. All isolates were sensitive to amphotericin B (MIC ≥ 1). Fluconazole resistance was 4.3% and 15.6% for all *C. albicans* and other species respectively. Resistance to itraconazole was found to be higher, which was 11.4% for *C. albicans*, and 26.5% for other species. Cross-resistance to itraconazole was determined in 12 of 13 (92.3%) isolates which were resistant to fluconazole.
Conclusions: The high resistance to azoles which can be considered as indicator of inappropriate antifungal usage should be paid attention. Empiric antifungal treatment by azoles which is routinely used by clinicians without differentiation between infection, colonisation and contamination, especially for Candida species isolated from urinary tract, threatens the efficacy and safety of antifungal therapy. Antifungal sensitivity tests should be used to determine the regional antifungal sensitivity patterns, to follow up of the patients with invasive infections like candidemia who need long term therapy and for determining the resistance and to choose alternative antifungal drug for those unresponsive and have recurrent mucosal disease.

Tropical and parasitic diseases

R2165

Loiasis with eyeworm and microfilaremia

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Objectives: Loiasis is a nematode parasitic infection endemic to sub-Saharan Western Africa. Native people from endemic areas emigrated to developed countries are often infected with this worm. We report a case of conjunctival loiasis in a young black male born in Guinea.
Case report: Patient was living in Spain for the last 2 years. He complained of eye pain and noticed a 'worm passing through the left eye'. He was attended in the ophtalmologic emergency care. Moderate eosinophilia (21%) was present in peripheral blood. Inflammatory reactions (Calabar swellings) were not present. After surgical and medical treatment, the patient was discharged from the hospital. At the present time, 10 months latter, he remains asymptomatic.
Results: The presence of migrating subconjunctival worm was observed, and removed surgically. It was identified as a female adult of Loa loa (55 × 5 mm). In blood samples collected during day-time, 300 μm long microfilariae were observed in direct wet smear. Hematoxylin stain showed the external sheath and Giemsa stain the nuclei extended to the end of the tail. Microfilariae discharged by the female worm were preserved in destilled water in the laboratory at room temperature. On day seven both free forms and eggs were present.
Conclusions: 1. The main interest of the case reported is the observation of all forms of Loa loa cycle in humans. 2. Country of origin and length of stay in the new residence will facilitate correct diagnosis and treatment.

R2166

Immunisation of Arabian sheep against haemonchosis with *Haemonchus contortus* intestinal homogenate

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Haemonchosis, a disease caused by Haemonchus contortus is of considerable economic importance in sheep cattle and goats throughout the world. Severe infections in sheep may cause considerable morbidity and mortality rates; even more, moderate infections in lambs, may give rise to body weight reduction and in adults cause various degrees of anaemia and decrease the animal products. Control depends on repeated application of anthelmintics and therefore there have been many attempts to develop a vaccine against this disease.In a trial, in Ahwaz area, the ability of an experimental vaccine made from the whole gut homogenate of Haemonchus contortus was evaluated. A group of 5 Arabian female lambs were immunized twice with 100 μm whole gut homogenate (WGH) of Haemonchus contortus diluted in 1 ml PBS and emulsified in 1 ml Freund's adjuvant. The other group (5 Arabian female lambs) received 1 ml PBS emulsified in 1 ml of the same adjuvant. The first immunization was performed intra muscularly in the semi-membranous muscles of each hind leg. Booster immunization administered subcutaneously on day 21. On day 33 each lamb was challenged with approximately 10,000 Haemonchus contortus third-stage larvae. From the first immunization until challenge the animal were bled with an interval of 10 day and then weekly from challenge until the end of study. Sera were tested by ELISA and

western-blotting. Lambs were necropsied 6 week post-challenge for recovery of nemetodes.Vaccinated lambs showed a 77% reduction in mean EPG, 75% reduction in mean male worm numbers, 80% reduction in mean female worm numbers and 78% reduction in mean total worm numbers. A significant difference in means optical density of sera in ELISA was noticed ($p = 0.001$). Proteins of Hemonchus contortus gut were seperated by SDS-PAGE. Then sera of vaccinated group indicated a high reactivity in western-blotting.

R2167

Clinical features and therapy of trichinosis in Belarus

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Objectives: Trichinosis is a rare helminthiasis, caused by the tissue-dwelling nematode Trichinella spiralis. The typical symptoms are acute onset, fever, myositis with myalgia, periorbital edema, eosinophilia and other allergic manifestations. We reviewed the cases observed at the Reference Center for Infectious Diseases of the Belarus Ministry of Public Health.
Methods: The observation group consisted of 15 patients with either mild or severe clinical trichinosis, admitted to the Infectious Diseases Hospital of the Medical State University over the years 2002–2004.
Results and Conclusion: The likelihood of the clinical diagnosis was enhanced by both anamnestic and epidemiological data, and serological confirmation was obtained by testing IgM with ELISA. 90% of the patients presented with a typical clinical picture of trichinosis, including fever, periorbital edema, myalgia (usually affecting the gastrocnemius muscle), and eosinophilia in the first days of disease. However, 5% of the patients showed eosinopenia (down to 2%) within the first days, which correlated with the severity of the disease. The clinical features of these latter patients also included myocarditis, neurologic disorders and respiratory failure.Treatment of trichinosis is known to be not satisfactory. In prescribing the antiparasitic therapy, attention should be paid to the phase of the disease, its severity and the eosinophyl blood count. Our approach was highly individualised and consisted of both etiologic and pathogenetic (or symptomatic) therapy. The etiologic therapy aimed at destructing intestinal worms, preventing the development of cyst walls around the larvae, and destructing the Trichinella larvae in the muscle fibers. We used mebendazol 0.3 g every 8 h for 10 days. This etiologic therapy was most effective in the incubation phase or in the first days of disease, when localisation of Trichinella still remained intestinal. Vice-versa, therapy with mebendazol during the muscular period of disease not only was less effective but could also lead to a further exacerbation of the symptoms. The symptomatic therapy included antihistaminic and non-steroid anti-inflammatory drugs. In the most severe invasive forms, we used glucocorticosteroids (prednisolon 20–80 mg/day for 5–7 days).

R2168

Intestinal parasites in native and foreign citizens of Athens during a two-year period

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Objectives: To determine the incidence of intestinal parasites in citizens of Athens and analyze the difference between the natives and immigrants coming from developing countries.
Methods: During a 2-year period (2003–2004), 1680 feacal samples (1399 from natives and 281 from foreigners) were examined in our hospital laboratory for the presence of parasitic pathogens. Of them, 592 were treated in the hospital and 1088 were outpatients. The 86.9% of outpatients were workers who were examined in order to obtain a health clearance certificate. All specimens were examined in direct microscopy using wet-mount and formalin-ether concentration methods.
Results: Of the 1680 specimens examined, 24 (1.4%) were found positive for pathogen intestinal parasites: *Giardia lamblia* 15, *Enterobious vermicularis* 5, *Heterophyes heterophyes* 1, *Ancylostoma duodenale* 1, *Entamoeba histolytica/dispar* 2. In 15 specimens (0.9%) *Blastocystis hominis* were detected and in 2 non pathogenic Amoebae (*Entamoeba coli* and *Iodamoeba butschlii*). The incidence of pathogen parasites was 0.7% for the Greeks and 5.0% for the foreign population.
Conclusion: The frequency of intestinal parasitic infection in Greece, is relatively low but not rare, especially during the recent years because many immigrants coming from developing countries have introduced new parasites. The alertness of public health services is critical in order to prevent parasite spreading from the carriers to the healthy population.

R2169

Control of coccidiosis in poultry

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An egg adapted gametocytes, *E. tenella*, vaccine(s) were used against coccidiosis in chickens. On day five chicks were divided into four groups and administered vaccine(s) orally viz; Vaccine I (gametocytes), Vaccine II (gametocytes inactivated), Vaccine III (gametocytes sonicated inactivated) and fourth group was served as control given normal saline. On day 15 immunized chicks were challenged with 60,000–70,000 sporulated oocysts of mixed species of Eimeria. A total of 126 birds were used in this study out of them 94 birds were scarified and 32 chicks died during challenge response. On day 21st post vaccination birds were subjected to postmortem and their lesions score were recorded. A maximum of 46 birds having lesions in intestine and caeca of Group-IV birds were observed while a minimum of 17 birds having lesions in intestine and caeca were observed in Group-III. There was non-significant difference ($P > 0.05$) in lesions score of Group-I, II and IV. Lesions scores in Group-III were significantly different ($P > 0.05$) from Group-I, II and IV.

R2170

Molecular characterisation of a HSP70 gene from *Entamoeba dispar*

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Entamoeba histolytica infects more than 480 million people annually worldwide which cause the mortality of about 50–110 thousand patients every year. Resent studies revealed that the agents of Amoebiasis which isolated from infected as well as non-infected individuals can be classified as two species; *Entamoeba histolytica* as infectious and *Entamoeba dispar* as non infectious. As these two species are morphologically similar to each other, the differentiation of them will need specific tests regarding iso-enzyme as well as modern molecular methods including PCR. In the present study, we tried to characterize the 70 kDa Heat Shock Protein of *Entamoeba dispar* at molecular leveland compare it with that from *Entamoeba histolytica*. With these findings, we will be able to gain more information about this protozoon and the new methods for distinguishing it from the infectious *Entamoeba histolytica*. For this purpose, we have isolated genomic DNA from *Entamoeba dispar* and used it as template in the PCR. This has been performed by using

designed primers from highly conserved parts of other HSP70 genes in eukaryotic cells. The result indicated some DNA binding patterns in agarose gel which may be some parts of HSP70 gene from *Entamoeba dispar*. The analyses of these genomic parts are still under investigation.

R2171

Experimental amoebiasis: immunological aspects

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Cellular immunity was studied in the course of immunization of animals and during their conseguent infection with *Entamoeba histolytica*, 81 reedleswe albino rats being employed. 4 strains of polyxenic culture *Ent.histolytica* were used isolated from patients and carriers. For immunization of rats, the killed antigen composed 50,000 trophozoies per 1 ml of the medium was administered subcutaneously 5 times at a week's interval. A week following the immunization the rats were infected through caecum with 0.5 ml of the live culture containing about 100,000 trophozoites. On days 6–9 p.i. all the rats appeared to be infected. On days 14–21 and 28 p.i.(the end of the observation), 93 and 97% of infected animals were registered respectively. The control animals were not infected. Up to the 6th day, 75% of young rats were infected. In most of the infected adult (91.3%) vegetative forms of *Ent.histolytica* were found in the caecum and moderate morphological changes were observed in 72.8% of these (hyperemia, oedema, haemorrhage, ulcer). In the submucous membrane of almost all the experimental animals vascular perivascular mononuclear infiltration was observed. With the help of the migration inhibition reaction of macrophages, it has been established that in the process of immunization and subseguent infection lymphocytes sensible to amoebic antigen appear in the rat's body. The danger the observation the higher the degree of body sensibilization. The evidence presented shows that the cellular mechanisms of immunity the of great significance in the pathogenesis of amoebiasis.

Sexually transmitted diseases (except HIV)

R2172

Study of *Chlamydia trachomatis* and *Chlamydia pneumoniae* as a cause reactive arthritis

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Objective: To study the role of *Chlamydia trachomatis* (Ct) and *Chlamydia pneumoniae* (Cp) in patients with reactive arthritis (ReA).
Methods: We examined 72 patients with ReA aged 22–774 years (45 women & 27 men) during January 2002 to October 2004. The control group consisted of 25 healthy persons same age with patients but without any arthritis problems. All specimens from urethra were tested by direct immunofluorescence (DAF), while serum samples by MIF test for specific IgA and IgG antibodies to *Chlamydia*.
Results: *Chlamydia trachomatis* and *Chlamydia pneumoniae* in 28 of 72 patients (38.7%) were detectived fulfilled diagnostic criteria of ReA. From the control group, Ct was found in only one case. Analytically, in three patients (4.1%) with acute ReA after an upper respiratory tract infection with Cp. The serological findings were as follow: IgA antibodies titres >1/20 and IgG >1/512. *Chlamydia trachomatis* was diagnosed in 25 patients (34.6%). Nineteen out of them had retinitis prior to arthritis. The diagnosis by DAF in 23 patients and in 2 by MIF.
Conclusions: (1) The data suggest that Ct and Cp were diagnosed in nearly a half (40%) of Rea patients. (2) Prior respiratory or urogenitical infection with the clinical presentation of arthritis and detection of *Chlamydia* may lead to the etiological agent of Reactive arthritis.

R2173

The genital infection as the trigger of development of a myoma of the uterus

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Objectives: The estimation of efficiency anti-inflammatory therapies in complex treatment of a myoma of an uterus.
Methods: There were surveyed 320 patients with a myoma of an uterus. The clinical, bacteriological, immunohistochemical, pathohistological, PCR-researches myometrium, endometrium, a serous cover of a uterus, myomatous units were carried out. The studying of anamnestic material by method ''the case-control''.
Results: The inflammatory diseases of genitals were preceded and accompanied in half of the patients of leiomyoma. In 60% various combinations of pelvic inflammatory disease. The method ''the case–control'' has revealed a high risk of development of a myoma of an uterus (the relative risk of occurrence by a parameter of the attitude of chances is 1.75) in patients with inflammatory diseases of genitals. The bacteriological research of 80 myomatous units, has revealed the associated microbic flora in them, in comparison with non changed fabrics of a uterus. Identification of flora by carrying out 400 PCR testifies to presence *Chlamydia trachomatis* (mainly in submucous) and *Ureaplasma urealyticum* (in intermuscular-subserous) myomatous units, even at their absence before operation in underlying departments of sexual ways. Pathohistological research of non struck with a myoma uteruses, removed concerning endometritis in a combination with purulent tubo-ovarian formations in three women of 24–25 years old, has shown formation of the reclaiming rudiments consisting from proliferated, concentrically located smoothly-muscular cells (SMC) around the inflammatory infiltrates in myometrium-leiomyomatoz. Immunohistochemical researches with PCNA have established its accumulation in nucleus SMC in inflammatory mononuclear infiltrates zones in myometrium at endometritis, that is considered by us as the trigger of a myoma of an uterus.
The conclusion: In our opinion, uterin fibroid embolization (UFE) is a prime method of a choice in treatment of a myoma of a uterus. In some cases there is a risk of secondary infection in degradation of unit or allocated myomatous detritus, that can demand hysterectomy. Taking into account all set forth above, before carrying out UFE, in our practice, we used: Fromilid 500 mg twice a day during 10 days + Tiberal 500 mg twice a day during 10 days. Using the given circuit, in complex treatment of a myoma of an uterus, has allowed to increase efficiency of conservative treatment in twice.

R2174

Comparative evaluation of effectiveness of urogenital clamidiosis treatment with antibiotics and ozonotherapy

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Objectives: The aim of this study is to examine the comparative evaluation of urogenital clamidiosis treatment with antibiotics and ozonotherapy in women of childbearing age.
Methods: were two groups of women of childbearing age from 20 to 45 years old with urogenital clamidiosis under investigation. I group of 37 patients were treated with classical antibiotics treatment regimen. II group of 31 got ozonotherapy as intravenous introduction of ozonized physiological solution 200 ml with concentration 2–3 mg/l, N°. 7–12 and ozonized oil was used locally. Estimation of the therapy results in both groups was held twice with the month interval with the use of enzyme immunodetection (EI) (diagnostic antibody titre 1:32 and more) and direct immunofluorescence (DI).
Results: Both groups of patients had identical results by serologic and bacterioscopic methods before the treatment. 87% of women in I group and 90% in II had positive results of DI. Median geometric of titre (MGT) in EI in I group was 6.224, in II – 6.006 ($P > 0.05$). Diagnostic antibody titres had 91.9% patients in I group and 93.6% in II ($P > 0.05$).After treatment, during the first control of DI positive results were noticed in 73% of patients in I group and 84% in II ($P \leq 0.05$). During the second control of DI elementary bodies of *C. trachomaris* were defined in 16.2% women in I group and II group they didn't revealed. Dynamic estimation of the serum examinations by EI during the first control discovered not authentic lowering of MGT in I group from 6.224 to 5.324 ($t = 1.84$) and authentic lowering of MGT in II group in 1.3 ($t = 2.57$). Second control showed lack of antibodies titres lowering dynamic in I group: MGT before treatment 6.224, MGT of second control –5.958 ($P > 0.05$) and positive dynamic patients of II group – MGT before treatment 6.006 and during second control 4.655 ($P \leq 0{,}05$). Control laboratories examinations discovered *C. trachomatis* eradication in 30% of patients in I group and in 67.7% in II ($t = 3.36$).
Conclusion: Classical antibiotics treatment of urogenital clamidiosis undoubtedly gives positive results. However, high effectiveness of ozonotherapy in urogenital clamidiosis treatment showed the possibility of using this method like alternative treatment.

R2175

Diagnosis of *Chlamydia trachomatis* infection in a sexually transmitted disease clinic using polymerase chain reaction in endocervical, urethral and rectal swab

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Objectives: The aim of this study is to show the incidence and the most important characteristic of *C. trachomatis* (CT) infection in a group of patients with sexual risk behaviours who are followed in a sexually transmitted disease clinic (STD).
Methods: Our study included 2412 patients, aged 30.1 (55% women and 44% men) attending in STD clinic from January 2002 at December 2003. Of all the patients attending, 50% have sexual risk behaviours (47% female commercial sex workers (FCSWs), 45% men who have sex with men (MHM), 8% men and women with a new sexual partner or more than one partner during the previous 3 months. All of them were attending for symptoms, periodic revisions and epidemiology control of sex partner. A total of 3136 specimens (1645 endocervical, 1189 urethral, and 302 rectal swab) were analysed. The samples were recovered following the STD system (Swab specimen Collection and Transport kit, Roche diagnostic system). The diagnosis was performance using PCR techniques with COBAS Amplicor CT system (Roche Molecular System). The patients with CT positive had come for follow-up examination 4 weeks after treatment for CT infection with a single oral 1.0 g doses of azithromycin.
Results: There were 139 patients with a positive results for CT. Of all samples, 155/3136 (4.5%) were positive [55 female (40%) and 84 male (60%)]. The incidence of CT was 5.7% (4.1% female and 7.8% male). The endocervical swabs were positive in 3.3%. Sexual risk behaviours were: 54.6% FCSWs, 40% women who did not have any risk factors for sexual transmitted infection, 4.5% promiscuous women. The 78.2% were asymptomatic. The urethral swabs were positive in 6%, and rectal swabs in 7%. Sexual risk behaviours were 52.4% MSM, 22.6% without risk factors, 25% promiscuous men. The 42.8% were asymptomatic. The detection of the CT infection was performance by screening programme for STD clinic.
Conclusion: 1. PCR is an sensitive and specific test for the detection of CT infections. 2. We recommended the rectal swab with the other samples (endocervical and urethral) in patients with sexual risk behaviors. 3. To reduce CT infections we recommended screening programme in STD clinic to asymptomatic infections.

R2176

Detection of *Chlamydia trachomatis* infection using leucocyte culture

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Complications of latent and chronic chlamydial infection is continuing to be a problem in medicine. The clinical onset of these cases is unexpectedly wide while the origin of complications is often concealed and detection of *Ch. trachomatis* plays important role to work out the problem-based treatment. The bone marrow and blood leucocytes culture system was adjusted to the investigation of *Ch. trachomatis* in vitro. This method helps to reveal concealed infection in chronic cases and observe behavior and kinetic of bacteria in cells and estimate the activity of infection according to the cell damage and dissemination of bacteria in cells. We investigated *Ch. trachomatis* infection in 25 active sportsmen with different unclear symptoms and revealed this infection in 20 of them. Besides in 15 sportsmen immune reactivity of organism according to the macrophage–lymphocyte rosette formation in vitro was significantly decreased while in the rest this index fell in the low range of the normal values. The sportsmen were treated by the combination of modern chemotherapeutical drugs (Josamycin, Asitromycin, Doxacyclin etc.) and immunomodulators. After clinically effective treatment immune reactivity of organism was improved and *Ch. trachomatis* bacteria was detectable in culture in 8 cases (though in some cells) pointing to the existence of bacteria in organism pointing to the necessity of further antichlamydial treatment.Our studies show that blood leucocyte culture is useful for the detection of chlamydial infection and for the estimation of immune reactivity of organism as well especially in clinically unclear cases.

R2177

Micro-organisms isolated from urethral exudates from males in a Spanish teaching hospital

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Objectives: The purpose of this study is to determine the most commonly isolated microorganisms from urethral exudates from male patients in a teaching Hospital of Madrid over a three-year period.
Material and methods: We performed a retrospective study from January 2001 to December 2003 of 595 urethral exudates from male patients with an average age of 39.57 years. Urethral samples were processed by conventional methods: a gram stain and a culture in an appropriated solid media (blood agar, chocolate agar, Tayer–Martin agar and a commercials kit for detection of mycoplasma) were performed. Isolates were identified by conventionals test and an automated system (Micro-Scan. Dade-Behring) or manual biochemical systems: API Strep, API 20NE, API NH (bio Merieux).
Results: Two-hundred and thirteen (36%) out of the 595 patients, were positive (average age: 38.95). Seventy-one (33%) were immigrants,118 patients (55%) came from primary care and 95 (45%) from Hospital. The microorganism most frequently isolated was *Ureaplasma urealyticum*, in 70 cases (33%), followed by *Neisseria gonorrhoeae* in 47 cases (22%). Other species commonly isolated were: *Streptococcus agalactiae* (12%), *Haemophilus* spp. (8%), *Escherichia coli* (5%), *Candida albicans* (4%), as well as other species of microorganisms. (15%).
Conclusion: An important number of patients with suspicion of sexual transmitted disease showed positive cultures. *Ureaplasma urealyticum* and *Neisseria gonorrhoeae* were the most commonly isolated microorganisms in urethral exudates.

Molecular virology (incl. diagnostics)

R2178

Automated DNA extraction influences the sensitivity of PCR-based molecular diagnostics

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Objectives: Direct detection of microbial DNA in clinical samples like blood or cerebrospinal fluid (CSF) requires a reliable DNA isolation procedure and a sensitive PCR amplification, due to low microbial loads in these clinical samples. In this respect optimal recovery of target DNA during extraction is necessary. In this study we compared the performance of DNA recovery of the automated Roche MagNA Pure Total Nucleic Acid Isolation Kit (M-extraction) and the manual Boom extraction method (Si-extraction).
Methods: DNA recovery experiments were conducted with PBS, CSF, plasma and whole blood (4–8 replicates for each sample matrix) for both methods. Other reconstruction experiments were performed with positive control DNA's for EBV and VZV (2 dilutions, tested in 5-fold) in PCR negative blood and analysed by PCR. Also serial diluted CMV was spiked in CMV negative blood (4 dilutions tested in 8-fold) and analysed by CMV PCR. Finally clinical blood samples were tested for EBV (n = 23) and CMV (n = 16) by both methods.
Results: Recovery experiments showed reduced recovery for M-extraction (~20%) compared to Si-extraction (>80%) for all matrices tested. Part of the missing DNA from the M-extraction could be retrieved from binding (~20%), washing (~5%) and silica matrix (~15%), leaving ~40% of the DNA irretrievable. Positive control DNA's showed between 7.6 and 14.2 and 1.9 and 7.9-fold lower PCR signals after M-extraction, compared to Si-extraction for EBV and VZV respectively. PCR analysis of spiked CMV showed reduced recovery and loss of sensitivity for M-extraction, resulting in 0% (0/8) and 88% (7/8) hit rates for the lowest spiked CMV loads, whereas Si-extraction obtained 50% (4/8) and 100% (8/8) hit rates. In clinical blood samples this resulted in 11 false negative and 1 invalid result(s) for M-extraction, compared to 1 false negative result for Si-extraction. Similar results have been obtained with other lots of the M-extraction reagents over a 4 year time period and with 2 MagNA Pure LC instruments.
Conclusion: The M-extraction seems to have a substantial lower DNA recovery compared to Si-extraction, leading to reduced sensitivity and possible false negative results with low microbial loads in molecular diagnostic methods. Before implementing the M-extraction the clinical consequences of the loss in sensitivity should first be considered, especially when maximal sensitivity is required. The quality of the DNA from both methods seems identical.

R2179

RT-PCR analysis of fulminantly dead Iranian patients with Crimean- Congo haemorrhagic fever symptoms and without antibody response to CCHF

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Objectives: CCHF is one of the most important zoonoses. The virus is from *Bunyaviridae* family and Nairovirus genus and has an RNA genome with 3 segments. CCHF is an arboviral disease transmitted by tick bite or by handling of infected blood, tissues or nosocomially. After the foundation of our lab in 2000, we worked on this matter in Iran and found 20, 62, 99, 49 and 17 IgM positive cases in 2000, 2001, 2002, 2003 and 2004, respectively. On the other hand as many patients died fulminantly with haemorrhagic syndromes and negative serological results and the cause of death was unknown for the physicians, so we decided to clear up the main cause of death by RT-PCR analysis for detection of CCHF genome in order to diagnose and treat the disease precisely.
Methods: In this study, from 7 June 2000 to 15 October 2004 three sequential serum samples were collected from each of 682 suspected patients throughout the country. In these sera, specific IgM and IgG antibodies against CCHF, Yellow Fever (YF), Rift Valley Fever (RVF) and Dengue2 (D2) have been quantified by Elisa method. Also by using RT-PCR method, the presence of the 2 fragments (226 and 536 bp)in the S segment of CCHF genome has been tested.
Results: The results show that among 682 suspected cases, 247 were IgM positive and had enough time for antibody generation because they died later or survived. By studying 83 fulminantly dead cases, 27 were IgM positive and from 56 cases who died fulminantly without enough time for IgM generation, 17 were

RT-PCR positive. This result shows the importance of molecular analysis because if only serological tests had been done on them, they had been considered negative but now we know that 30.35% (17/56) of them are RT-PCR positive. All of the sera were negative for YF, RVF and D2.
Conclusion: Our results show that CCHF is the most important agent of Viral Haemorrhagic Fevers in Iran. The great number of IgM positive cases indicates that the patients have been infected recently by the virus. As many patients die fulminantly and have no time for antibody generation, by using RT-PCR in all of these IgM negative dead cases, we can determine the cause of death of these suspected patients. The rapid diagnosis of the disease by detection of virus genome is an effective help for the physicians to start on time supportive and antiviral treatment and prevent predictable deaths. Our work shows the cause of some unknown deaths and regards its benefits and importance we decide to continue this research.

R2180

Rapid identification of human T-cell leukaemia virus type I using a PCR method

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Background: The human T-cell leukaemia virus type I (HTLV-I), is the etiological agent of adult T-cell leukaemia–lymphoma (ATLL) and HTLV-I associated mielopathy/tropical spastic paraparsis (HAM/TSP). In HIV coinfected patients only rare cases of cutaneous T-cell lymphoma. HTLV-I infect T cells and reverse transcribe its RNA into DNA, witch integrates into the host cell genoma. The aim of our study was to evaluate a PCR technique for the diagnosis of HTLV-I infection in clinical samples of peripheral blood mononuclear cells (PBMCs).
Materials and Methods: A PCR approach was adopted to detect HTLV-1 infection in clinical samples of peripheral blood mononuclear cells (PBMCs) from subjects with leukaemia and/or lymphoma from Basurto Hospital. The PCR was designed using primers from the pol and tax genes of human T cell leukaemia virus type I. The assay reliably detected a single copy of HTLV-I proviral genome in DNA from 1 x 105 PBMCs. We also included in each sample the HLA DQ alpha gene to check the presence of PCR inhibitors and the quality of DNA extraction. As positive control we used HTLV-I infected MT-2 cell line maintained in RPMI, supplemented with FBS, glutamine, penicillin and streptomycin.
Results: After analyzing 30 patients, all of then presented the band of the HLA DQ alpha gene (242 bp), indicating that the PCR had been carried out under good conditions. Our results showed that the PCR quickly solved the diagnostic query, detecting the presence of proviral HTLV-I DNA in two of 30 patients in both genes (188 bp for pol gene, and 156 bp for tax gene).
Conclusion: The main advantage of this method is the rapidity (5–6 hours) of the performance of the assay and its sensitivity is comparable with other HTLV-I PCR formats. Both genes, pol and tax, could be used to evaluate HTLV-I viral infection in PBMCs clinical samples.

R2181

Quantitation of CMV, EBV and HBV using new sensitive real-time PCR assays

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Objective: New therapeutic options have led to considerable improvements in viral infection management and it is also clear that such treatment requires careful consideration and follow-up. Monitoring of viral load in immuno-compromised patients applying a pre-emptive disease management strategy is important to manage opportunistic infections and prevent disease. In order to meet the growing clinical demand for these patients, three sensitive assays have been developed for measuring CMV, EBV and HBV viral DNA in serum, plasma or whole blood.
Method: Real-time PCR quantitation utilising the Scorpion™ technology provides sensitive and rapid methods for determination of CMV (affigene® CMV trender), EBV (affigene® EBV trender) or HBV (affigene® HBV trender). An internal control (IC) is included in each sample to indicate for the effects of inhibition, and control for sample preparation, amplification and detection. A manual sample preparation protocol with input volumes from 50 to 1000 ul (serum, plasma or whole blood) is used.
Results: Performance testing demonstrated: affigene® CMV trender: assay sensitivity (95% positivity-rate) of ~100 copies/mL and at least 6-log dynamic range for CMV clinical specimens. Equivalent quantitation of CMV strains has been shown using clinical specimens.affigene® EBV trender: assay sensitivity (95% positivity-rate) of <500 copies/mL and at least 6-log dynamic range for EBV clinical specimens. Equivalent quantitation of EBV genotypes A and B has been shown using clinical specimens. affigene® HBV trender: assay sensitivity (95% positivity-rate) of <4 U/mL and a 8-log dynamic range for HBV clinical specimens. Equivalent quantitation of HBV genotypes A–H has been shown using clinical specimens and quantitated genotype plasmids. Normal blood donors were negative for HBV by serology. No cross-reactivity for potential cross-reactive pathogens has been observed in any of the assays. Correlation between titers generated with all three assays correlate well with the 2002–2004 QCMD/VQC sample panels.
Conclusion: The affigene® trender tests provide robust methods for monitoring viral DNA levels in immuno-compromised and transplanted patients. The assays' performance is well suited for monitoring patients being treated with the new efficacious therapies for viral diseases and for studies designed to determine the relationships between viral load and the disease state of the emergence of drug resistance.

R2182

Development and validation of the first open system CE marked real-time PCR kit for the detection and quantitation of hepatitis B virus

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Objectives: Artus GmbH has developed and established the first CE marked quantitative Hepatitis B Virus (HBV) detection Kit for use with the LightCycler Instrument (Roche). The assay was validated utilizing an efficient, CE marked viral DNA isolation system (QIAamp DSP Virus Kit, Qiagen). One of the objectives was to establish a highly sensitive and reliable in vitro diagnostic Kit, which can be run on open systems.
Methods: All validation experiments were done by using plasma samples, spiked with known amounts of HBV particles. Extraction and amplification were done using the QIAamp DSP Virus Kit and the LightCycler Instrument, respectively. For the determination of the viral load the HBV LC PCR Kit quantitation standards were calibrated against the 1st International Standard preparation (WHO 97/746). Therefore, the quantification results are defined as international units (IU). Genotype detection studies were done with the HBV Genotype Panel (15 Member, Teragenix). Each genotype was tested in a 0.5 log dilution series 4 times. The correlation of quantification

results was determined by analysing 117 clinical samples with the Cobas Amplicor HBV Monitor System (Roche) and the HBV LC PCR Kit in parallel.
Results: The detection limit was determined to be 5.8 IU/ml at a detection rate of >95%. The linear range of the assay was determined to cover concentrations from 20 IU/ml to at least 4 x 10 E9 IU/ml. In contrast to other HBV detection systems (e.g. Cobas TaqMan, Roche) the HBV LC PCR Kit was able to detect all members of the HBV Genotype Panel with a sensitivity below 100 IU/ml. The diagnostic sensitivity and specificity were determined to be >99%. The quantitative correlation (1.0 log range) with the Cobas Amplicor HBV Monitor system was determined to be 95.91%.
Conclusion: It has been shown, that the HBV LC PCR Kit developed and established by artus GmbH performs in respect of sensitivity, specificity, quantification, reliability and turn-around time (about 3 hours) at least as good or better than all other currently available HBV detection and quantification assay.In addition the HBV LC PCR Kit is the first HBV detection and quantitation assay designed to work on open systems, demonstrating the usability of research grade sample preparation systems and real-time PCR instruments for clinical in vitro diagnostics. The HBV LC PCR Kit is in complete accordance to the EU IVD Directive 98/79/EC.

Viral diseases (not HIV, Herpes, Hepatitis)

R2183

Parainfluenzae tybe B meningitis with autonomic nerve involvement: a case report

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Objective: The most frequent causes of aseptic meningitis are viral meningitis. The causative agent cannot be shown by routine methods of staining and culturing of cerebrospinal fluid in viral meningitis, lymphositic pleocytosis with increase in protein levels and decrease in glucose levels may be seen. Mumps virus, enteroviruses, HSV are frequent causes of viral meningitis, while parainfluenzae virus is a rare cause.
Methods and results: In this study, a 26 year-old male patient who applied with fever, headache, and disuri, and had lymphositic pleocytosis, increase in protein, and decrease in glucose levels of his cerebrospinal fluid, and who was diagnosed by determining specific antibodies against parainfluenzae virus type B as viral meningitis, is reported. He had symptoms like globe vesicale, hypertension resistant to therapy, tachycardia, hyperthermy, and dry skin which showed involvement of the autonomic nerve system. Autonomic nerve system pathologies like acute pandisautonomic neuropathy can be seen following infections like rubella, infectious mononucleosis. In order to show autonom neuropathy MUAP (Motor Unit Analysing Potential) test was performed. Cardiac autonom nerve involvement was shown by determination of loss of changeability in P-R interval by breathing.
Conclusion: The patient recovered spontaneously and was discharged from the hospital after 40 days.

R2184

Epidemiology of human metapneumovirus in Western Austria

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Human Metapneumovirus (HMPV) is a newly discovered Paramyxovirus which causes respiratory infections. In this study we tested 266 respiratory samples from the local paediatrics department and from adult transplant patients for active replication of HMPV by a combined cell culture/PCR method. Most of the HMPV cases clustered within 2 major outbreaks in October/November and March, whereas only few isolations were obtained from December to the end of February, in most cases from immunosuppressed patients. Sequencing analysis of the isolated viruses revealed that a single strain predominated the 2 outbreaks whereas a clearly different genotype was detected in the 'interepidemic' period. As the same strains were detected in small children (< 2a) and immunosuppressed adults, active transmission within the population can be assumed. Prolonged HMPV shedding was observed in some patients after lung transplantation, although also these patients despite immunosuppression seemed to be able to clear the virus after some months.

R2185

Cytomegalovirus infection and biliary atresia in a newborn infant

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A 2-month-old female infant was admitted to the paediatric clinic because of progressive and unexplained jaundice and light coloured stools. Symptoms began 20 days before admission. On examination, pediatricians found distened abdomen, hepatomegaly and enlargement of the spleen. The ultrasound test detected a tiny gall bladder, while during the HIDA scan test the radioactive dye could not flew through the billiary system. Blood tests showed the following: PT and PTT times were prolonged, repeatedly blood cultures were negative and serologic tests ruled out an acute infection with A, B, C and E hepatitis viruses, HIV, HSV, VZV, EBV, Rubella viruses, Toxoplasma gondi and Coxiella burnetii. CMV serologic study was based on the perfomance of ELISA (DiaSorin s.r.l.) and IFA (PANBIO, inc) methods. Sera samples were collected on the 1st, 5th, 8th day after admission. CMV IgG antibodies were found positive by ELISA test (54 Au/ml, 60 Au/ml, 66 Au/ml, respectively). In all the tested samples CMV IgM antibodies were found positive by both ELISA (+) and IFA tests (1:40, 1:80, 1:80 respectively). At the same times, PCR analysis was carried out (COBAS AMPLICOR CMV MONITOR test) and detected CMV-DNA in blood and urine samples. Only the first blood sample was positive (5.2×10^2 copies/ml), while two urine samples were CMV positive (3.45×10^3, 2.69×10^4 copies/ml). The baby's mother was CMV-IgG (+) 236 Au/ml and CMV-IgM (-). CMV-DNA has not been detected in her blood and urine samples. Under antiviral treatment with ganciclovir first the blood on the 5th day, and then urine samples on the 8th day became negative. However, this did not affect the progressive damage of the liver. Portal hypertension and hepatic coma eventually occurred.
Conclusion: CMV perinatal or congenital infection may participated in the etiopathogenesis of the the neonatal cholestasis. The cause is difficult to be determined, since there was no history of maternal CMV infection and the serology of the mother was unknown before and during

pregnancy and at the time of the birth. At that time, the serology of the infant was also unknown and CMV-DNA was detected 2 months later.

R2186

Seasonal distribution of gastroenteritis viruses in Bosnia and Herzegovina during 2002/2003

I. Salimovic- Besic, M. Hukic, E. Dervovic *(Sarajevo, BIH)*

Objective: The relative importance of the different enteropathogens depends on a variety factors. Some of them are geographical location and season of study.
Methods: 547 stool specimens of patients with acute gastroenteritis were examined for viruses by direct electron microscopy using negative staining method and by qualitative direct EIA for the detection of rotaviruses group A, adenoviruses and astroviruses.
Results: Data were analysed monthly from July 2002 till June 2003 (included). During the Summer- Autumn time (July–September 2002) the major viral causative agent of gastroenteritis was the diverse group of caliciviruses. The peak of calicivirus infection we observed in August (68.4%). In October, the rotavirus infection reached the same percentage as infection caused by calicivirus (40%). In the next months, especially in the Winter–Spring time, the rotavirus infection was the most common and the peak of infection was in March (82.6%). In this study the rotavirus infection was observed in a 52.3%, calicivirus in 28.1%, adenovirus in 14%, astrovirus in 13% and coronavirus infection in 10% of positive samples.
Conclusion: In Bosnia and Herzegovina, analysis of seasonal distribution of gastroenteritis viruses for 2002/03 has shown to be important for rotavirus and calicivirus infections. In Summer–Autumn period the calicivirus infection was the major causative agent of virus gastroenteritis while in Winter–Spring time it was rotavirus infection. Annual 2002/03 results for our country have shown the rotavirus infection as the most common causative agent of gastrointestinal tract infection.

R2187

Enterovirus meningitis: study from 1995 to 2003

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Objectives: The aim of this report is to analyze the evolution of the clinical and epidemiological characters of Enterovirus meningitis in the Miguel Servet University Hospital in Zaragoza during the period 1995–2003.
Methods: Since 1995 until 2003, 65 cases of Enterovirus meningitis were detected in Miguel Servet Hospital. Samples of cerebrospinal fluid (CSF) were performed on fibroblast MRC-5 and human rhabdomiosarcoma cells. Positive cultures were identified with monoclonal antibodies and since 2000 Enterovirus RNA was detected through RT-polymerase chain reaction. Sixty three clinical records were revised.
Results: Isolation of Enterovirus in CSF was positive in 65 of 926 of the samples studied in our laboratory (7.0%). Forty-eight (74%) of these patients were males and 17 were females (26%). Most patients were under 14 years and 11 of them (17.2%) were infants younger than one year. Enterovirus was isolated only in three adults (4.8%).The evolution time until hospital admission was less than 24 h in 57 cases (90.5%) and longer than two days in 6 patients (9.5%). Hospitalization was required in all the patients. Six of them were discharged before 48 h (9.5%) and 26 remained in the hospital more than 5 days (41.2%).The most common symptoms were fever, headache and meningeal symptoms. Antibiotic treatment was given to 39 patients (61.9%) and only in 6 cases (9.5%) it was removed before of the fourth day. The rest of the patients received antibiotic treatment until viral culture result was known. The evolution of the patients was favourable without sequels.
Conclusions: Enterovirus are the most important cause of viral meningitis in our environment with a high incidence in infants. A seasonal distribution has been found in Enteroviruses meningitis; the highest incidence was recorded from May to June (Spring). The knowledge of this seasonal distribution is a diagnostic indicator, which can help to predict infection in these months and adequate the treatment.

R2188

Telomerase activity in cutaneous and anogenital warts: a preliminary study

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Objective: To evaluate telomerase activitiy in cutaneus and anogenital warts.
Methods: Normal skin, genital and cutaneous lesion specimens were collected from 6, 9 and 14 patients, respectively. Telomerase activity was detected by classical immunohistochemstry by using telomerase catalytic unit (hTERT) antibody. Specimens were stained with monoclonal antibody (Novocastra).
Results: Staining pattern was located in basal and suprabasal location in normal skin specimens. However, telomerase activity in basal, supra basal and spinose layers was detected in all anogenital wart specimens but this staining pattern was detected only 7of 14 cutaneous specimens. Mean telomerase activity of the specimens was 66.9%, 61.7%, 27.6% in anogenital wart, cutaneus wart and normal skin specimens, respectively. No association in telomerase activity was determined between anogenital and cutaneus warts. The telomerase activity was significantly higher in HPV infection groups (cutaneus and anogenital) than in normal skin.
Conclusion: The increase in the activation of telomerase by HPV found in this study is consistent with all other proliferative lesions.

AIDS and HIV infection

R2189

Efficacy of acute HCV treatment with peg-interferon and ribavirin in HIV infected patients

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Objectives: Mortality related to HIV infection has significantly decreased due to the implementation of highly active antiretroviral therapy (HAART). But, morbidity associated with Hepatitis C (HCV) is increasingly affecting the HIV/HCV co-infected population. It's essential to perform clinical studies to identify the efficacy, safety and tolerability of interferon and ribavirin in the co-infected population.
Methods: Efficacy, safety and tolerability of peg-interferon 1.5 mcg/kg/weekly and ribavirin 800–1000 mg/d for 24 weeks was assessed in a prospective open labeled study in HIV patients with acute Hepatitis C. Quantitative HCV RNA, HIV RNA and

CD4 count as well as blood count and liver enzymes were assessed at baseline and at weeks 4, 12, 24, 48. All patients had a CD4 count >200 × 10/ml and HIV viral load <200 cp/ml. Recruitment of patients is ongoing with 17 patients enrolled to date.
Results: Infection was predominantly acquired through intravenous drug usage (87%). Other groups were heterosexuals (8%) and homosexuals (5%). The majority was virologically stable on HAART (53%); the remainder did not require HAART. 31% patients were infected with genotype 1/4 and 69% with genotype 2/3. 79% were male and mean age was 29 years (range 19–39). At 4 weeks early biochemical and virological response (normalization of liver transaminases and negative HCV RNA) was observed in 11 (65%) patients, and at 12 weeks 15 (88%) patients were HCV RNA negative. Two patients (12%) did not complete therapy up to 12 weeks due to severe depression. At the end of treatment 15 (88%) patients were still HCV RNA negative, and 9 (53%) patients who overcame 48 weeks are always HCV RNA negative.
Conclusion: After 24 weeks of acute HCV treatment with peg-interferon and ribavirin 15(88%) of treated patients were HCV RNA negative. Peg-interferon and ribavirin show good antiviral efficacy in HIV/HCV co-infected patients. Discontinuation rate is low, but depression is a major problem in this cohort with a high proportion of drug users.

R2190

Infective endocarditis in a group of patients at risk for HIV infection: analysis of 63 episodes

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Objectives: To study epidemiological, clinical, microbiological and evaluative characteristics of infective endocarditis (IE) in a group of patients at risk for HIV infection and to determine the influence of HIV virus in the patients' evolution.
Methods: All IE (defined according Durack criteria) diagnosed between 1996 and 2004 have been studied. Data were collected with regard to the clinical, laboratory and demographics characteristics of patients as well as results of blood cultures and data on clinical outcome. Statistical study was done with SPSS program (11.0).
Results: There were diagnosed 63 episodes of IE between 55 patients. Only one of them was not intavenous drug adict (IVDA) and most were men (81%). Mean age was 33 years (20–49) and 20 (32%) had had a previous episode of IE. There was HIV infection in 56 patients (89%), 26 of them had AIDS and mean CD4+ was 248 mm^3 (4-810). Only 12 (19%) were receiving highly active antiretroviral therapy (HAART) and 49 (78%) had also HVC infection. The onset was acute in 43 cases (68%). All the patients were febrile, 48 (76%) had respiratory symptoms and 33 (52%) cardiac murmur. Chest X-ray was normal only in nine cases (14%) and septic embolisms were observed in 28 (44%). The vegetations were detected by two-dimensional echocardiography and tricuspid was the most frequent affected valve (53 cases; 84%). Mitral valve endocarditis was diagnosed in five cases (8%), aortic in 6 (9.5%) and pulmonary in 3 (5%). There was valvular insufficiency in 39 cases. Blood culture was negative in 28 episodes (44%), there was three cases of polymicrobial IE (4.8%) and S. aureus was the most frequently isolated organism (19 episodes; 30%). Patients were treated with antibiotics during 2–4 weeks, but 7 (11%) needed treatment for 6 weeks. Therapy was depended on the organism's susceptibility but initially the subjects were empirically treated with cloxacillin with or without tobramycin. Ten patients, all with left sided IE (16%), developed cardiac insufficiency, 7 (11%) renal insufficiency, 2 (3%) arterial emboli and 8 (12.7%), all with HIV infection, dead.
Conclusions: Most of the cases of IE in patients with HIV infection are seen in subjects without HAART, probably because IE is more related with drug abuser than with HIV, CD4+ lymphocyte count or HAART use. For this reason IE may be an important disease with an elevated mortality rate in patients with HIV infection, overall they will probably be active IVDA without HAART.

R2191

Global trends in infectious diseases in HIV-infected patients in HAART era

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Background: The incidence and prevalence of different microorganisms and infection sites in HIV-infected patients remains controversial. The aim of this study was to evaluate the global presence of infectious diseases during the HAART era.
Methods: We retrospectively studied all HIV-AIDS records from 1996 to 2003 at a teaching-urban hospital in Barcelona (Spain). All microbiological positive cultures and infection sites were reviewed from 1996 to 2003. Contamination was ruled out based on clinical grounds. Statistical analysis was performed with standardized methods.
Results: We analysed 1502 HIV infected patients. A total of 623 (41%) patients showed one or more infections, with 1681 positive microbiological cultures (371 patients had more than one infection). 429 (68.8%) were men. Mean age was 40 + 9 (range: 18–79). 89% of patients were on highly active antiretroviral therapy. The most frequent isolates were: *E. coli* 159 (9.5%), *M. tuberculosis* 132 (7.9%), *S. aureus* 106 (6.1%), *Herpes simplex* 84 (5.0%), *S. pneumoniae* 74 (4.4%), *Campylobacter* sp 72 (4.3%), *Pneumocystis carinii* 68 (4.0%), *Pseudomonas aeruginosa* 59 (3.5%), *S. epidermidis* 56 (3.3%), *Salmonella* sp 50 (3.0%). The most frequent clinical syndromes were: Bacteremia 262 (15.5%) cases (*S. epidermidis* 16%, *E. Coli* 13%, *S. pneumoniae* 12%), respiratory infections (whit positive BAL culture) 213 (12.7%) cases (*Pneumocystis carinii* 32.5%, *Candida* sp 17.7%, *M. tuberculosis* 7.2%, *S. pneumoniae* 5.3%), urinary infections 231 (13.7%) (*E. coli* 47%, *Enterococcus* sp 11.4%) and diarrhoea was present in 200 (11.9%) cases (*Campylobacter* sp 37.9%, *Entamoeba* 14.9% *Cryptosporidium* 14.4%, *Giardia* 13.8%, and *Salmonella* sp 12.1%).
Conclusions: 1. Almost half the patients suffered one infection during the study period. 2. The most frequent isolated microorganisms were *E. coli* and *M. tuberculosis* and *S. aureus*. 3. The most frequently involved sites were blood, respiratory, urinary and gastrointestinal tracts.

R2192

Post-kala-azar dermal leishmaniasis during highly active anti-retroviral therapy in an HIV-infected patient. Case report

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Introduction: Post-kala-azar dermal leishmaniasis (PKDL) is a complication of visceral leishmaniasis (VL) and manifests as multiple hypocromic macules, erythematous papules or nodules arising in the skin during or most often after treatment of VL. PKDL is very uncommon in HIV-positive patients and just few cases have so far been documented. We describe the occurrence of PKDL in an HIV-infected patient unresponsive to liposomal amphotericin B and successfully treated with *N*-metilglucamine antimoniate (Glucantim®).

Case report: An HIV infected patient in HAART with ddI-d4T-Lop/Rit in June 2003 started complaining of asthenia and failure to gain weight. The blood tests showed: CD4 50/mm3, undetectable HIV-RNA, PMN 680/mm^3, Hb 9.4 g/dL and PLT 161,000/mm^3. The search for opportunistic infection was negative, but a serologic test (IFI) for Leishmania spp. was positive at a titre of 1:2560 and a PCR on peripheral blood was positive for Leishmania infantum. The patient refused bone marrow aspiration. A presumptive diagnosis of VL was made. Liposomal amphotericin B at a 3 mg/kg dose was administered daily for 12 days and weekly later on, with initial benefit.One month later, a maculopapular, nonpruritic, pink rash on the trunk appeared. The rash gradually worsened over time and in October 2003 a skin biopsy was performed. A granulomatous infiltrate was seen, with histiocytosis, PMNs, plasmacells, and amastygotes. The PCR performed on the skin sections was positive for Leishmania infantum. A diagnosis of PKDL was made. The patient continued to receive liposomal amphotericin B 3 mg/kg once a week. Because of the rash worsening, in January 2004 a new course of liposomal amphotericin B at a 5 mg/kg qd dose for 10 days was tried, unsuccessfully. In April a course of Glucantim® was started, at the dose of 100 mg/kg i.m. qd. After a transient increase in amylase and lipase plasma levels, the dose was adjusted to 75 mg/kg daily and was administered for4 weeks. The treatment was well tolerated. At stop therapy the skin lesions were flattened and began to turn pale. The patient is still in good conditions after a 6 months follow-up.

Conclusions: Although very uncommon, PKDL must be considered in HIV-positive patients treated for VL and presenting a skin rash, especially during HAART. Even liposomal amphotericin B, which is considered the most effective anti-Leishmania drug, can sometimes need to be substituted by antimonials for PKDL treatment.

R2193

Temporal pattern of infectious complications in HIV-infected patients

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Background: The aim of this study was to determine and evaluate pattern changes over the last 9 years regarding the presence of infectious diseases in HIV infected patients during the HAART era.

Methods: We retrospectively analysed all infectious episodes in all HIV infected patients from 1996 to 2003. Infectious disease was defined as isolation of a pathogenic bacteria together with consistent clinical findings. No serological data were assessed. We analysed the temporal trend of the most prevalent infections, in search of significant differences to compare their annual prevalence using contingency tables mediating Chi-square analysis

Results: 1677 infectious samples in 628 patients were analysed during this period. Significant results are shown in the following table:

YEAR	96	97	98	99	00	01	02	03	Total	*P* value
Candida	38	37	34	29	33	30	39	23	263	0.3
E. coli	6	21	18	12	24	29	24	25	159	0.001
H. simplex	19	9	8	10	9	8	11	10	84	0.3
M. tuberculosis	13	24	16	30	17	13	17	2	132	0.001
Aspergillus	8	2	2	11	1	1	2	2	29	0.001
Campylobacter	12	12	4	8	8	8	10	10	72	0.8
Citomegalovirus	20	7	5	0	2	3	1	6	44	0.001
Cryptosporidium	7	3	2	6	4	3	2	0	27	0.1
Enterococo	3	6	5	3	7	5	5	6	40	0.8
Klebsiella	0	2	1	0	2	1	4	6	16	0.05
Pnemocystis	7	12	5	11	8	8	8	9	68	0.9
P. aeruginosa	19	8	4	7	4	5	6	6	59	0.003
S. pneumoniae	5	11	9	10	8	8	14	9	74	0.7
Salmonella sp.	10	7	4	6	3	7	7	6	50	0.7
S. epidermidis	7	7	4	9	7	5	7	10	56	0.9
Pyogenes	0	0	2	1	0	2	8	3	16	0.001
M. xenopi	3	6	4	3	5	2	3	5	31	0.8

Conclusions: *E. coli* , *Klebsiella* sp, and *Streptococcus pyogenes* showed an increasing prevalence over time, whereas prevalence of. *M. tuberculosis*, *Aspergillus* sp, *Citomegalovirus* and *P. Aeruginosa*, showed a progressive and significant decrease.

R2194

Potential risk factors and specific management of pancreatic abnormalities in HIV-infected patients taking combination antiretroviral therapy

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Introduction: Epidemiological-clinical features of pancreatic abnormalities are expected to change after the introduction of highly active antiretroviral therapy (HAART).

Methods: The frequency, risk factors and clinical-therapeutic features of pancreatic alterations were assessed in an observational case–control study.

Results: 920 patients (p) were evaluated for pancreatic abnormalities in a case–control study including the entire follow-up period of each considered p;128 p with high and prolonged laboratory anomalies were assessed to outline the profile of pancreatic disease before and during the HAART era. Compared with controls, the 334 p (36.3%) who experienced >1 episode of confirmed pancreatic laboratory abnormality had a longer duration of seropositivity, exposure to protease inhibitors, a more frequent immunodeficiency, AIDS diagnosis, liver-biliary disease, and hypertriglyceridemia, while no relation was found with antiretroviral use, and the duration of nucleoside analogue use. Among these 334 p, high and prolonged laboratory alterations eventually associated with signs of organ involvement occurred in 128 p, and were related to the administration of ddI, d4T, 3TC, pentamidine, cotrimoxazole, or antitubercular therapy, substance or alcohol abuse, opportunistic infections, liver-biliary disease, a protease inhibitor-based HAART, and hypertriglyceridemia. However, no difference was noticed between the 32 p with clinical and/or imaging evidence of pancreatic involvement and the remaining 96 asymptomatic p, as to the same risk factors. Although recurrences of enzyme alterations involved >70% of p, in only 33.8% of cases a change of antiretroviral or antimicrobial therapy became needed. An acute but uncomplicated pancreatitis occurred in 7 p of 26 overall symptomatic ones. A 2–4-week gabexate and/or octreotide administration (performed in 59 p of 128), achieved a significant laboratory, clinical, and imaging cure or improvement in 71.2% of p, with a better success rate of combined vs single therapy; a reduced tendency to disease recurrences, and an ameliorated tolerability of antiretrovirals, were also noticed.

Conclusion: Epidemiological and pathogenetic studies are needed to assess pancreatic abnormalities especially in the HAART

era and their consequences on continued anti-HIV and antimicrobial therapy. The antiretroviral management and the indication to gabexate and/or octreotide administration in the different clinical-laboratory situations, deserves extensive, controlled studies.

R2195

Occurrence of cholangiocarcinoma after AIDS-related cholangitis

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Objective: Increased survival among patients with AIDS has led to a recent increase of neoplasms. Cholangiocarcinoma (CC) is a rare form of malignancy arising from the bile duct epithelium, that may be associated with history of chronic inflammation of the biliary tract. We describe here the first series of five patients with AIDS-associated CC.
Methods: Retrospective multicentric study among all 54 Departments of Infectious Diseases of French University Hospitals.
Results: All patients were men of Caucasian origin. At CC diagnosis, median age was 48 yrs (range 38–61 yrs), median known duration of HIV infection was 13 years (range 0–17). Four patients were at CDC stage C of infection, with a median time between AIDS and CC diagnoses of 13 years (0–17). Three patients were treated by HAART with a median duration of 1 year (range 0–8 years). Median CD4+ lymphocyte count was 170/mm^3 (range 1–600/mm^3) and median viral load was 32,000 copies RNA/ml (range <50–47, 700). All five patients were lacking the most important risk factors for CC (sclerosing cholangitis and liver fluke infection), but three had an history of AIDS-related cholangitis (including one related to cytomegalovirus). Median survival was 7.5 months (range 2 month-9 yrs). Only one patient could have complete tumour resection with prolonged survival (9 years).
Conclusions: 1. Patients with AIDS may develop CC, especially those with long lasting infection and previous AIDS-related cholangitis; 2. Patients with history of AIDS-related cholangitis should therefore be closely monitored, since early diagnosis is key for surgical excision which is associated with increased survival.

R2196

Outcome of invasive pulmonary aspergillosis under highly active antiretroviral therapy in a HIV-infected patient without appropriate antifungal drugs

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Background: Invasive pulmonary aspergillosis (IPA) is the most common manifestation of aspergillus infection in HIV patients, associated with an extremely high mortality rate.
Aim: To present IPA in a HIV infected patient recovered by immune reconstitution under antiretroviral therapy, without appropriate antifungal drugs.
Case report: A 15-years teen-ager, male, was admitted in our hospital for a 2 months history of weight loss, weakness, anorexia, fever, persistent cough and thoracic pains and related more previous episodes of pneumonia, diarrhoea and allergy. He had fever, height and weight deficit, labial herpes, white plaque tongue, tachycardia, normal pulmonary sounds, inflammatory syndrome (ESR = 85–110/h, fibrinogen = 560 mg%, CRP = 6.5 mg/dL), high values of Ig G (2200 mg/dL), leucocytes and lymphocytes count decreased (3800/mm^3, respectively 570/mm^3). Chest radiograph showed an inhomogeneous opacity with a poorly defined nodular appearance, about 4–5 cm diameter in left upper pulmonary lobe, extended to hila by multiple micro nodules, 5–10 mm, and high-resolution chest computed topography indicated a 4.5/5.2 cm parenchyma nodule with a surrounding halo of ground glass, suggesting an IPA. High levels of the Aspergillus antigen galactomannan detected by enzyme-linked immunosorbent assay (ELISA) in two consecutive serum samples confirmed the diagnosis. ELISA and Western Blot tests for HIV were positive, CD4 count 140 cells/mm^3, HIV-RNA levels 68,000 copies/mL and he was diagnosed as nosocomial-transmitted HIV infection, CDC stage C3. HAART was started with lamivudine + efavirenz + indinavir with a very good adherence to the treatment. He did not receive any antifungal therapy because he presented intolerance to Amphotericin B and no another antimycotic drugs were available at that moment. After 3 months of HAART, re-evaluation showed weight growth, inflammatory syndrome absented, WBC count 6500/mm^3, lymphocytes count 1855/mm^3 (27%), CD4 count increased 612/mm^3, viral load <400 copies/mL, radiographic and CT abnormalities resolution; 2 months later radiological manifestations absolutely disappeared and Aspergillus antigen galactomannan levels was undetectable. The patient remained stable for 5 years with the same HAART regimen.
Conclusions: We obtained IPA recovery by immune reconstitution after 5 months of HAART, even in absence of any antifungal treatment. HAART starting may improve the clinical outcome in HIV-infected patients with IPA.

R2197

Prediction of potential antigenic domains of HIV-1 Pol protein and evaluation their immunoreactivity

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Objectives: The aim of this study is prediction and evaluation diagnostic relevance of potential antigenic epitopes of HIV-1 polymerase protein (protease; reverse transcriptase; integrase).
Methods: Five potential antigenic epitopes of HIV-1 pol protein have been predicted by bioinformatics analysis. Recombinant genes encoded selected amino acids sequences have been assembled by PCR from synthetic oligonucleotides and expressed in *E. coli* as hybrid proteins with Glutathione S-transferase. Proteins were tested by enzyme immunoassay against a panel of human serum specimens positive for anti HIV (n = 228) and specimens from normal blood donors (n = 200). Status of samples has been confirmed by 'New Lav Blot' (Bio-Rad, USA).
Results: Potential antigenic determinants were predicted at position 1–51 aa, 198–347 aa, 242–347aa, 352–411 aa and 922–1002 aa pol protein. Only protein comprising 922–1002 aa of pol protein (integrase) was immunoreactive and detected IgG antibody in 87% anti-HIV positive serum samples. Average of signal to cutoff ratio is 8.5. None of specimens from normal blood donors were tested as positive with this recombinant protein. Other theoretically selected amino acids sequences demonstrated no immunoreactivity. This may be connected with the conformational nature of other predicted epitopes.
Conclusion: The results indicated that recombinant protein comprising one of the theoretically predicted antigenic epitope(s) of HIV-1 pol protein demonstrated a significant diagnostic potential and can be used for the development EIA for the detection of anti-HIV activity in serum specimens.

R2198

A progressive study of mycobacterium isolations in HIV+ patients (1991–2004)

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Objectives: We studied the mycobacterium isolation frequency and resistance to antituberculous drugs in HIV+ patients, examinated in National Mycobacterium Reference Laboratory of Athens, during the years 1991–2004.
Methods: From 1212 HIV+ patients studied, 156 mycobacterium strains were isolated from various clinical sources. Laboratory testing included Ziehl Neelsen strain, cultures performed classically and automatically by the use of Bactec and MGIT systems, detection of *M. tuberculosis* rRNA in clinical specimens by the Amplified Mycobacterium Tuberculosis Direct Test AMTD (Gen Probe), identification by the nucleic acid hybridization method of Accuprobe (Gen Probe) and Innolipa V2, (Immunogenetics) and finally, susceptibility testing, by the method of proportion on solid medium Löwenstein Jensen and in liquid medium by the use of Bactec and MGIT automated systems Innolipa RTF TB (Immunogenetics) for the detection of rpo B gene was also performed.
Results: From 1212 HIV+ patients examinated for TB, 1029 (84.9%) were men and 183 (15.1%) women. Mycobacterium was isolated in 156 (12.8%) patients. In 91 (58.3%) patients *M. tuberculosis* was isolated, while in 65 (41.7/%) *M. avium*. In particular, *M. tuberculosis* was isolated in 77 (58.8%) men and in 14 (56%) women, while *M. avium* in 54 (41.2%) men and in 11 (44%) women. *M. tuberculosis* was mainly isolated from lower respiratory tract, urine and CSF, while *M. avium* from blood and bone marrow. Among the 91 *M. tuberculosis* isolations, 87 (95,6%) were susceptible to all antituberculous drugs.
Conclusions: Mycobacterium isolation frequency in HIV+ patients is progressively decreasing. *M. tuberculosis* is predominating in comparison to *M. avium*. In *M. tuberculosis* isolations not any significant resistance to antituberculous drugs was detected.

R2199

Clinico-evolutive aspects of tuberculosis in horizontally HIV-infected patients

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Objectives: Evaluating the incidence, clinical forms and evolution, of tuberculosis (TB) in horizontally HIV/infected patients.Materials and methods: Retrospective study 01 Nov1994–01 Nov 2004 on a cumulative lot of 305 HIV+ patients in evidence of Regional Center of Monitoring and Evaluation HIV/SIDA Infection Craiova, Romania. The assessment was clinical, immunological, imagistic, bacteriological and histopathological.
Results: 87 cases with TB (28.55%): 68 pulmonary forms (88.3%), 7 ganglionary forms (8%), seven cases meningoencephalitis (8%), 4 bacillary pleurisy (4.6%), three cases intestinal TB (3.4%), 2 TB pericarditis (2.3%); three patients with multiple determinations. In 18 cases (20.7%) of TB, the diagnosis of HIV infection was established concomitantly with TB diagnosis. Pulmonary forms were: primary – 35 cases (51.4%), miliary – six cases (8.8%), infiltrative-ulcerative – seven cases (10.3%), infiltrative-nodulary – five cases (7.4%). Bacteriologic confirmation was found in 10.7% and histopathological in all cases with ganglionary TB. Main characteristics of patients with HIV-TB coinfection: average age: 16.9 years, average CD4 = 176.4 cells/mmc; M/F = 48/39. Evolutions with tuberculostatic and antiretrovirals treatments: favorable in 65 cases (74.7%), recidives – nine cases (20.7%), paradoxal reactions – nine cases (20.7%), deceases – 13 cases (14.9%).
Conclusions: TB remain one of most frequent opportunistic infections in HIV+ patients with advanced immunodepression having a potential severe evolution.

R2200

Atazanavir early access programme: safety data from an HIV infected Greek population

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Objectives: To report our experience regarding safety from the Atazanavir early expanded programme ran in our clinic from March 2003 to October 2004. We registered Grades 3 and 4 toxicities according to ACTG table of Grading Severity of Adult Adverse Experiences.
Methods: We enrolled 19 patients (CDC stage: 9A, 4B, 6C), 84.2% male, median age 43 years, median time on prior ART 95 months, median CD4 564 cells/ml, 36.8% with undetectable VL and 84% placed on a TDF-containing regimen. Reasons for starting on ATV/RTV were 47.4% hyperlipidemia, 26.3% virological failure and 26.3% toxicities. 10.5% were coinfected (1 HBV, 1 HCV). 4/19 initially received ATV 400 mg daily according to the protocol, changing 6 months later to ATV / RTV. Median time on ATV was 6.5 months.
Results: 5/19 patients had mild to moderate bilirubin elevations (2/5 were on IDV) before enrollment. After the initiation of ATV/RTV 15/19 patients (78.9%) experienced grade 3 or 4 hyperbilirubinemia (due to elevation of indirect bilirubin), of whom 7 grade 3 (36.8%) and 8 grade 4 (42.1%). Jaundice/scleral icterus was noticed in 10/19 (52.6%). 14/15 patients (93.3%) manifested hyperbilirubinemia within 12 weeks. 4/19 patients (21%) discontinued ATV: 3/4 due to protocol requirements, and 1/4 due to patient's request (scleral icterus, esthetic reasons). No other side effects were noted (transaminase elevations, rash, gastrointestinal disorders, dizziness, myalgia or ECG changes). Total bilirubin was returned to normal in all four patients after discontinuation of ATV.
Conclusions: ATV was safe and well tolerated. Almost 8/10 patients experienced severe hyperbilirubinemia (4/10 grade 4). 1/2 patients experienced jaundice/ scleral icterus. No relation between the above side effects and coinfection with HBV and HCV was noted. 1/5 discontinued ATV, mainly due to protocol requirements. ATV seems to be a more potent inhibitor of the uridine diphosphate-glucoronosyl-transferase than IDV. These data in our study revealed a significantly higher percentage of severe hyperbilirubinemia and jaundice from ATV use than previous studies.

R2201

The epidemiologic and clinical characteristics of HIV/AIDS patients in a tertiary hospital, Turkey

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Objective: To describe the epidemiological and clinical findings of 87 Acquired Immune Deficiency Syndrome (AIDS) patients from Turkey.
Methods: All the patients had been followed in Ankara Numune Education and Research Hospital between 1993 and 2004.
Results: Seventy-two per cent of the patients were male, mean age at the diagnosis was 36 (min 13, max 71). Mean years of

survival was 3.4, and maximum years of survival was 11. Thirty-one of the patients died, of those 26 died at their first admission. The most common professions were, drivers and workers. Eighty per cent of the women were housewives. Only one of the patients was university graduated. Heterosexual intercourse was the most common (86%) route of transmission. Blood transfusion, and IV drug use was the other routes. One male patient declared men with men sexual relation at the first interview, and 5 did after more detailed interview. Median CD4 count at the diagnosis was 198 (min 5, max 1051), and the mean viral load at the diagnosis was 122, 000 (min 400, max 107), 36% of the patients were grouped in C3 category at their first admission. The most commonly detected opportunistic infections were Candidal infections (0.1 per patient year), Mycobacterium tuberculosis (0.034 per patient year), and CMV (0.015 per patient year).

Conclusions: Considering the low education status among the patients, the role of the education in preventing HIV infection should be re-emphasized. Appropriate education programmes should be developed to prevent the transmission of HIV infection. A significant number of patients were diagnosed at very late stages. The physicians and other health care workers should be educated on the clinical pictures of HIV/ AIDS to diagnose the cases earlier.

R2202

AIDS, a risk disaster for the African Great Lakes region

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Africa bears the heaviest burden of HIV/AIDS. According to the UNAIDS (2003), more than 28 millions are contaminated. The prevailing remains stable.African states already faced with many developmental difficulties must by all means avoid the total explosion of this bomb (HIV/ AIDS).Southern African still has this disaster. The African Great lakes region constitutes countries such UGANDA, Tanzania, Kenya, Burundi, Rwanda, Zambia and DRC. These Countries are noticing new infections following a massive displacement of (refugees) population due to ethnic conflicts, political, economic and social crisis (lands, poverty, identity and other problems).According to research conducted by UMHE/GREAT LAKES, ANDA-ONLUS for Central And Eastern Africa, out or 12,000 young students (boys/girls) of the sub-regions, +20% of girls agreed to have succumbed to sexual violence from soldiers from one or another regime.+80% have no idea on voluntaring testing and antiretroviral , +70% are engaged in unprotected sexual relations +75% fear being pregnant than HIV/AIDS . +2% accepted tocontribute towards funds for solidarity for students and young HIV/AIDS victims.No one ignores that the Great Lakes region is led by Armies, Warlords, troops that 40% are infected by HIV/ AIDS.+50% Of other young girls did not want to testify in sexual violence from the men in uniform fearing stigmatization and reject.+60% of young would like to know their status but there is lack of appropriate structures.A study conducted in the East of DRC (North–South Kivu and Maniema), for about 10,000,000 habitants, there exist six structures of voluntary testing but with a weak capacity of less than 500 volunteers per year.In south –Kivu for example, there is only one existing structure taking care of patients of HIV/AIDS with a capacity of 20 Patients per year out of a population of +4,500,000 habitants.Rape is part of principal causes of HIV/ AIDS expansion in the sub-region. Sexual Violence have become a current denomination in the region for a decade now.The African Great Lakes Region is undergoing a disaster without precedent.The future of the region might be auctioned. Efforts for a stable development risk being trapped by the explosion of HIV/ AIDS. We are requiring once more, action for all in order to save human lives in danger in the great region.

Hepatitis

R2203

Prevalence of serum HBV markers among 13,581 women of child-bearing age in Greece

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Objectives: To evaluate the seroprevalence of hepatitis B surface antigen (HBsAg) in a large multinational group of women at gestational age and to examine the presence of hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe) as well as antibody to hepatitis C virus (anti-HCV) in HbsAg(+) ones.

Methods: Between August 2003 and August 2004 a total of 13,581 women at gestational age (range 16–45 years) were prospectively evaluated. HBsAg, HBeAg, anti-HBe as well as anti-HCV were detected using routine commercially available enzyme immunoassays (Abbott Laboratories, Abbott Park, Illinois, USA). All women of the study population were screened for HBsAg whereas the rest serum markers were evaluated only in HBsAg(+) ones.

Results: The majority of the study population were from Greece (70.31%) whereas 15.96% of them were from Albania, 7.06% from countries of Eastern Europe (Russia, Romania, Bulgaria), 4.09% from countries of Africa, 0.69% from countries of Asia, 0.99% from countries of Northwestern Europe, 0.46% from Australia and 0.43% from countries of North America. Overall, 157 of 13581 females (1.156%) were HBsAg(+) and the vast majority of them (112/157, 71.3%) were Albanian. None of the females from countries of North America, Northwestern Europe and Australia were HbsAg(+). Among women from Albania the seroprevalence of HBsAg was 5.1%, representing the higher rate among the national groups of the study population, followed by 4.2% in Asian women and 1.14% in women from Eastern European countries. The prevalence of HbsAg among African (0.36%) and Greek women (0.29%) was very low. Overall, only 4.45% of HbsAg(+) women were also HbeAg(+) whereas the vast majority of them were HbeAg(-)/antiHbe(+).Only 3 of 157 women (1.91%) with chronic HBV infection were HBV/HCV coinfected.

Conclusion: The overall seroprevalence rate of HBsAg is relatively low among women at gestational age in Greece but is higher enough among specific populations (Albanian, Asian). The HBeAg(−)/antiHBe(+) status is a finding observed in the vast majority of HBsAg(+) women of our study population, suggesting possibly that only a small subgroup of them exhibit an extremely high risk of vertical transmission of the infection.

R2204

A comparative study of C-reactive protein serum values in chronic hepatitis Band C and correlation with the progression of the disease

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Objectives: Our study was conducted to control hospitalized patients for chronic hepatitis infection B and C and to estimate the clinical significance of C-reactive protein (CRP) serum levels, the relationship between CRP and alanine aminotransferase (ALT) concentrations in serum and the correlation of CRP with the hepatic inflammation and damage in these patients.
Methods: During a ten month interval 100 hospitalized patients were found with chronic viral hepatitis. There were two groups of patients, Group A: 70 patients with chronic hepatitis B and Group B: 30 patients with chronic hepatitis C. Serum samples from them were examined for the determination of CRP and ALT levels. The detection of surface antigen HBsAg and HCV-antibodies was performed by MEIA methodology (AXSYM-ABBOTT) and the diagnosis proved by liver's biopsy. The CRP values were determined using the nephelometric method (Dade-Behring) taking as normal values 0–5 mg/lt and ALT values were determined by IFCC method (Technicon RA 1000 Medicon) taking as normal values 5–40 U/L.
Results: In Group A of the 70 patients, 24 (34.3%) had pathological CRP levels that ranged from 15 to 174 mg/lt, 23(32.8%) had high ALT levels that ranged from 58 to 6110 U/L and in 18 of them (79.7%) were found high CRP concentrations fluctuated simultaneously with ALT serum levels and ranged from 35 to 174 mg/lt.In Group B of the 30 patients, 6 (20%) had high CRP levels that ranged from 13 to 83 mg/lt, 7 (23.3%) had high ALT levels that ranged from 56 to 89 U/L and in all of them (7/7) the CRP concentrations were normal.
Conclusion: This study showed a significant association between elevated CRP and ALT serum levels in chronic hepatitis B. In contrast no correlation was observed in chronic hepatitis C. In conclusion the combination of CRP and ALT values could be a useful marker for the progression of the disease in chronic hepatitis B but not in chronic hepatits C.

R2205

Treatment of chronic hepatitis C in haemophiliac patients

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Objectives: Chronic hepatitis C infection is very common among haemophiliacs in the developed World.
Methods: Retrospective evaluation of the treatment results in haemophiliacs with chronic hepatitis C, all infected with genotype 1b. Twelve patients were treated with interferon-alfa monotherapy, 21 patients with interferon-alfa and ribavirin, and 3 patients with pegylated interferon and ribavirin, all for 48 weeks.
Results: Sustained virologic response (defined as an undetectable serum HCV RNA level 24 weeks after treatment was completed) was not achieved in any of 12 patients treated with interferon-alfa alone. Combination therapy with interferon-alfa and ribavirin was associated with better results: 4/10 (40%) patients still untreated with interferon-alfa, 2/4 (50%) relapsers, and 2/7 (29%) non-responders to previous interferon-alfa monotherapy achieved sustained virologic responses. Combination therapy with pegylated interferon and ribavirin has been used in 3 patients. Sustained response was achieved in one patient who had relapsed after treatment with interferon-alfa and ribavirin and in 1 of 2 non-responders to this combination therapy. There were no serious adverse events and it was not necessary to reduce dosages or even cease therapy prematurely.
Conclusions: The efficacy and tolerability of antiviral treatment in haemophiliacs did not differ from other patients with chronic hepatitis C.

R2206

Prevalence of hepatitis B virus genotypes in chronic infected patients

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Objectives: Hepatitis B Virus (HBV) genotypes have been associated with specific patterns of liver disease and treatment response. The aim of this study were to determine the prevalence of HBV genotypes, serum HBV-DNA level and severity of liver disease in chronic infected patients.
Methods: A total of 87 HBV infected patients were enrolled, 63 males and 24 females. The mean age was 46 years (range 28–77 years). Fourteen of them were co-infected with human immunodeficiency virus (HIV). Sera were tested for HBV genotyping by reverse hybridization line probe assay INNO-LiPA HBV Genotyping (Innogenetics) after a PCR sensitive amplification protocol. Quantitative HBV-DNA levels were determined using Cobas Amplicor Monitor kits (Roche). Detection of Hepatitis B e antigen (HbeAg) in patient serum samples also was performed using the ETI-EBK enzyme-linked immunosorbent assay (DiaSorin).
Results: All HBV genotypes (A–G) were found. The genotypes A, D, E and F were common and constituted 22%, 36%, 8% and 12% of the total patient population. Genotypes B, C and G were uncommon and represented less than 2% each one. Fifteen patients were infected with two HBV genotypes. On the HIV co-infected patients genotypes A, D and mixed (A + G) were prevalent. Only in 14 of the 87 patients was detected the presence of serum HbeAg, ten of them were HIV-infected, and it was associated with HBV genotypes A (30%), D (20%) and mixed genotype A + G (30%). Among the patients HbeAg negative genotypes D, A and F were more frequent 37%, 20% and 12% respectively. Analysis of baseline serum HBV–DNA levels in HBeAg positive patients revealed that were higher than 106 excepted in two of the three mixed genotypes. Genotypes B, C and G were associated with high levels of serum HBV-DNA. The lower level of serum HBV–DNA was detected in a patient with genotype D and negative HbeAg.
Conclusion: Our data indicate that HBV genotypes A, D and mixed A + G were associated with a higher prevalence of HbeAg, and HIV-infected patients. Genotypes B and C presented higher serum HBV-DNA levels in HbeAg negative patients.

R2207

Molecular phylogenetic analysis of Iranian HDV complete genome

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Objectives: Hepatitis D (delta) virus (HDV) is a subviral pathogen agent and a satellite of Hepatitis B virus. Three distinct genotypes of HDV are described. So far at least 60 full genome HDV sequencing have been reported from all over the world. There is limited genetic information on Middle East's HDV isolates and no data about isolates from Iran where delta infection is endemic. In this study complete HDV genome sequences isolated from an Iranian patient has been reported.

Methods: RNA extraction was done on serum sample from an IVDU who was chronic HBsAg carrier and anti-HD ELISA positive. Twelve pairs of primers were used to amplify six partially overlapping fragments, covering the entire HDV genome by RT-nested PCR. PCR products were purified and subjected to automatic cycle-sequencing procedure. Editing of raw data was accomplished by the Generunner and Chromas softwares. Alignment analysis of complete genome and HDAg amino acid sequences were carried out on IR-1 and eleven reference sequences. These analyses were done by ClustalW method, ClustalX software and subjected for phylogenetic analysis by applying neighbour-joining model for tree construction.
Results: This new isolate (IR-I) contained 1676 nucleotides encoding 214 a.a. of HDAg and belongs to genotype I. IR-I revealed the most sequence similarity to an Italian isolate (92.6%) whereas IR-I HDAg showed the most homology with those of Lebanese isolate. Tree branching and bootstrap values confirmed these findings. In addition IR-1 showed the same pattern of diversity throughout complete genome and its deduced HDAg shown by other HDV isolates.
Conclusion: In this study, the location of an Iranian HDV isolate was determined among other worldwide isolates for the first time. It might be explored that IR-I, Italian and Lebanese HDV isolates are from same origin. Data analysis also confirmed genetic variability and heterogeneity of HDV species isolated from different geographical areas.

R2208

Immunocomplexes in HCV-infected patients

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Hepatitis C virus infection is widespread and considered the main cause of chronic hepatitis in Western countries. Many studies conducted in the last decade have shown that the outcome of HCV infection is determined by the interaction between the virus and the host immune system. Recent acquisitions suggest that in persistent HCV infection the immune response may contribute to hepatocellular injury. To this regard HCV-directed antibodies (Ab) might be involved in the development of immunocomplexes (IC), that may favour the infection of usually not susceptible cells, and of immunocorrelated diseases. The present study is aimed at evaluating the development of IC and, eventually, their relevance in HCV-infection clinical outcome.
Methods: The presence of IC was evaluated in 44 chronic and in 6 acute naïve HCV-infected patients with different genotypes. To verify the presence of IC, patient's plasma samples were sequentially absorbed on protein A coated beads and then analysed to quantify the number of HCV-RNA copies absorbed (or not) to the beads. HCV-RNA was monitored using the Amplicor HCV Monitor kit, while HCV genotype was obtained by INNO-LIPA HCV II assay. The study of the Ab was carried out by INNO-LIA HCV Ab III and the reactivity for E2 protein was also monitored by in house-made ELISA.
Results and conclusions: The results suggest that in chronic HCV patients most of the HCV-RNA (ranging from 80 to 99.9%) is bound to Ab in IC with a higher percentage in patients infected with 1b genotype. No correlation between 'baseline' plasma viral load and IC development was observed suggesting that the frequency of IC does not depend on plasma viraemia levels. The analysis of antibodies revealed that the IC do not contain anti-E2 Ab, even if these antibodies can be detected in the plasma of all subjects. In contrast newly HCV-infected patients developed no or low amount of IC that, interestingly, increases within time (0, 1, 3 and 5 months of follow-up) only in patients who developed chronic infection. Again this phenomenon does not seem to be related to the presence on anti-E2 antibody. These data suggest that the presence of IC is a peculiarity of HCV chronic infection; the pathogenetic relevance of such a phenomenon is now under study.

R2209

Identification of mutations in hepatitis B virus under lamivudine therapy

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Objective: The aim of this study was to determine the prevalence of HBV genotypes and viral loads in the serum of patients with chronic hepatitis B and also the variants that may appear in the HBV polymerase region (in and around YMDD region) due to prolonged lamivudine treatment.
Methods: A group of 100 patients from Northern Greece (70 men, 30 women, mean age 45.5 ± 10.0 year) with chronic hepatitis B was tested. All patients were treated with lamivudine for more than six months. The mean duration of the antiretroviral therapy was 15 (±2.3) months. Serum HBV-DNA levels (viral load) were measured with quantitive PCR (COBAS TaqMan HBV Test), genotypes and polymerase gene mutations were determined using the reverse hybridization in a line probe assay (INNO-LiPA HBV genotyping, INNO-LiPA HBV DR kits, respectively). Three kind of mutations were examined in and around YMDD region.1st substitution of leucine by methionine at position 180 (L180M), 2nd substitution of methionine by valine or isoleucine at position 204 (M204V/I) and 3rd substitution of valine or leucine or methionine by isoleucine at position 207 (V/L/M207/I).
Results: The majority of patients (90%) showed infection with genotype D, genotype A was the second most common (9%) and only 1 patient had mixed type (D and G). Polymerase mutants were identified in 49 patients (78%).The M204V/I mutant was observed in all (100%) YMDD variants of both genotypes D and A. The L180M mutant was detected in 4/43 (8%) patients with genotype D and in 3/5 (60%) patients with genotype A. There were no patients in whom mutation V/L/M207/I occurred. HBV-DNA was detectable in 64% of patients (baseline > 35 copies/ml). The prevalences of variants and viral loads were respectively the following: low viral load (10^2–10^3 copies/ml) 25% mutants 75%, medium viral load (10^4–10^5 copies/ml) 30% mutants 60%, high viral load (>10^5 copies/ml) 45% mutants 90%.
Conclusions: Genotype D is the most common in the area of Northern Greece. The M204V/I mutation is the most frequent among genotypes D and A. Although the appearance of mutants seems to have no correlation with HBV genotypes and viral load, the type of change is propably relayed.

R2210

Phylogenetic analysis of hepatitis A virus isolated in Korea

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The test was conducted to find infection of Hepatitis A virus (HAV) and the genotypes against twenty-six of the patients with fatigue and nausea. Fifteen isolates (57.7%) were amplified by RT-PCR using HAV specific primer, and then compared to 168 bp nucleic acid sequences in VP1/2A junction region for the genotypes. Based on 79% similarity, 13 out of 14 isolates were

clustered with genotype IA and 1 out of 14 isolate was separated to IIIA. Above 13 isolates found in genotype IA were sporadic to Japan, China, and Korean strains which are grouped determined by it's geographic region with over 95% similarity. The history of patients informed the high relationship of endemic or family transmission, and it also showed the changes according to risk age group followed by the economic growth as 14~46 years-old patients.

R2211

Secondary acute viral hepatitis

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Introduction: There are some diseases caused by viruses that secondary affect liver. These viruses are: Epstein Barr, Cytomegaloviruses, adenoviruses, Echo, Coxsachie, herpes simplex, varicella zoster, and rubella virus.
Objective: To evaluate the clinical and biological evolution of some secondary acute hepatitis.
Material and method: Study about 53 patients hospitalized with secondary acute viral hepatitis in Children Infectious Diseases Department of Clinical Infectious Diseases Hospital of Constanta during a period of one year (2003). The etiological diagnosis was established on clinical and paraclinical data (serological markers – ELISA method). In order to establish a complete diagnosis we checked the following syndromes: cytolitic, hepatoprive and billiary retention. Also we studied parameters such as age, sex, residence and period of hospitalization.
Results: From the total of 53 patients the most of the cases with secondary acute viral hepatitis were patients with infectious mononucleosis – 25 cases, followed by patients with rubella – 17 cases, by patients with cytomegalovirus infections – 9 cases, and by *varicella zoster* – 2 cases. Patient's age was between 2 and 17 years. Sex ratio shown a predominant feminine injury (M:F = 1:2.53). For residence point of view the majority of children were from urban area (31 patients from 53). The cytolysis syndrome was presented with a slight elevation of ALT (2–5 N) in majority of cases (except one case with infection with a severe form of hepatitis), such as billiary retention syndrome. The hepatoprive syndrome was light and medium in all cases except the patient already mentioned with *varicella zoster* infection in an immunodepressed person. There were no colestatic forms and no chronically cases or deceases.
Conclusions: The secondary acute viral hepatitis presented a favorable evolution in almost all our cases. In the context of some viral diseases hepatic injury is frequent but slight, sometimes asymptomatic. In these diseases generally the liver affection is underestimated in our area.

R2212

Hepatitis B virus genotypes in Belgium

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Objectives: Several studies have demonstrated that pathogenic and therapeutic differences exist among hepatitis B virus (HBV) genotypes. It appeared sensible, therefore, to establish the prevalence of the different HBV genotypes in Belgian patients with chronic hepatitis B.
Methods: The study population consisted of two groups of patients: Flemish blood donors and patients whose sera were collected at the gastro-enterology units of three university hospitals in Antwerp, Brussels and Ghent. HBV genotyping was carried out with the Inno-Lipa HBV Genotyping assay (Innogenetics NV, Belgium) on 128 samples where chronic hepatitis B infection and HBV DNA were detected.
Results: Of the 93 serum samples from blood donors 49 (53%) samples were infected with genotype A and 34 (37%) samples were infected with genotype D. Mixed genotypes A and D were found in 7 (8%) samples. In the group of patients from the gastro-enterology units, 19 (54%) of the 35 were infected with genotype A and 11 (31%) patients had genotype D. Here also, mixed genotypes A and D were found, specifically in 3 (9%) samples. In total, only 2 samples could not be genotyped by the Inno-Lipa assay, despite the detection of HBV-DNA. No significant difference was observed with respect to the distribution of the genotypes A, D and A/D between the 2 groups of patients.

Distribution of genotypes among blood donors and patients from the gastroenterology units, with chromic hepatitis B. Genotype H could not be assessed with the line-probe assay (Inno-Lipa HBV Genotyping Innogenetics NV, Belgium)

Genotype	Blood donors		Patients from gastro-enterology units	
	N	%	N	%
Total	93	100	35	100
Genotype A	49	53	19	54
Genotype B	1	1	0	0
Genotype C	0	0	0	0
Genotype D	34	37	11	31
Genotype E	0	0	1	3
Genotype F	1	1	0	0
Genotype G	0	0	0	0
Genotype A/D	7	8	3	9
Unclassified	1	1	1	3

Conclusion: The results indicate that genotypes A and D are the predominant genotypes in Belgian patients with chronic hepatitis B infection.

R2213

Response to interferon-alpha in chronic hepatitis B with and without precore mutant strain

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Objectives: Chronic hepatitis B is an important public health problem worldwide and in Turkey. In Turkey chronic hepatitis B were mostly precore mutant strains. We investigated whether the presence of precore mutant affects the response to interferon-alpha in patients with chronic hepatitis B.
Study: Chronic hepatitis B patients who were admitted to the Infectious Diseases and Clinical Microbiology polyclinic in the last eight months were included in this study. Thirteen with hepatitis B e antigen-positive, hepatitis B e antibody-negative and ten with hepatitis B e antigen-negative, hepatitis B e antibody-positive (precore mutants) chronic hepatitis patients were treated with natural interferon-alpha 24 months. Before treatment, all patients had liver biopsy to grade and stage liver disease. Liver biopsy showed chronic hepatitis (necroinflammatory score 4), there was no cirrhosis and nobody had interferon therapy before. Serum HBV DNA level criteria to begin the therapy was 104 copies/ml for precore mutant strains and 105 copies/ml for non-mutant strains.
Results: Serum HBV DNA level before treatment was not different between the two groups ($p = 0.053$). At the end of treatment, serum HBV-DNA was decreased to undetectable levels in 30% of mutant group and 23% of the non-mutant group. The decrease of serum HBV-DNA level was found significant ($z = -2.66$; $p = 0.008$) in mutant group and not significant ($z = -1083$; $p = 0.279$) in non-mutant group. Six months after treatment, the percentage of cases with a decrease in the transaminase level to

within the normal range was significantly higher in mutant group ($p = 0.028$) than in non-mutant group ($p = 0.108$).
Conclusions: Chronic hepatitis with precore mutant strain seems more responsive to IFN-alpha. The reason of not expecting result may be the serum HBV-DNA of four patients in non-mutant group showed increase in spite of the treatment and none of the patients in the mutant group.

R2214

Neutrophil oxidative metabolism in children with chronic hepatitis C

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A growing number of data suggest the role of reactive oxygen species (ROS) in the pathogenesis of chronic type c hepatitis. Activated neutrophils, macrophages and Kupffer cells are basic sources of ROS in the course of the inflammatory process in the liver. The aim of the study was to assess the metabolism of neutrophil oxygen in children with chronic hepatitis C.
Material and methods: The study comprised 14 children with chronic hepatitis C (group I), 4 children with positive serum anti-HCV antibodies and negative HCV-RNA as well as normal ALT activity (group II) and 6 healthy children 9 (group III). Production of reactive oxygen species by neutrophils using the method of chemiluminescence (CL) with luminol (MLX Microtitier Plate Luminometr, Dynex, USA) was assessed. We estimated chemiluminescence response (CL max and CL total) of neutrophiles both unstimulated and stimulated by formyl-methionyl-leucyl-phenyloalanine (fMLP), opsonized zymosan (OZ), phorbol-myristate-acetate (PMA) without and after priming with tumor necrosis factor alpha (TNF-?) was estimated.
Results: CL max f MLP stimulated without preactivation was significantly lower in group I and II than in group III ($p < 0.04$, $p < 0.06$, respectively). CL max OZ stimulated was significantly higher in group I in comparison to group II ($p < 0.04$) and it tended to be higher in group I than in group III. CL max PMA stimulated was lower in group I compared to group III ($p < 0.08$). CL total fMLP stimulated was lower in group II ($p < 0.07$) compared to group I and it tended to be lower in group I and II compared to group III. CL total OZ stimulated was significantly higher in group I than in group II ($p < 0.05$) and group III ($p < 0.03$). CL max and CL total OZ stimulated and preactivated with TNFa was significantly lower in group I compared to group III ($p < 0.02$ and $p < 0.04$, respectively).
Summing up: In the course of chronic hepatitis C in children, both neutrophil preactivation and inhibition of neutrophil release of reactive oxygen species, which occur as a result of different mechanisms, are observed.

R2215

One year combined treatment with ribavirine and intron A in children with chronic hepatitis C

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The aim of the study was to assess the effect of 12-month therapy with IFN-a and Ribavirine in children with chronic hepatitis C.
Material and methods: The study comprised 22 children treated with Intron A administered subcutaneously at a dose of 3 M.U. Three times a week and Ribavirine orally 15 mg/kg body weight daily forthe period of 12 months. In the course of the treatment, ALT activity, haemoglobin, leucocyte and thrombocyte were determined. HCV-RNA in serum was performed at the beginning, after 6 month and after the therapy.
Results: After 12 months of combined treatment with IFN-alpha and Ribavirine the elimination of HCV-RNA and normalisation of ALT activity were observed in 14 children. In 2 children the normalisation of ALT activity without elimination of HCV-RNA was observed. Six children did not respond to 12-month combined therapy. A lower mean activity of ALT at the beginning of the treatment was observed in children who fully responded to the combined therapy as compared to those who did not respond to the treatment. The following side effects were found in all the treated children: fever, headache, abdominal pain loss appetite, hair loss, myalgia and hypersomnia. In 12 children transient leucopenia, in 6 thrombocytopenia and in 5 anaemia were detected.
Conclusions: 1. Full response to the combined therapy (HCV RNA elimination and ALT normalisation) was obtained in about 64% of the treated children.
2. Numerous side effects observed in the course of the combined therapy did not cause the interruption of the treatment.

Herpes virus

R2216

Human herpesvirus 8 and associated neoplasms

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Objective: Human herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi sarcoma. HHV-8 has been associated to other diseases such as primary effusion lymphoma, Castleman disease, bone marrow failure and oral cavity plasmablastic lymphomas (PBL) in HIV+. Epstein-Barr virus (EBV) is known as a cofactor in AIDS-related lymphomas. We report the case of an HIV+ male which presented with two HHV-8 related neoplasms: a colonic PBL and a sigmoid Kaposi sarcoma. To our knowledge this is the first case of colonic PBL in the literature, as well as the first case where these two malignancies are associated.
Case: A 41-year-old male had a 3-month history of liquid diarrhoea, fever and malaise. He was known HIV+ for 11 years and had not previously received HAART. Physical examination was normal except for a facial lipoatrophy and small cervical lymphoadenopathies (<1 cm). Hemoglobin 9.9 g/dl, WBC 5030/mm^3, platelets 227000/mm^3, ESR 29 mm/h, creatinine 0.89 mg/dl, GOT 61 U/l, GPT 55 U/l, LDH 545 U/l, beta-2-microglobuline 3.58 mcg/ml, CRP 4.82 mg/dl; CD4 135/mcl, HIV-1 viral load 302,000 copies/ml. PCR for EBV in the cerebrospinal fluid was positive. A colonoscopy showed a purplish lesion in the sigmoid colon and an ulcerated mass in the caecum; both were biopsied. The first one was informed as a Kaposi sarcoma and the second one as a PBL. A bone marrow biopsy showed lymphomatous infiltration. D4T, 3TC and lopinavir/ritonavir was started, as well as CHOP chemotherapy. The latter was stopped after the third course due to progressive deterioration. The patient developed a palsy of the III and VII cranial nerves. Opportunistic infections were ruled out and a spinal tap revealed lymphomatous meningeal involvement. He died 6 months after the initial referral.

Conclusion: HHV-8 is involved in the pathogenesis of both malignancies, Kaposi sarcoma and PBL. Nevertheless, neither this association nor the peculiar localization of the PBL (right colon) have been described. Only few other extra-oral sites have been found: nose, lung, mediastinum, stomach, testicle, bone and ano-rectum. Diagnostic procedures with regard to HHV-8 infection are not routinely performed. Repeated detection of HHV-8 has been described to increase the risk of active Kaposi sarcoma. We hypothesize that extra-oral PBL might also be related to HHV-8 as a cofactor, similar to EBV. We suggest that a better understanding of the mechanisms of the infection could provide prevention and treatment strategies.

R2217

Epstein-Barr virus infection in patients with gastric carcinoma

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Objective: The studies aimed at evaluation of serological markers of Epstein-Barr virus (EBV) infection and presence of EBV DNA in the gastric carcinoma (GC) material.
Methods: The studies were performed on 21 patients, aged 42–68 years, in whom advanced GC was diagnosed by histological methods. Sera of the patients were tested for EBV DNA (Sharp Signal System; Digene), IgG-anti-EA antibodies (ETI-EA-G; DiaSorin), antibodies directed to VCA (ETI-VCA-M, ETI-VCA-G; DiaSorin) and IgG-anti-EBNA antibodies (ETI-EBNA-G; DiaSorin). In parallel, in the GC material EBV-encoded small RNA (EBER) particles were detected using in situ hybridization (ISH) and ISH Detection Kit (DakoCytomation).
Results: Basing on serological markers (serum IgG-anti-VCA and IgG-anti-EBNA antibodies), past EBV infection was documented in 20 patients. In one patient no anti-EBV antibodies were detected. In 12 of the patients positive reaction for EBER was documented in the nuclei of carcinoma cells, while 11 of them demonstrated presence of EBV DNA in the serum. The 9 remaining patients manifested neither positive signals in EBER-ISH nor presence of EBV DNA in the serum.
Conclusions: The obtained results point to involvement of EBV in development of GC confirming a multifactorial etiology of the disease.

R2218

The prevention and treatment of neonatal herpesviruses infections

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Objectives: In this report, the authors have presented clinical picture, course and therapy used in 13 infants with congenital VZV infection, 12 infants with congenital herpes simplex infection and 18 infants with CMV infection hospitalised at the Department of Paediatric Infectious Diseases in Brno within January 2000–November 2004.
Methods: The diagnosis of congenital HSV, VZV and 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 isolation of viruses from skin lesion, urine or occasionally from cerebrospinal fluid, detection of VZV, HSV and CMV DNA in the CSF, blood, or surface specimen, and/or by detection of viral antigen and by means of serological examinations.
Results: There were no death in HSV and VZV study group. 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).
Conclusion: The early diagnosis and management of congenital and perinatal infectious in neonate is important and this will depend on the clinical recognition of the condition. Unfortunately, early diagnosis is problematic. Many of these conditions have non-specific manifestations. As most maternal infections are asymptomatic, repeated serological screening of all susceptibile seronegative women would be required throughout pregnancy. This difficulty exemplified by neonatal herpes infection when early diagnoses and treatment is essential to prevent possible adverse consequences. Early initiation of therapy is of outmost importance every case of herpesviruses infection should be treated as soon as possible. Antiviral therapies have dramatically improved survival rates.

R2219

Evaluation of herpes simplex virus-1 latency in trigeminal ganglia of ocular and systemic infected mice

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Objective: Experimental herpes simplex virus type 1 infection in mice parallels that which occurs in humans. During primary infections, viral replication at the surfaces mucosal is detectable for a 10- to 12-day period. Within 24 h post infection (p.i) HSV infections are common and produce not only a primary infection, but also latent and recurrent infections. Following primary infection of herpes simplex virus type-1, the virus gain access to the termini of sensory neurons, transported in retrograde direction to the neuron cell bodies in sensory ganglia, replicates, spreads to other neurons, and establishes a lifelong latent infection in a portion of the neurons.
Method: In this study we used PCR analysis for detection of HSV-1 latency in inoculated BALB/c mice. Thirty five to forty days after intraocular (IO) and intraperitoneal (IP) challenges in different groups, the surviving mice were euthanized, trigeminal ganglia (TG) were harvested, mouse TG was individually homogenized and cell pellet was used to detect viral DNA by PCR analysis .The TG pellet after washing with PBS, were suspended in Tris-EDTA containing .1%SDS and protienase K. After incubation in 55 for 5 h, viral DNA was extracted and PCR performed using two primers of thimidine kinase gene of HSV-1 that generated a 398 bp product.
Results: We show that the rate of virus latency in IO infected mice is significantly higher than IP infected mice.
Conclusion: Primary HSV infection of any of the 3 branches (ophthalmic, maxillary, mandibular) of cranial nerve V can lead to latent infection of nerve cells in the trigeminal ganglion then, after IO challenge we have more per cent of latency in comparison to IP challenge.

R2220

Evaluation of the antigenaemia assay in early diagnosis of active cytomegalovirus infection in hospitalised patients

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Objective: To evaluate the detection of Cytomegalovirus (HCMV) pp65 antigen and serologic response in patients with active HCMV infection.

Methods: We examined 36 hospitalized patients with suspect HCMV infections (8 children and 28 adults) and 25 blood donors as control group. HCMV pp65 antigen in blood leukocytes were detected and specific IgM and IgG antibodies in sera wera determined by DF and EIA, respectively. The number of blood leukocytes was >2000/ml.
Results: Active HCMV infection was diagnosed in22 adults and 6 children (77.7%) by pp65 antigen detection ,while in control group antigenaemia not found. In all cases IgM antibodies were positive in 31 patients (86.1%), IgG antibodies were positive . In 5 children (13.8%) was found seroconversion of IgG .In control group, Igm antibodies were negative, while in 18 of them (72%) igG antibodies were found positive. From the 6 children with active HCMV infection 2 had mononucleosis-like syndrome and 4 acute febrile infection. Adults in respectively from the 22 patients, 6 had mononucleosis-like syndrome, 4 acute febrile infection and 12 undergoing immunosuppressive therapy. The specificity and the sensitivity of antigenaemia assay for diagnosis active infection were 100% and 87.5%, respectively.
Conclusions: In this study the pp65 antigen assay seems to be simple, rapid, inexpensive test for early diagnosis of active HCMV infection.

R2221

Identification of two major human cytomegalovirus gB genotypes in Taiwan

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Objectives: Genotypic preference of human cytomegalovirus (HCMV) can be found in a certain geographic area. To determine the predominant CMV genotype(s) in Taiwan, an island located in the southeastern Asia, we performed this study.
Methods: We used restriction fragment length polymorphism (RFLP) analysis to determine the subtypes of the HCMV gB gene, which encodes a viral membrane glycoprotein. A total of 101 clinical HCMV isolates collected island-wide were tested to determine their genotypes.
Results: We found two major CMV gB genotypes, gB1 (52%) and gB3 (33%), in the paediatric patients. There were 9% of mixed gB types 1 and 3 and 6% of unknown types. None of them belonged to gB types 2 and 4. The reason that results in the significant difference of gB genotypic preference is unclear.
Conclusions: Two major HCMV gB genotypes, gB thpe 1 and 3, are found. RFLP may serve as a convenient method for genotypic analysis of CMV clinical isolates.

R2222

IgG response to herpes simplex virus type 2 gG and gD recombinant proteins among adults and children

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Objectives: Recent studies have shown that both HSV-2 glycoprotein D (gD2) and glycoprotein G (gG2) have high potential as diagnostic reagents for the reliable detection of the type-specific IgG. The purpose of this study was to evaluate specific IgG response to HSV-2 gG2 and gD2 among child and adult populations and to investigate the protein-specific pattern of low avidity IgG reactivity.
Methods: A total of 160 serum samples from adults (normal blood donors) and 55 serum samples from children (age range 2–3 years-old) were analysed by type-specific HSV2 IgG enzyme immunoassay (DS-EIA-ANTI-HSV2-IgG, Russia), based on recombinant HSV 2 gG2 (525–578aa) and gD2 (266–394 aa) proteins. All HSV-2 IgG positive samples were additionally tested for presence of IgG to gD2 (anti gD2) and gG2 (anti gG2) individually. The determination of IgG avidity was performed with 8M urea as a dissociative agent.
Results: 73 out of 160 (45.6%) serum samples from normal blood donors (adults) and 14 out of 55 (25.5%) serum samples from children were positive for HSV2 antibodies by EIA. Protein-specific distribution of IgG activity was significantly different in terms of both IgG level and IgG avidity for two investigated groups (Table 1, Table 2).

Table 1. Frequency of IgG antibodies to gD2 and gG2

HSV2-positive samples	Frequency of IgG antibodies to gD2 only	Frequency of IgG antibodies to gG2 only	Frequency of IgG antibodies to gG2 and gD2
Adults (n = 160)			
73	50 (68.5%)	5 (6.8%)	18 (24.7%)
Children 2–3 years (n = 55)			
14	9(64.3%)	3(21.4%)	2(14.3%)

Table 2. Frequency of low-activity antibodies to gD2 and gG2

HSV2-positive samples	Frequency of low avidity antibodies	Frequency of low avidity antibodies to gD2 only	Frequency of low avidity antibodies to gG2 only	Frequency of low avidity antibodies to gG2and gD2
Adults (n = 160)				
73	12 (16.4%)	11 (15%)	1 (1.4%)	0
Children 2–3 years (n = 55)				
14	6 (42.9%)	2 (14.3%)	3 (21.4%)	1 (7.1%)

Conclusion: Specific IgG response to HSV 2 gG2 and to gD2 differs among various age groups. Frequency of low-avidity antibodies in children significantly exceeds frequency of low-avidity antibodies in adults. Furthermore, low-avidity anti gG2 was found predominant in children, and low-avidity anti gD2 in adults.Serologic assay based on the only protein (gG2 or gD2) has a potential for false negative result. It may be especially important for primary HSV2 infection detection.

R2223

Qualitative DNA PCR for early detection of symptomatic CMV infection after renal transplant

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Objectives: One of the most perspective methods in CMV infection diagnostics is detection of viral DNA by the polymerase chain reaction PCR. It is very actual for early differential diagnostics of the arising acute rejection crisis and CMV clinical syndrome after transplantation. Treatment strategy of these clinical syndromes is different: in the crisis of acute rejection, immunosuppressive treatment is intensified, while on the onset of the CMV clinical syndrome-minimised with additional administration of specific antiviral treatment.
Methods: There was carried out a prospective laboratory-clinical study of patients after renal transplantation (duration – 3 months). Blood samples for the CMV serological tests were collected once every 10–14 days. In a retrospective way, for the tests by the DNA PCR there were selected 24 kidney recipients with manifesting clinical symptoms over the all first months following transplantation. By the repeated serological tests (ELISA, indirect immunofluorescence), for the patients with manifesting clinical symptoms there was found a diagnostic increase in antibody titres indicating active CMV infection.
Results: The CMV clinical syndrome manifesting in self by fever and other typical clinical signs was determined for 13 persons. In 11 cases the CMV infection was asymptomatic. A positive PCR

test was obtained for 12 recipients out of 13 with symptomatic CMV infection and only for 5 out of 11-with asymptomatic infection. A negative PCR test was obtained for 6 patients with the asymptomatic infection and only for 1 with the clinical manifestation of the CMV infection. Thus, using the qualitative PCR method when only a positive or negative result is obtained without estimation of the amount of viral nucleic acids, the symptomatic CMV infection with early onset may be identified in 92.3% of cases (sensitivity of methods), though a positive result in also obtained in 29.4% of cases of the asymptomatic CMV infection. Therefore, specificity of method in as low as 54.4%.

Conclusion: The positive prognostic value of the PCR test while seeking to have early detection of the symptomatic CMV infection requiring appropriate treatment is as low as 70.6%. Somewhat more valuable is the negative prognostic value of the PCR test-in the presence of a negative result, in 85.5% of cases there is no clinical manifestation of the CMV infection, and in such cases a specific antiviral treatment is not needed.

Emerging infectious diseases

R2224

Serological evidence of coinfection with *Borrelia burgdorferi* and agent of human granulocytic ehrlichiosis in forestry workers in north western Poland

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Objectives: Tick-borne diseases-Lyme disease (L.d.)and human granulocytic ehrlichiosis (HGE) can be a cause of high morbidity in some groups of population, especially in forestry workers, exposed for tick bites. Accordingly to some papers, coinfection with Borrelia burgdorferi (B.b.) and agent of HGE may alter the immune response and severity of L.d. The aim of this study was to assess prevalence of anti-B.b. and anti-HGE antibodies in serum of forestry workers in North Western Poland.

Methods: Eighty three samples of sera of forestry workers were tested to detect anti-B.b. and anti-HGE antibodies using ELISA (anti-B.b.) and IFA (anti-HGE) tests.IgG class of antibodies anti-HGE and IgG and IgM classes of antiborrelial antibodies were examined.

Results: Presence of anti-HGE IgG antibodies were confirmed in 15 tested serum samples (15/83, 18.1%). Anti-B.b.antibodies were found in 13/83 samples (15.7%). Among 13 patients infected with B.b. 10 (77%) had IgG class of antiborrelial antibodies and 3 (23%) had IgM class antibodies. In analysed group there were only 2 serum samples with presence of both anti-B.b. and anti-HGE antibodies (2/83, 2.4%)

Conclusions: 1. Conducted study revealed presence of infection with B.b. and agent of HGE among examined forestry workers in North Western Poland.

2. Serological study confirmed possibility of coinfection with these two pathogens.

3. Patients with L.d.should be tested to detect potential coinfection with HGE.

R2225

Brucellosis in municipality of Kërçovë during 1983–2000

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Objective: To present clinical features and serological data of brucellosis in the municipal of Kercove, Republic of Macedonia.

Methods: patients with brucellosis followed between January 1983–December 2000 were retrospectively evaluated. Initial evaluation included complete blood count urinalysis and serological tests (standard tube Brucella agglutination test-STA). Diagnosis of brucellosis was made by following criteria: (a) history of exposure, (b) compatible clinical picture of disease, (c) brucella serum agglutination titre more then 1/160. The method of work is statistical method.

Results: In the above mentioned period were registered 133 cases with brucellosis, 28 of which are female and 105 male. The average age was 41 ± 30 years. The most frequent features in the patients disease of acute Brucellosis are: fever 92 (69.17%); joints pain (mostly in the spine and in the hips) 107 (80.45%); sweat 91 (68.42%); loss of appetite 60 (45.11%); headache 68 (51.12%); hepatosplenomegaly 14 (10.52%). In the diagnosis of human brucellosis the best results were shown with the Rose Bengal test, which was positive 100% of patients with acute brucellosis, then with the Coombs test positive in 96.99%. The test by Wright with 93.23%.

Conclusion: Brucellosis remain a major zoonosis worldwide. The most frequent features in the patients disease of acute Brucellosis are: joint pain(80.45%), fever (69.17%), sweat (68.42%). In the diagnosis of human brucellosis the best results were shown with the Rose Bengal test, which was positive 100% of patients with acute brucellosis.

R2226

Prevalence of antibody against varicella zoster virus in Iran

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Objectives: Varicella zoster virus is a highly contagious virus that affects people worldwide. The seroepidemiology of infection due to varicella-zoster (VZV) was investigated in 426 Iranian children aged from under 2 to 14 years and adults (healthy male blood donors and pregnant women).

Methods: The enzyme linked immunosorbent assay (ELISA) method was used to assess the presence of anti-VZV antibody.

Results: Age-specific prevalence of IgG antibodies VZV showed a progressive increase with age in both males and females with no obvious sex-related variation in the level. The overall prevalence of antibodies was 51% for VZV in children whereas about 88% of the adults.

Conclusion: These data show that almost 50% of the children in Iran have not been infected with VZV. The differences in age-specific prevalence of varicella may be due to the climate that may also decrease the survival of virus in tropical regions.

R2227

Risk factors of Crimean-Congo haemorrhagic fever outbreak in Central Anatolia: a case control study

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Objectives: An outbreak of CCHF evolved through 2002–2003 in Central Anatolia in Turkey. To demonstrate the risk factors and potential sources or dissemination vehicles of this outbreak a case control study in the region was undertaken.

Methods: Cases were the patients those admitted to tertiary care hospitals in 2003 with an illness consistent with CCHF and a positive IgM or RT-PCR test result. Two controls per case were selected randomly among those living in the same villages but not experienced a similar illness during the last 3 years. As being potential confounding parameters, age (+10 years) and gender were controlled. A questionnaire was filled by home visits at the region while a blood sample was obtained for serologic screening of specific IgG and IgM antibodies.Statistical analysis was performed by the software NCSS (Number Cruncher Statistical Systems, ver. 2004). Dichotomous variables were compared by Chi-square or Fisher's Exact Test where applicable. Statistical comparisons were always two tailed.
Results: A total of 62 cases were reachable. Accordingly, 124 controls were selected. Among the risk factors compared only 'tick exposure before the unset of disease' was significant (OR, 13.33; CIs, 6.39–27.81). Cases those experienced ticks bites mostly described domestic animals and rural areas out of villages for tick exposure (data not shown). Serologic screening between cases and controls were significant, as well. These comparisons and demographic characteristics, such as age and gender were shown in Table 1.

Table 1. Some demographic features and one significant risk factor (tick bite) and serologic torts between cases and controls

	Cases (n=52)	Controls (n=124)	*P*
Age (mean [SD])	42.4 (17.0)	43.9 (16.9)	
Male sex	29 (46.8)	58 (46.8)	
Animal keeping	56 (90.3)	99 (79.8)	0.0943
Tick bite	43 (59.4)	13 (14.5)	<0.001[a]
IgG	59 (96.7)	15 (12.1)	<0.001[b]
IgM	47 (78.3)	3 (2.4)	<0.001

Discussion: This study demonstrated that tick bite was the single significant risk factor responsible for this outbreak. Animal keeping, buying and selling is widespread in the region (83.3%). Domestic animals could be a source for tick exposure. However, some cases described certain areas out of villages for thick exposure, as well. One important point was that, cases were mostly scattered. Except few examples, they were mostly from different houses. If the live stocks are the source of infected ticks we would found clusters from same houses. The significant difference in the serologic survey between cases and controls supported this view as well. This study, in other words, indicated that rural areas with a heavy tick infestation must be the most likely source of this outbreak. However, more studies in the region are warranted to explore the source of this outbreak and eventually to control it.

R2228

High prevalence of *Helicobacter pylori* infection in Tunisia

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Objectives: *Helicobacter pylori* (HP) infection is widespread in developing countries. We performed a survey to evaluate the prevalence of this infection in a Tunisian population including all age brackets.
Methods: During February and Mars 2002, patients (414) referred to our hospital laboratory for blood tests were asked to reply to a questionnaire and give a blood sample for a test measuring out the anti-HP antibodies (anti-HP EIA QUANT, Cobas Core II, Roche).234 asymptomatic volunteer blood donors (AVBD) were also included (mean age: 22 yr).
Results: 414 patients were included (52 % male) with mean age of 31 yr (extr: 1–80).94% of these patients were of average social standing. 9.2% were complaining of dyspepsia and 14.2% reported symptoms of dyspepsia in the household.The seroprevalence of HP antibodies was 9% in the young children (1–5 yr), 40% in the older children (6–15 yr) and 60% in patients between 16 and 20 yr of age. In patients older than 20 yr, seroprevalence was 88% (279/317) comparable to AVBD: 86.3% (202/243). Except for the age, there was no other factor (gender, origin, social standing, dyspeptic symptoms) influencing the HP infection seroprevalence.
Conclusions: Seroprevalence of HP infection is high (79.3%) in our mainly asymptomatic population. Infection increases during childhood and adolescence, reaching 88% in adults. This high prevalence raises the question of whether we should continue to systematically look for HP infection in patients with upper gastroenterological disorders.

R2229

A case of acute melioidosis in a traveller

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Burkkolderia pseudomallei is the causative agent of melioidosis and a potential biological weapon. This anthropozoonosis is endemic in regions of southeast Asia and northern Australia and sporadic in Europe. Bacteraemia in melioidosis is associated with high mortality.We report a case of acute melioidosis in a traveler. A 52-year-old man was admitted to hospital with fever, asthenia and vomiting. He had recently traveled in Bangladesh and Vietnam. He had a documented non-insulino-dependent diabetes. On clinical examination he presented fever and abdominal pain. The diagnostic of malaria was excluded after laboratory investigation. CT abdominal scan showed hepatic and splenic abscesses and the diagnosis of visceral amebiasis was discussed although amebic serology was negative. Of four blood-culture bottles, one aerobic bottle was positive with Gram-negative bacilli identified as Burkholderia cepacia by Vitek 2 (very good identification, T-index 0.66). In regard to the doubtful diagnosis of visceral amebiasis and the unusual susceptibility of the isolate to imipenem, a new identification was made by 16S RNAr sequencing and API20-NE that identified a Burkholderia pseudomallei. An appropriate antimicrobial treatment was then prescribed for a total duration of 12 weeks (ceftazidime i.v and association of trimethoprim-sulfamethoxazol/doxycycline). Clinical outcome was favorable.This case emphasizes: 1/the possibility of misidentification of Burkholderia pseudomallei in laboratories that are unfamiliar with this microorganism; 2/the importance of an acute diagnosis of melioidosis to treat correctly this severe disease and to prevent relapses; 3/the necessity to consider the diagnosis of melioidosis in travelers.

R2230

Acute gastroenteritis caused by *Aeromonas jandaei* – importance of identification to the species level

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Objectives: Aeromonads have recently been accounted into a new emergent pathogens. They can cause wound infection or septicaemia as well as gastroenteritis. Until now, the epidemiology is not known too much and lately described species still appear. According to our experience the identification of clinical

isolates of mesophilic aeromonads only as *A. hydrophila*, *A. caviae*, or *A. sobria* complex is no longer accurate. With proper identification system even so-called rare species can occur in clinical samples.
Methods: 160 isolates of aeromonads originated mostly in stool samples. Biochemical identification was done by ENTEROtest24 (Pliva-Lachema), results were assumed by software TNW for oxidase positive fermenting rods. Cellular fatty acid analysis was performed by MIS Sherlock (MIDI, Inc., USA).
Results: Genus specific tests such as resistance to vibriostatic agent O/129 (150 μg) and growth in broth culture with addition of NaCl (0%,1%,6%) confirmed, that ENTEROtest24 ensured good distinction of genera Vibrio and Aeromonas. In combination with TNW software ENTEROtest24 was useful for identification of common aeromonads. Nevertheless, due to permanent changes in taxonomy the supplementary conventional biochemical tests are recommended. Routine species identification of Aeromonas isolates by biochemical testing and fatty acid analysis proved the presence of species from complexes *A. hydrophila* (*A. hydrophila*, *A. bestiarum*), *A. caviae* (*A. caviae*, *A. eucrenophila*, *A. media*) and *A. sobria* (*A. veronii*, *A. sobria*). Beside these common ones we noticed also *Aeromonas jandaei* isolate from stool sample. Diagnosis of acute gastroenteritis was evident (vomiting, watery dirrhoea. Isolate was identified by ENTEROtest24 as excellent (T index 0.882, ID 91.39%) and comparison of cellular fatty acid profile confirmed the identification. The same major and also minor fatty acids as by A. jandaei CCM 4355T (C16:1w7c alcohol, C16:0N alcohol, C17:0 10 methyl) were presented by our isolate.
Conclusion: There are enough aeromonads in clinical samples and there are also less frequent Aeromonas species occuring in our region. Use of an appropriate identification system can easily warn microbiologist of a rare species. Only proper identification of each isolate will provide us with information, whether all described mesophilic Aeromonas species are involved in diarrhoeal disease.Our project is being supported by IGA of Ministry of Health of the Czech Republic, Id.code: NR/8011-2.

R2231

Q-fever febrile illness and pneumonia in a rural town in the Balkans

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Objectives: In the spring of 2004 an increase was noted inpatients with acute fever and pneumonia in a small rural town in the Balcans. The diagnosis Q fever was established and the epidemics was limited. 51% of the outbreak cohort had specific antibody titres to phase II C.burnetii antigen >1:64, that confirmed a recent infection. The objectives of our study were to establish the clinical, epidemiological and laboratory parameters of Q fever in this part of Bulgaria and to draw conclusions concerning the early diagnosis, treatment and prevention of the infection in the endemic regions.
Methods: To establish the diagnosis of Q-fever all patients had a complete physical examination, epidemiological data were collected. Atypical pneumonia was documented. Seroconvertion was considered in patients demonstrating titres of antibody (IgG > 200 and IgM > 50) to phase II C.burnetii.
Results: 51% of seropositive cases presented acute self-limited illness, 39%-atypical pneumonia. Common clinical symptoms were found and appropriate antibiotic regiments indicated.
Conclusions: The apparent reason for the outbreak was the inhalation of infected aerosols. The large number of infected domestic animals in the town may have been the cause. The character of flue-like illnesses and pneumonias during May implies a point source. The early diagnosis of Q fever in risk regions can be helped by epidemiological data on morbidity due to influenza-like illnesses and atypical pneumonia. In such conditions, physicians must treat with appropriate antibiotics before serological confirmation of the diagnosis of Q fever. The public health, veterinary and municipal authorities must work together to educate the population about the basic principles of Q fever prevention which includes restricting contact between people and cattle and improving infection control in the places where animals are bred.
Discussion: The apparent reason for the outbreak of atypical pneumonia due to *C. burnetii* was the inhalation of infected aerosols.The large number of infected domestic animals in the town may have been the cause. The early diagnosis of Q fever in risk regions can be helped by epidemiological data on morbidity due to influenza-like illnesses and atypical pneumonia. In such conditions, physicians must treat with appropriate antibiotics before serological confirmation of the diagnosis of Q fever.

Vaccines

R2232

Production, extraction and evaluation of *Neisseria meningitidis* serogroup B outer membrane vesicle containing PorA

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Capsular polysaccharides from *Neisseria meningitids* groups A, C, W135 and Y induce protective bactericidal antibodies in humans, having been used as vaccines for almost two decades. In contrast, group B capsular polysaccharide vaccines have proven to be essentially nonimmunogenic and for this reason's serogroup B is presently the major cause of meningococcal disease in most developing countries and this, combined with the lack of immunogenicity of the B polysaccharide, has necessitated development of alternative vaccines based upon the outer membrane proteins and lipooligosaccharides. Major surface components, such as the outer membrane porin PorA (class 1 protein), are nowadays considered as potential vaccine candidates. Sergroup B *N. menigitidis* strains CSBPI, G245 obtained from the Collection of Standard Bacteria Pasteure Institute of Iran for farther investigation the cultivation medium was meningococci medium according to Frantz, with main compotent: L-glutamic acid, L-cystein, glucose and yeast extract. Cell's were cultivated at 36oC for 18h.Outer membrane vesicle-containing class1(PorA) were prepared and purified according to the Deoxycholate Ultracentrifuge-Differentiation Technique (DUDT) of fermenter grown bacteria. Briefly *N. meningitides* CSBPI, G245 Was cultivated in Frantz medium supplemented with 0.2% yeast extract dialysate in 40 l fermenter contact-Flow b.v. Bilthoven unit system until early stationary phase and OMV-PorA extract by tris-buffer, EDTA and 0.5% w/v

deoxycholate. Purification was done by sequential centrifugation steps at 20,000 g for 30 min. Following ultracentrifugation at 125,000 g for 2 h, the pelleted OMV-PorA were homogenized in PBS. OMV-PorA were ultrasonically treated to disperse the vesicles and were attached to Formvar/Carbon coated Nickel Grids. After preparation step's, the Grids were examined in a Zisse CEM 902A electron microscope. In negative contrast staining and electron microscopy size of OMV-ProA ranged 50–150 nm. Intactness of vesicles in these preparation ranged 70–90% of the vesicle.Protein concentration was measured according to method of lowry et al. The samples were analysed on 10% poly acrylamide gels in the presence of 2% (w/v) SDS. After electrophoresis protein were stained by 0.1% (w/v) coomassie Brilliant Blue staining and the relative amount of OMV-PorA were determined. The results of lowry method was 6 mg protein per 1 ml of sample. When analysed on SDS-PAGE can be separated and their MW can be calculated.

R2233

Immunological evaluation of OMP-F vesicle of native Iranian *Pseudomonas aeruginosa* CSBPI: 16-190 as a protective vaccine in mice model

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300 *Pseudomonas aeruginosa* strains were isolated from patients admitted in four Tehran hospitals. Using standard O-specific typing sera, they were all grouped in 16 serotypes out of 17 known *Pseudomonas aeruginosa*. The serotypes were lyophilized and each was given a code according to the Collection of Standard Bacteria Pasteur Institute of Iran (CSBPI) for farther investigations. Among all clinical samples, CSBPI: 16-190 was the most prevalent *Pseudomonas aeruginosa* serotype which showed a high agglutination titre (4+, 320) against homologous O-specific typing sera. This serotype was selected for extraction of *Pseudomonas aeruginosa* major outer membrane vesicles (OMP-F). OMP-F vesicles were extracted and purified according to the Deoxycholate Ultracentrifuge-Differentiation Technique. Purity and molecular weight of OMP-F was determined by SDS-PAGE and ability of OMP-F vesicles to induce high titre of antibody in rabbit was shown as a sharp antibody-antigen precipitation line in gel double diffusion test. Passive immunization of mice with anti-rabbit OMP-F antisera, induced a high level of protection when the mice were post-challenged with 2×LD50 of live *Pseudomonas aeruginosa* CSBPI: 16-190. Besides, active immunization of mice with 50 μg of OMP-F, could protect mice against 2×LD50 of live homologous (100% protection) and 15 heterologous native Iranian *Pseudomonas aeruginosa* serotypes with 50–100% level of protection. OMP-F was Pyrogen-free in rabbit pyrogenic test and did not produce any detectable abnormal toxicity in mice and guinea pigs. Moreover, OMP-F did not show any skin sensitization when 25, 50, 100, 250 and 500 μg were injected intradermally.The above investigations indicate that purified OMP-F of CSBPI: 16-190 can be regarded as a safe protective immunogen in vaccinothrapy against all *Pseudomonas* aeruginosa immunotype isolated in Iran.

R2234

Expression and purification of *Herpes simplex* virus type 2 glycoprotein D in insect cells using the Baculovirus expression system

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Objectives: *Herpes simplex* virus type 2 (HSV-2) infects mucocutaneously and causes genital infection in humans, followed by the establishment of a latent infection in the sensory ganglia and can reactivate for life. A need for effective vaccine remains the preferred strategy for control of HSV-2 infection. Glycoprotein D (gD) of HSV has been focused on as subunit vaccine due to its primary role in inducing cellular and humoral immune responses to a herpes infection. In this study, HSV-2 gD was expressed in insect cells using the Bac-to-Bac Baculovirus Expression System in order to apply as a subunit vaccine in animal models.
Methods: The HSV-2 gD of an Iranian isolate was amplified by PCR and cloned into pFastBac plasmid. The recombinant plasmid was transformed into *E. coli* DH10Bac cells containing bacmid, a baculovirus shuttle vector, for the site-specific transposition of the gD2 gene. The recombinant bacmid was confirmed by PCR, transfected into Sf9 cells, cultured in serum-free Grace's medium at 27 °C and monitored daily for observation of cytopathic effects. After 72 h, the baculovirus was harvested from the cell culture medium. The viral stock was amplified by reinfecting insect cells and titrated as pfu. The Sf9 cells were infected with gD2-baculovirus and incubated at 27 ordm;C for various times to express the recombinant protein of interest. The cells were pelleted by centrifugation, lysed and subjected to SDS-PAGE before and after purification. The gels were analysed by western blotting using a monoclonal anti-gD2 antibody.
Results: The gD gene of HSV-2 was cloned after confirming by sequencing. The protein production in insect cells was shown by western blot analysis. The results revealed that Glycosylated and nonglycosylated forms of gD2 were expressed in insect cells as cell-associated proteins by 72 hours post infection.
Conclusion: Baculovirus expression system provides correct folding of recombinant protein as well as disulfide bond formation, oligomerization and other important post-translational modification. Consequently, the expressed protein exhibits the proper biological activity and function. The pure gD2 can be used as a subunit vaccine in animal models.

R2235

Seroprevalence of Rubella antibodies among young population in Tehran, Iran

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Objectives: Congenital rubella is a preventable disease, which has largely been controlled through immunization. The aim of this study is to examine the prevalence of antibodies against rubella in Iran, before and after the largest mass campaign for measles and rubella (MR) vaccination of 33,000,000 people with an age rang of 5–25 years-old in 2004.
Methods: A total of 400 serum samples before and after vaccination were collected and assayed using hemagglutination inhibition test (HIT). HIT considered as a reliable index of the immunity level to RV infection.
Results: The data revealed that 95% of target population becomes seropositive after vaccination, while 94.25% of them had rubella antibodies before MR vaccination.
Conclusion: Rubella is an infectious viral disease, which is endemic in Iran. The more detailed epidemiological studies are still needed to perform a national immunization programme. Based on the results, rubella virus is circulating among population in Iran, so it is recommended to selective vaccination of girls near the age of marriage.

R2236

Analysis of anti-flu vaccinations among patients of a family practice: four-year observation

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Objectives: Estimation of anti-flu vaccinations, accomplishment in chosen groups of patients.
Material and methods: Retrospective analysis included case records of FP patients vaccinated agains the flu in 2000–2003. Basing on WHO recommendations assessment involved: (1) patients over the age of 50, (2) patients over the age of 65, (3) diabetic patients, (4) patients suffering from chronic, obturative respiratory tract diseases, (5) children and adolescent patients under the age of 18, and (6) the other parients over the age of 18.
Results: Among 3430 patients of FP over the age of 50, 81 (i.e. 2.29%) were vaccinated in the year 2000. In the following years: 3.38%/4.97%/4.17%, respectively. In 2000, 39 out of 959 patients over the age of 65 i.e 2.76% were vaccinated. In 2001–2003 this percentage was: 7.71%/12.06%/12.48%. Among diabetic patients, in 2000–2003 were vaccinated: 5.3%/9.6%/11.38%/ 12.83%. Patients suffering from asthma and chronic, obturative respiratory tract diseases were vaccinated in:4.8%/8.02%/ 8.78%/7.91%. In 2000, among 1553 patients under the age of 18, 42 were vaccinated i.e 2.7%. In 2001/2002/2003 this percentage was: 2.42%/4.11%/2.60%. Among patients over the age of 18, in whom anti-flu vaccination is not recommended, percentage of vaccinated patients was: 3.11%/1.85%/3.23%/ 2.27%. Among vaccinated agains the flu at least three times during 2000–2003, we vaccinated: 22% of patients over the age of 50, 59% over the age of 65, 67% of diabetic patients, 90% of those suffering from astma, 32% of patients under the age of 18 and 28% over the age of 18%.
Conclusion: Hitherto existing system of anti-flu vaccination promotion is not efficient enough, despite the advertisements in media and information in FP.

R2237

Comparison of two types of recombinant live-attenuated *Salmonella* strains expressing HBc-ag in experiments

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Objectives: One of the method of approach for chronic hepatitis B treatment is activation of T-cell immunity. For this purpose therapeutic vaccine containing HBc-ag can be used. Live-attenuated *Salmonella* are able to deliver both HBc-ag, encoded in prokaryotically expressed plasmid, and HBc-ag, encoded in eukaryotically expressed plasmid. Therefore such mentioned above two types of recombinant *Salmonella* strains were examined in experiments in mice and cell culture of human monocytes in vitro.
Methods: Attenuated cya (lack adenylatecyclase) suppressor (CRP) mutant of Salmonella Enteritidis was used as a vechicle for both HBc-ag encoded in prokaryotically expressed plasmid pKHBc (vector's vaccine), and HBc-ag encoded in eukaryotically expressed plasmid pcDNA3.1pKHBc (DNA-vaccine carried in Salmonella). Mice were inoculated per rectum with suppositories containing 1,000,000 cells of examined Salmonella strains. Salmonella multiplication in Payer's patches, spleen, liver, lungs, and determination the titre of specific anti – HBc-ag antibodies were examined. Culture of human monocytes in vitro was used for examination of interaction Salmonella strains with phagocytes.
Results: All strains possessed ability to multiply in Payer's patches more then 7 days. In contrast to other Salmonella strains, strain carrying pcDNA3.1pKHBc (DNA-vaccine in Salmonella) did not possessed ability to multiply in parenteral lymphoid tissue. It did not also induce production of specific anti HBC-ag antibodies. Examination of interaction Salmonella strains with culture of human monocytes in vitro was shown that strains free from HBc-ag synthesis possessed ability to multiply in phagocytes. The number of Salmonella cells reached 40 cells per phagocyte. Synthesis of HBc-ag limited Salmonella multiplication. Effect was more expressed in Salmonella carrying DNA-vaccine.
Conclusion: Salmonella strain carrying DNA-vaccine expressing HBc-ag , possesses limited persistence in mice. HBc-ag increases bactericidal activity of phagocytes.

R2238

Humoral response to *Staphylococcus aureus* polypeptides in patients treated with autovaccine

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Objectives: The studies aimed at evaluation of anti-*S. aureus* humoral response in patients treated with autovaccine, prepared from killed *S. aureus* strains isolated from the patients.
Methods: The studies were performed on 15 strains of *S. aureus* isolated from patients with chronic inflammatory processes in the throat and/or skin and demonstrated to be of staphylococcal aetiology. Surface proteins were isolated by Laemmli buffer and were electrophoretically separated (SDS-PAGE), stained with Coomassie blue or transferred onto nitrocellulose membrane for immunoblotting with patient sera, obtained in two time points, i.e. before and after applying the autovaccine. The antigen/ antibody reactions were visualized employing the alkaline phosphatase method. Protein profiles for individual *S. aureus* strains and immunobloting results were analysed using GelD-OK software (Syngen Biotech).
Results: Using Western-immunoblotting, the IgG lass antibodies were fund to react to *S. aureus* surface proteins of 14–140 kDa molecular weight while IgM class antibodies reacted with proteins of molecular weight of 25–94 kDa. In studies on the autovaccines IgM antibodies were detected directed toward individual polypeptides in 12 patients while no IgM class antibodies could be detected in the remaining 3 patients. In the subsequent time point of the study (after administration of autovaccine), in 12 patients IgM class immune response was found to resemble that documented before while the remaining, previously negative 3 patients this time carried IgM antibodies specific toward individual polypeptides. As far as IgG class humoral reaction was concerned, the response was comparable in the two time points of the study.
Conclusion: The obtained results suggest that therapeutic effect of *S. aureus* autovaccine is not related to the respective humoral response.

Infection in transplant recipients

R2239

Bacterial and fungical colonisation of the bronchial tree in outpatient lung donors. An advance in lung transplantation

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Objectives: Out hospital non-heart-beating lung donors (NHBLD) may be advantageous in terms of microbiological colonization of the bronchial tree because of limited ventilation time and no ICU environment exposure.
Methods: Microbiological results of 8 out hospital NHBLD and 31 brain-dead lung donors (BDLD) performed in the same period of time (november 2002–may 2004) were systematically reviewed in three categories: donor's bronchial swabs and bronchial aspirates (BAS) and receptor's BAS prior and after transplantation. Any microbiological isolated microorganism was considered significant excluded upper airways flora.
Results: Only 1 out of 8 (12.5%) cultures of the out hospital NHDLD a significant microorganism could be isolated. In the case of the BDLD 17 cultures out 31 donors (54.8%) were positive for significant colonization. Colonization was statistically different in both groups ($p = 0.049$).Respect to the recipient's results, in 5 of the 8 NHBLD (62.5%) significant colonization was evidenced compared with the 8 of 31 (25.8%) microbiological isolates in the case of BDLD receptors. No statistically significative differences could be found in recipients colonization ($p = 0.09$). BAS after transplantation was microbiologically significant in 5 of the 8 (62.5%) cases of transplantations from a NHBLD. In the case of the transplantations from BDLDs 19 of 31 (61.2%) presented a significant isolate.In the case of BDLD transplantation a statistically significative ($p = 0.012$) increase in the colonization after transplantation was found due to BDLDs (from 25.8% to 61.2%) in contrast with NHBLD transplantation in which NHBLD did not increase the proportion of significant cultures (62.5% in both cases).
Conclusions: According to our series NHBLD are statistically less colonized than BDLD. A difference in the proportion of colonization in out hospital NHBLD in the era of prophylactic antibiotherapy could be adventageous in terms of decreasing infections after transplantation, using less prophylactic antibiotics, and avoiding the appearance of antibiotic resistant microorganisms.

R2240

PTLD following allogeneic HSCT: a single-centre experience in Korea

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Objectives: Post-transplant lymphoproliferative diseases (PTLD) is a well recognized complication after HSCT. However, it has been difficult to characterize because the incidence is <1% and clinical presentation is diverse. Incidence and characterisitcs of PTLD after HSCT have not been reported in Korea. This study is aimed to evlauate and characterize PTLD after HSCT in a single institution.
Methods: Medical records of adult patients who underwent allogeneic HSCT in the Catholic HSCT centre from in January 1993–December 2003 were retrospectively analysed.
Results: There were 7 patients who had PTLD for 10 years. The mean age was 33 ± 11.6 years, male to female ratio was 6–1. The underlying hematologic diseases were 3 chronic myeloid leukaemia, 2 acute myelocytic leukaemia, 1 myelodysplasic syndrome, 1 severe aplastic anemia. The types of HSCT were 4 of matched sibling donor (MSD) HSCT, 2 of matched unrelated donor (MUD) HSCT, and 1 of haploidenticl HSCT. Five of these patients (71.4%) experienced episodes of acute GVHD and received steroid therapy. PTLD developed at mean 145.3 days (range, 83 ∼ 244) after HSCT. Clinical symptoms were diverse depending on the involved sites, but cervical lymphadenopathy was frequently observed (5 out of 7). Theincidence of PTLD has been increasing: no proven cases before 1996, 1 case in 1996, 3 in 2001, 1 in 2002, 2 in 2003. The pathology review showed that 6 cases had B-cell origin PTLD and one patient had T-cell origin, who had haploidentical HSCT and rapidly progressed to death. In situ hybridization for EBER was positve in 4 cases. Treatments of PTLD were 1 acyclovir therapy, 2 reduction of immunosuppressants plus acyclovir therapy, 2 reduction of immunosuppressants plus donor lymphocyte infusion (DLI), 2 reduction of immunosuppressants plus acyclovir therapy plus DLI. Five of seven patients (71.4%) reponded to the treatment. Three patients died, and 2 of them died patially or directly due to PTLD, which developed in 2003 in these cases.
Conclusion: The incidence of PTLD is low in allogeneic HSCT patients, but has been increasing recently probably because the rate of HLA mismatched HSCT or MUD HSCT, which needs more profound immunosuppression, has increased. Therefore, we should be careful of development of PTLD in patients after HSCT, who have risks of PTLD.

Paediatric infections

R2241

The prevelance of respiratory viruses in hospitalised children in Amman, Jordan

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Objectives: To detemine the prevelance, seasonal distribution and to draw out the clinical signs and symptoms associated with the following respiratory viruses:Respiratory syncytial virus(RSV),Human parainfluenza viruses (PIV) type 1,2 and 3,Influenza viruses A&B and Adenoviruses(Ad).
Methods: A total of 200 nasopharyngeal aspirates were obtained from hospitalized children (below 2 years old), admitted to Islamic hospital in Amman-Jordan, between september 2002 and March 2004. These specimens were analysed for the presence of some respiratory viruses using the direct and indirect immunoflouresence techniques.
Results: 27% of enrolled patients have proved viral respiratory infection. The etiological agents associated with these infections were RSV(12%), Ad.(11.5%) and PIV-3(3%). Seasonal distribution of respiratory viruses and their correlation with the climatic factors (temperature, rainfall, and humidity) were determined. The peak of RSV incidince was during February 2003 and January 2004 and showed a significant correlation with the climatic factors. Ad infections peaked during December of2002

and 2003, and PIV-3 infections restricted to march 2003 and January 2004.However,both Ad andPIV-3 did not show any significant correlation with the cilamatic factors.Males were affected more than females, and most infections were associated with lower age groups(less than 1 year). Croup (laryngotracheobronchitis) was only associated withHPIV-3,Bronchiolitis with RSV, bronchopneumonia, pharyngitis and tonsillitis were mostly associated with Ad infections. According to the clinical and radiological findings, RSV infection can be distinguished clinically fromPIV-3 and Ad infections by the higher rates of hypoxemia, retractions, tachypnoea, hyperinflation, and interstitial infiltrates.however.PIV-3and Ad infections cannot be distinguished on the same basis.

Conclusion: This study has demonstrated the prevelance of three othe major respiratory viruses infecting Jordanian children below the age of 2 years, and causing their hospitalzation. The study stated the seasonabilty of repiratoy viruses and their associted illnesses, as well as the major clinical manifestations associated with each virus. The knowledge of local climatic factors together with the characterstic pattern of these viruses may help in the prediction of the beginning and end of epidemics and thus also help in the planninig of preventive programmes.

R2242

Tuberculous meningitis in sucklings

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Objective: To study the tuberculous meningitis in sucklings, from the clinical , laboratory findings and therapy point of view.

Material and Method: retrospective study of tuberculous meningitis of 23 infants under 1 year of age, admitted in the Infectious Diseases Hospital of Iasi-Romania, in the period 2000–2004.

Results: The were identified in medium 29% new cases in children per year, comparatively with adults (14.8%). From these 23 cases,60% were boys and 26% of the group of age 1–4 months. The onset was incidious in 72,84% , with meningeal syndrome in 91% cases and conscience troubles in 8% cases. The CSF was clear in 95% cases, with high values of proteins and low values of glucose and chlorus. *M. tuberculosis* was isolated in culture in 28% cases and 14.66% by directly exam. The therapy was then with quadruple association of antituberculous drugs, with favourable evolution 65% cases, 8 deaths being recorded. The factors of bad prognoses still remain: the little age, the hydrocephalus (5 cases), malnutrition (3 cases), and coma from the beginning (2 cases).

Conclusion: *Tuberculous meningitis* still remain a disease with a high incidence and severe evolution in sucklings, despite the progress of diagnostic methods and therapy.

R2243

Clinical course of urinary tract infections in early infancy

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Objective: Urinary tract infections are the most common bacterial infections in infants. The aim of the study was the clinical analysis of infants younger than 60 days of age with urinary tract infection.

Methods: We reviewed the medical records of 54 infants younger than 60 days-old hospitalized with a urine tract infection at our hospital during a 2 year period. Diagnosis was confirmed by positive urine culture. All urine specimens obtained by suprapubic aspiration. Clinical presentation, laboratory data, management, imaging studies and outcome were reviewed.

Results: Forty six of patients were males (85%). The most common symptom was fever (65%). Other clinical manifestations were: weight loss (11%), vomiting (9.2%), failure to thrive (7.4%), diarrhoea (3.7%) and jaundice (3.7%). The most common pathogen was *Escherichia coli* (60%). Other isolated microorganisms included *Klebsiella pneumoniae* (24%), *Enterobacter cloacae* (11%) and *proteus* (5%). Abnormal urinalysis results were noted in 65% of infants. Leucocytosis, high C-reactive protein concentration and elevated erythrocyte sedimentation were noted in 45%, 58% and 43%, respectively. 24 infants had abnormal renal ultrasound results (46%). In all cases intravenous treatment with a combination of ampicillin or cephalosporin and aminoglycoside was efficient. Voiding cystourethrogram detected vesicoureteral reflux in 13 infants(24%). Renal scanning revealed scarring in 3.7%.

Conclusion: Early diagnosis and prompt management of urine tract infection in infants are very important to prevent serious complications and preserve renal function.

R2244

Colonisation of bronchial secretions in intubated patients in paediatric intensive care unit in Athens, Greece

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Endotracheal intubation is generally performed to facilitate the treatment of respiratory failure or to control the airways during shock, sepsis, status epilepticus or cranioencephalic trauma. Intubated patients present frequently colonization and infections of the lower respiratory track.

Aim: The aim of our study was to determine the frequency and etiology of infections or colonization of the lower respiratory tract in intubated children in intensive care unit.

Material and method: 22 intubated children (15 boys and 7 girls) were studied, aged from 2 months to 16 years-old, treated in I.C.U. during the year 2002. From the study were excluded the chronic cases with frequent re-admissions. The clinical and laboratory infection indexes were evaluated, such as culture of bronchial secretions, C.R.P., procalcitonine, blood culture, chest X-R, high fever and acrocyanosis.Bronchial secretions were taken for culture, by means of endotracheal suction the first day of intubation and after second day and finally the day of removal of the tube.The samples cultured quantitatively in blood, M.C., chocolate and Sabouraux agar, in aerobic and anaerobic conditions.

Results: From the 22 children, infection developed in 6 (26%). Infection appeared in the 3rd day of intubation, whereas colonization after the first 24 h.The bacteria isolated the first 24 h of intubation were mainly *H. influenzae* 9 srains (25%), *M. catarrhalis* 6 (17%) and *S. aureus* 5 (14%), whereas in 48 h predominated *S. aureus* with 10 strains (30%), *Pseudomona*s spp. 7 (21%), *A. baumanii* 4 (13%) and *Candida* spp. 4 (13%).

Conclusions: 1. Children intubated for more than three days are prone to infection. 2. Colonization of bacteria of the environment may happen the 2nd 24 h of intubation. 3. Children of I.C.U. colonized mainly by Gram-bacteria, *S. aureus* and *Candida* spp. 4. Knowledge of factors contributing to pulmonary infection in intubated patients is important for more effective treatment.

R2245

Nasopharyngeal colonisation in healthy children in Greece

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Colonization of nasopharynx by potential respiratory pathogens is established in early childhood, although this varies greatly according to locality, individual and social factors.

Aim: The aim of this study was to investigate the prevalence of the nasopharyngeal carriage of respiratory pathogens and identify factors affecting colonization in healthy children in an area of Athens inhabited mainly by ethnic minorities.

Material and method: From January 2003 up to May 2004 with exception of summer months, 392 nasopharyngeal swabs from equal in number healthy children were obtained. 38 of the children were under the age of 6 (group A) and the rest (354) from 6 to 10 years-old (group B). 45% of the above children either had not been or incompletely vaccinated against *H. Influenzae* type b (Hib). The samples were cultured in blood and chocolate agar for 48 h. Identification of the isolates and sensitivity to antibiotics was performed by disk diffusion method following the guidelines of NCCLS.

Results: Out of 392 swabs, 285 (73%) were found positive to one or more pathogens. From these in 74 (26%) were isolated 2 bacteria and in 8 (3%) three. The pathogens isolated were 307 (71%) *H. influenzae*, 38 (8,8%) *M. catarrhalis*, 24 (5,5%) *Candida* spp., 15 (3,5%) *S. pyogenes* (GAS), 8 (1,8%) *Neisseriae* spp., 4 (0,9%) *S. aureus*, 1 (0,2%) Streptococcus Group C and 36 (8,3%) Gram-bacteria. From the 38 group A children, 27 (59%) were found positive for H. influenzae, 6 (13%) for *M. catarrhalis*, 1 (2%) for GAS, 1 (2%) for *Candida* spp., 1 (2%) for *S. aureus* and 10 (22%) for Gram-bacteria. 9 swabs (24%) were positive for two pathogens and 1 (5%) for three. Significant resistance had H. influenzae to Cotrimo (31%), *M. catarrhalis* to Ampicillin (76%) and *S. aureus* to Penicillin (100%).

Conclusions: *H. influenzae* is the prevalent colonist in the nasopharynx of the examined children, possibly due to the fact that these had not been at all or partially vaccinated against Hib. Group A children had been colonized in higher frequency by Gram-bacteria. Rhinopharyngeal colonization by respiratory pathogens on these children might have a strong relationship with the socioeconomic level and the way of living of these populations. Therefore, antimicrobial restrictive guidelines should be tailored to local microbiologic sceneries.