Pathogenesis

R1885

Concurrent nasopharyngeal squamous cell carcinoma and lymphoproliferative disorder during HIV infection. Possible pathogenetic interactions among retroviral infection, Epstein-Barr virus infection, and inhalatory cocaine abuse

R. Manfredi, S. Sabbatani, U. Gianelli, F. Chiodo *(Bologna, Milan, IT)*

Introduction: Only two human cells types allow the replication of EBV: B-lymphocytes and squamous epithelium. Burkitt lymphoma recognizes a pathogenetic role for both EBV and multiple co-factors influenced by geographic issues, too. The nasopharyngeal carcinoma predominates in South-Eastern Asia where the exposure to some inhalants (i.e. phenoles) seems more frequent, but antibodies against the early EBV antigen VCA are commonly found, as well as the isolation of EBV genomic sequences from neoplastic cells. The staging of rhinopharyngeal carcinoma also includes a poorly differentiated variety with a prominent lymphoid infiltrate, which may raise the suspect of an extranodal lymphoma, although the resident T-cells are not malignant. During HIV disease, malignant lymphomas and EBV-associated lymphoproliferative disorders prove more frequent than in the general population, but nasopharingeal carcinoma remains an extremely infrequent occurrence with only anecdotal cases reported.
Case report: Our patient, HIV-infected since 20 yr and with a long history of inhalatory cocaine abuse, 18 months ago developed a remarkable laterocervical lymphadenopathy, whose histopathologic study disclosed a polymorphic EBV-associated B-cell lymphoproliferative disorder. A TC scan concurrently showed a right nasopharyngeal mass and detected multiple cervical, axillary, and ilar-mediastinal lymphadenopathies. A biopsy of rhinopharingeal lesion disclosed a poorly differentiated squamous carcinoma also EBV-positive at biomolecular testing. A cytotoxic chemotherapy was performed with ten consecutive cycles of cisplatinum-fluorouracil-bleomycin, associated with HAART, which achieved disease remission. A CT re-staging performed four months later showed sparse parapharyngeal and paravertebral infiltrates so that the suspected recurrence prompted a still ongoing radiotherapy course, while the immune recovery was not attained, as expressed by a CD4+ count of 83 cells/μL despite HIV virologic control.
Conclusions: Our patient suffered from two distinct malignant and pre-malignant disorders with some intriguing pathogenetic associations mainly linked to EBV but also HIV and related immunodeficiency and perhaps the chronically inhaled cocaine. Both squamous carcinoma and lymphoproliferative disorder of the nasopharynx are more frequent in the immunocompromised host and deserve further pathogenetic investigation to clarify and eventual shared or addictive role of some viral and environmental risk factors.

R1886

Community-acquired septicaemic pneumonia caused by a multiresistant *Staphylococcus aureus* strain, resulting in multiple organ involvement exacerbated by extensive immune activation

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Background: Antibiotic-resistant Gram-positive cocci are of increasing concern, and immune activation promped by microbial products (bacterial superantigens) may play a major role in the pathogenesis of disseminated, life-threatening *S. aureus* infection.
Methods: An exceptional case report of community-acquired, severe infection caused by a methicillin-resistant *S. aureus* strain, responsible for pneumonia, septic shock, and scattered septic embolism, and accompanied by diffuse polyvisceritis and thrombophlebitis as signs of an extensive immune system activation, was observed in a otherwise healthy 40-year-old man.
Results: The striking features of *S. aureus* polyvisceral disease (pneumonia, sepsis, and pulmonary and hepato-splenic septic embolism) were associated with multiple immune-mediated focal manifestations (massive pleuric-pericardial effusion, mycocarditis, and multiple lower limb thrombophlebitis). *In vitro* resistance to all beta-lactams, fluoroquinolones, macrolides, and aminoglycosides, apparently did not justify the clinical-microbiological failure of a glycopeptide-based combination therapy. Only the administration of linezolid-rifampicin-tetracycline together with intensive care support, achieved a slowly progressive ameliorement, while the polyvisceritis (associated by an immune-activation syndrome documented by increased CD4+, CD34+ and CD4-CD8- T-lymphocyte subsets), caused directive disease at multiple body sites, and required a prolonged high-dose steroid therapy. A complete clinical, laboratory, and instrumental recovery was reached only three months after admission.
Conclusions: This report raises multiple questions about the epidemiology, pathogenesis, manifestations, and management of complicated *S. aureus* infection, with special focus on the immune system activation triggered by microbial antigens, and the therapeutic role of steroids and novel antibiotics targeted against resistant Gram-positive cocci.

R1887

Status of interleukin-5 and interleukin-13 in peripheral blood of adults with bronchial asthma

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Objectives: To explore the possible role of IL-5 and IL-13 in the pathophysiology of asthma, and to define the source of these cytokines in peripheral blood.
Methods: Forty-two patients with acute asthma, 25 patients with asymptomatic asthma (4 weeks or more without exacerbation) and 20 healthy controls were subjected to quantitative measurements of serum IL-5 and IL-13. In addition, IL-5 and IL-13 mRNA expression in peripheral blood mononuclear cells was defined by reverse transcription-polymerase chain reaction (RT-PCR). Spirometric parameters including: forced vital capacity (FVC), forced expiratory volume in one second (FEV1), forced expiratory flow rate at 25%, 50%, and 75% of the vital capacity (FEF 25, 50, 75) and peak expiratory flow rate (PEFR) were recoded in acute asthma patients.
Results: Serum IL-5 and IL-13 levels were significantly higher in patients with acute asthma than asymptomatic asthma and healthy controls, and in asymptomatic asthma patients than healthy controls. Serum IL-5 and IL-13 levels were significantly and positively correlated with each other in patients with acute asthma but significantly and inversely correlated with the spirometric parameters. The frequencies of IL-5 and IL-13

mRNA in PBMC from patients with acute asthma (90.9% and 81.8% respectively) were significantly higher than that from patients with asymptomatic asthma (27.3% P = 0.0024 and 36.4% P = 0.03 respectively) and healthy controls (0.0%, P = 0.00003 and 0.0%, P = 0.0002 respectively). However, the difference between asymptomatic asthmatic and healthy controls was insignificant regarding the frequency of positive PBMC- based IL-5 mRNA expression but significant regarding the frequency of positive PBMC- based IL-13 mRNA expression (P = 0.034).

Conclusion: Serum levels of IL-5 and IL-13 may be useful in monitoring of bronchial asthma particularly as they correlated significantly with severity of the disease as indicated by Spirometric parameters. PBMC contribute to serum levels of IL-5 and IL-13 and that spill over from the airways might be an additional source of these cytokines in serum of patients with acute asthma.

R1888

The study of cellular and tissue mechanisms of homeostasis of the system ''helminth – host'' during muscular trichinellosis and the phytoorigin antihelminthic effect

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Objectives: The purpose of our work was the study of the influence of phytoantihelminthic on the structure and functional activity of tissues of larva of *trichinella* and on the structure and micromorphofunctional condition of muscles and barrier-organs of their hosts during mixed invasion by larvas of *T. spiralis* and *T. pseudospiralis*.

Methods: The experiment was undertaken to test rats of Wistar line.The animals were infected with per.os. of *T. spiralis* and *T. pseudospiralis* larvas. The infection dose was 10 larvas of each species for 1 gram body weight of rat.The following medicinal herbs were used as phytoantihelminthics in the ratio of 2:2:1– *Plantago major* L., *Satureja Montana* L. and *Ranunculus acris* L. Animals received the substance in food in a dose of 50 mg/kg body weight. The animals were lanced on 35th day after being infected.

Results: Since 16-th day after infection destruction of the larvas of the trichinell of both species and destruction of capsules round the larvas of the *T. spiralis* were observed among the infected animals. The intracapsular sarcoplasm completely changed into coarse-grained mass. In most of larvas of *T. spiralis* and *T. pseudospiralis* the tincterial properties of cuticle broke and the latter became hard-knobbed and teared away from the hypoderm here and there. Metachromasia of reproductive glands and of digestive system was observed. On specimen the dead larvas of *T. pseudospiralis* revealed. In muscles, in livers and intestines of rats the factors of pathogenesis were weakening and the characteristic lines of the compensatory and the regenerative processes were observed. In muscular tissue the connective-tissue cicatrixes were formated, sudstituting the destruction of the muscular fibres and their fragments. In the liver partial recovery of structure and architectonics of trabecules were observed. In the result of phytotherapy as a whole the infiltration of parenchyma of liver by leucocytal elements descended. In muscles, in livers and in intestines of rats as a whole the synthesis of RNA and formation of glycogen noticeably became intensive.

Conclusion: The phytoantihelminthic detained encapsulation of larvas of *T.spiralis* caused death of particular number of larvas of *trichinella* of both species. This work is of interest for the development and for application of plant origin antihelminthic during the therapy of helminthosies especially trichinellosis.

R1889

Soft tissue infections by *Stenotrophomonas maltophilia* in immunocompromised rats. Implication of bacterial translocation

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Objectives: The aim of the present study was to investigate the role of bacterial translocation in the overall mortality in an animal model of soft tissue infection by *Stenotrophomonas maltophilia*.

Methods: Soft tissue infection was induced in twenty-seven immunocompromised Wistar rats. Neutropenia was achieved by the intramuscular infusion of cyclophosphamide at a dose of 100 mg/kg on day 1 and 150 mg/kg on day 3. Animals were challenged in the left thigh by an 8log10 cfu/kg inoculum of *S. maltophilia* on day 5 and randomised into three groups. Group A consisted of eight rats observed for survival; group B of 10 animals sacrificed two hours post inoculation and group C of nine rats sacrificed four hours post inoculation. Through a midline incision 4 ml of blood were drawn from the inferior vena cava vein of rats in groups B and C for quantitative culture upon euthanasia; liver, spleen and lung samples were collected and quantitatively cultured. For the evaluation of culture results, stool specimens were obtained from five healthy rats.

Results: Mean ± SE survival of group A was 34.6 ± 5.56 hours; all animals in this group died within 51 hours. Stool specimens from healthy rats yielded *Enterococcus* spp, *Escherichia coli*, and *Enterobacter cloacae*. Mean log10 (±SE) bacterial counts in blood and tissue cultures of rats in groups B and C are shown in the table. The same pathogens isolated in blood samples were also isolated from respective tissue samples.

Group	Microbe	Blood	Liver	Spleen	Lung	%
B	*S. maltophilia*	4.4+/-0.68	4.6+/-0.67	5.6+/-0.42	4.4+/-0.31	50.0
	Enterococcus spp	4.7+/-0.24	6.1+/-0.85	5.7+/-0.92	5.7+/-0.62	30.0
	E. cloacae	4.7+/-1.33	4.7+/-0.80	5.2+/-0.75	5.9+/-1.10	40.0
C	*Enterococcus spp*	5.5+/-0.79	4.8+/-0.69	4.7+/-0.54	4.2+/-0.29	77.8
	E. cloacae	5.8+/-1.20	4.6+/-1.63	4.4+/-0.58	5.9+/-0.82	22.2

*denotes the percentage of rats that yielded each pathogen.

Conclusion: Soft tissue infection in immunocompromised rats results in rapid spread of *S. maltophilia* and translocation of intestinal flora to liver, spleen and lung two hours post inoculation, whereas four hours post inoculation only growth of normal intestinal flora is observed. Bacterial translocation may account for mortality from soft tissue infection by *S. maltophilia* in rats.

R1890

Serum and stool levels of interleukin-1beta, interleukin – 1ra, interleukin-6, interleukin-10, interleukin-12, tumour-necrosis factor-alpha and interferon-gamma in patients with salmonellosis

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Objectives: The information on the types of cytokines produced in serum and stool of patients with salmonellosis is still very little. Elucidation of the cytokine cascade can improve

understanding the mechanisms of inflammation, induced by Salmonella, and may allow the identification of leading pathogenetic links in the disease. The purpose of this study was to investigate the level of some cytokines (IL-1beta, IL-1ra, IL-6, IL-10, IL-12, TNF-alpha and IFN-gamma) in serum and stool of patients at the acute stage of salmonellosis.
Methods: The study included 48 patients with culture confirmed gastro-intestinal salmonellosis. Cytokine concentrations were determined by ELISA (BioSource, Belgium).
Results: Serum levels of IL-1beta, IL-1ra, IL-6, IL-10, IL-12, TNF-alpha and IFN-gamma were significantly elevated in patients in comparison with the healthy controls. In the stool IL-12, TNF-alpha and IFN-gamma were not increased, IL-10 was high and corresponded with the serum concentration whereas IL-1 beta, IL-1ra and IL-6 were more sharply raised than in the sera.
Conclusion: Dissemination of Salmonella in the gut stimulates local production of pro-inflammatory cytokines IL-1beta, IL-6 and IL-1ra. Their elevated serum concentrations mirrored the intestinal inflammation.

R1891

Characterisation and comparison between the proteomes of *Brucella abortus*, *B. melitensis* and *B. suis* by 2-dimensional HPLC mass spectrometry

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Objectives: Brucellosis is caused by a Gram-negative intracellular pathogen of the genus Brucella. This genus consists of at least six distinct species; of which, *B. abortus*, *B. suis* and *B. melitensis* are considered the most pathogenic to man as well as their principle hosts of cattle, pigs and goats respectively. A common cause of infections in humans is due to the consumption of un-pasteurised dairy produce such as cheese and milk. New identification and speciation methods are required to replace bacterial culture and traditional bio-typing which is both time consuming and hazardous. The aim of the present study was to determine differences in the proteomes of three *Brucella* species that may account for host specification and could be utilized as diagnostic targets.
Methods: Recent isolates of *B. abortus*, *B. melitensis*, and *B. suis* were cultured on serum dextrose agar for three days at 37°C and 10% CO_2. Bacterial cells were harvested into PBS and inactivated with methanol. The proteomes were extracted with urea and detergent and digested (100 mg) with trypsin. The tryptic peptides were separated in the first dimension by strong cation exchange chromatography and in the second by reverse phase HPLC with on line mass analysis. Proteins were identified and proteomes compiled with SEQUEST. The proteomes were further compared based on the spectrum count as a measure of relative protein expression using Microsoft Access.
Results: The proteomes of *B. abortus*, *melitensis* and *suis* contained 470, 554, 493, proteins respectively following analysis by 2D-HPLC-MSn. Over 281 proteins were detected in all three species of *Brucella*; of these 65 were only evident in the proteomes of *B. abortus*, 119 in *B. melitensis* and 72 in *B. suis*. The most predominant proteins in the proteome of *B. abortus* were heat shock proteins (GroEL), outer membrane proteins (Omp2b) and elongation factors (Tuf1). Similar proteins were evident in the other species.
Conclusions: This study extends previous proteomic analyses by 2D gel electrophoresis and provides new insights into microbial specific differences between three species of *Brucella* at the protein level. Further studies are required to determine the biological relevance of the differences observed in protein expression.

R1892

Serotypes of *Salmonella* and antibiotic resistance in outpatients in a Greek district hospital

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Objective: A comparative study of the resistance patterns and serotypes of enteric Salmonella isolated in the same number of outpatients in Corfu General Hospital (Greece) between A period: 2001–2003 and B: 2004–2005.
Methods: 88 enteric Salmonella strains were isolated of 1080 coprocultures the three years 2001–03. 137 enteric *Salmonella* strains were isolated of 1093 coprocultures the two last years 2004–2005 and 58% of the strains were isolated from children in both periods. The stools were inoculated in Selenit broth and SM agar (BioMerieux). The identification and susceptibility were performed by VITEK II (BioMerieux). The disk diffusion method of Kirby Bawer was used when necessary. The serotyping was tested in Salmonella-Shigella Center of the Univercity in Athens.
Results: The serotypes of Salmonella isolated in A and B period, were: A: *S. enteritidis* 68, *S. typhymurium* 12, other 8 B: *S. enteritidis* 110, typhymurium 13, other 14. All the isolated strains were 100% susceptible to AMC, SAM, CXT, S/T, C, NA and CIP, except AM (85%).
Conclusions: 1. In the last 2 years there is a 62% increase of isolated strains than in the first 3 years, in the same number of coprocultures. 2. *S. enteritidis* was the serotype isolated most frequent in both periods. 3. Children are susceptible to *Salmonella*. 4. The resistantance rates are low. The rational use of antibiotics and the foodborn infection surveillance are essential to prevent the resistant Salmonella strains emergency.

R1893

Serratia marcescens causing bloodstream and urinary tract infections and their abilities of penetration and cytotoxicity

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Objectives: Most infections associated with *Serratia marcescens* are urinary tract infections (UTIs) or bloodstream infections (BSIs), the pathogenesis of which remains poorly understood. To address this question, the present study was conducted using methods from both clinical and laboratory approaches to elucidate whether any specific factor(s) may have contributed to the occurrence of invasive infections associated with the bacterium.
Methods: A total of 38 patients, 19 each with BSIs or UTIs caused by *S. marcescens*, were retrospectively selected for further analysis. Clinical features of the patients and results of laboratory investigation among the associated isolates were analysed using a STATA System for Windows (version 8.2).
Results: Clinical characteristics of the patients, including gender, age, and underlying diseases, were similar in both groups. Fever and mortality occurred more frequently in the BSI group. Urinary isolates were generally more resistant to multiple antimicrobial agents and possessed plasmids of various sizes. Genotyping analysis by infrequent-restriction-site PCR revealed 18 genotypes, 12 of which were found in the BSI group

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and 10 in the UTI group. Pigment production was found in three isolates from each group, respectively. Virulence factors, such as penetration and cytotoxicity, were assayed by using Madin-Darby canine kidney (MDCK) epithelial cells grown on filters. After 6-h incubation of the bacteria with the cell monolayer, significantly more blood (94.7%) than urinary (52.6%) isolates were detected in the basolateral medium. A close association between the penetrative ability of bacteria and their cytotoxicity to MDCK cells was noted. Although several significant factors were found in the univariate analysis, only the abilities of penetration and cytotoxicity retained the significant association in the final multivariate model.
Conclusion: The ability to penetrate through epithelial barriers as well as the concurrent cytotoxicity may be important factors for *S. marcescens* to cause invasive infections.

R1894

The prevalence of *Listeria monocytogenes* in neonetal sepsis in a Tajrish hospital, Iran during 2003–04

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Introduction: Neonatal sepsis is a clinical syndrome characterized by systemic signs and symptoms and bacteraemia during the first month of life. *Listeria monocytogenes* is one of the newborn sepsis causes. The incidence is relatively low (one to eight cases /1000 live birth), yet the risk mortality is approximately 25%. Recent seroepidemiologic studies show that the infection is foodborn.
Materials & methods: This study was performed in Tajrish hospital during 2003–04. Between 1680 hospitalized neonatals 910 cases, which suffered from sepsis were admitted to NICU. These newborn were evaluated according to: birth weight, sex, blood group, clinical and laboratory evidences and recent antibiotics therapy.
Results: Between 910 sepsis cases, 7 (0.76%) of them were *Listeria* positive according to positive blood culture or positive CSF culture. Out of these 7 patients 4 (57%) were male and 3 (42%) were female. Among them 5 (72%) had low birth weight and 2 (28%) were weighted more than 2500 gram. The most frequent symptoms were, positive CPR: 6 (85%), Positive Icter: 5 (71%), Thrombocytopenia: 5(71%), PCRP & low PLT: 3(42%) and Leukopenia: 2(28%). Through these patients 14% were Ampicillin resistance, 28% were Gentamicin resistance and 14% were Amikacin resistance.

R1895

Anti-Yersinia antibodies in patients with Behçet's disease

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Objectives: As the etiopathogenesis of Behçet's disease (BD) is still unknown, we planned to investigate the relationship between Behçet's disease and Yersinia infection because: a) Yersinia virulence factor-YopP- distrupt ubiquitin in a way that might lead to the induction of an autoimmune response, b) Yersinia HSP60 is involved in the pathogenesis of uveitis in Behçet's patients, c) clinical findings of Yersinia infection resembles Behçet's disease findings such as uveitis, erythema nodosum, arthritis, and finally d) Behçet's disease clinically mimic the seronegative spondyloartropathies, mainly reactive arthritis developing after enteric or urinary tract infection.
Methods: Yersinia IgG, IgM and IgA antibodies were tested by quantitative micro ELISA in the sera of patients with Behçet's disease (n = 101), rheumatoid arthritis (RA) patients as disease controls (n = 38) and healthy controls (HC) (n = 43).
Results: Serum Yersinia IgM antibody levels were significantly elevated in BD (13.6 + 0.93 U/ml) compared to RA (7.97 + 3.55 U/ml) and HC (7.34 + 3.11 U/ml) cases ($p = 0.000$). The quantitative test results are grouped as positive, low titre positive and negative. Yersinia IgA, IgM, IgG seropositivity (>24 U/ml) in BD and RA were 4%, 4%, 15% and 13%, 0%, 18%, respectively. Yersinia IgA, IgM, IgG antibody positivity in HC group were 7%, 0%, 11%, respectively. Yersinia IgG and IgA antibody levels were not significantly different between groups. No correlation was found between Yersinia antibody titers and duration of disease, immunosuppressive therapy or activation in BD.
Conclusion: These results do not suggest a role for *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* infection in the etiopathogenesis of Behçet's disease. However as recent data suggest that Yersinia HSP60 – virulence factor is involved in the pathogenesis of uveitis in Behçet's patients and YopP might lead to the induction of an autoimmune anti-ubiquitin response, the role of Yersinia infection in the etiopathogenesis of Behçet's disease should be clarified in future studies.

R1896

The effect of laser on morphology of protoscoleces of hydatid cyst

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Objectives: According to application of laser in medicine and its various effects on body tissues. The present work was design to exhibit a method for killing hydatid cyst protoscoleces, using energy of laser (fluencies) with pulse dye laser device *in vitro* condition.
Materials & methods: Liver and lungs infected with hydatid cyst were collected from a slaughter house. They were immediately transferred to laboratory. The cysts were opened under the sterile condition then the hydatid fluid as well as protoscoleces of the cysts were transferred into a sterile dishes. 1000 of live and fresh protoscolese in identical volume, were subjected to different fluencies of laser ranging from 2 to 9.8 J/cm^2 for different time periods. The morphology and viability of the parasite was tested under light microscope.
Results: The results indicated that, although 2–4.5 J/cm^2 fluencies of laser have not effect on the morphology of protoscoleces, but dehydration, contraction, early evagination, granulation and death of the parasite were observed following inducing with 4.6 to 9.8 J/cm^2.

R1897

The role of infecting agents and systemic markers of inflammation in COPD patients

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COPD patients (pts) have an active local and systemic inflammatory response due to infection/colonization. Systemic markers of inflammation were investigated during a stable phase of their illness, because continued inflammation could affect their general health and be involved in disease progression.
Aim: To describe the clinical/microbiological/biochemical characteristics of COPD pts.

Study population: 70 COPD pts (II and III st.) were studied from February 2004 till November 2005.
Methods: Clinical data (CD) in stable period (SP) and during acute exacerbation (AE); lung function (LF); serum systemic markers of inflammatory (TNF-alpha, sICAM, GM-CSF); sputum cultures (SC).
Results: Pts were grouped according to the results of SC in SP: 1-st gr. – 43 (61.4%) pts without pathogens; 2-nd gr. – 27 (38.6%) pts with pathogens: 7 pts – *Ps. aeruginosa*, 7 – *Kl. pneumonia*, 5 – *E. coli*, 3 – *St. aureus*, 2 – *Str. pneumonia*; 3 – mixture. CD and LF were significantly poor in the 2-nd gr. There were no significant differences between the groups during AE: there were isolated *H. influenzae* and *Str. pneumonia* in 30%. TNF-alpha and sICAM were elevated and correlated with the extend of disease, poor LF and SC. But there were no significant differences between groups for GM-CSF.
Conclusions: 1. SB correlated with CD and LF. 2. The main role of the bacterial infection/colonization in the SP of COPD pts are Gram-negative bacilli. 3. The great role of AE COPD are *H. influenzae* and *Str. pneumonia*. 4. The level of some inflammatory agents may determine disease progression.

R1898

Susceptible clone of *Staphylococcus aureus* dormant in the human host?

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Objectives: A fully susceptible strain of *Staphylococcus aureus* isolated from a purulent lesion in the leg of a 77-year old diabetic patient was the trigger for this study. There had been no trauma to the infected area. However, it had been the site of osteomyelitis, more than six decades ago, when the patient was a child. The isolate was fully susceptible to all antimicrobial agents tested: penicillin, cloxacillin, clindamycin, erythromycin, gentamicin, rifampicin, tetracycline, ciprofloxacin, chloramphenicol, cotrimoxazol, mupirocin, tobramycin, linezolid, teicoplanin and vancomycin. We sought to investigate the genetic background of this isolate, one among the 2.4% fully susceptible isolates collected in the hospital in 2004, and whether it could represent the osteomyelitis strain that remained dormant in the host only to surface after the development of diabetes.
Methods: We analyzed the colonization status of the patient and studied the initial isolate and 53 *S. aureus* strains recovered from osteomyelitis and other bone-related infections in different hospitals or from carriage. Strains were characterized by multilocus sequence typing (MLST) and spa typing. Presence of the Panton-Valentine leukocydin (PVL) and collagen-binding protein (cna) genes and polymorphisms in the repeat region of the clumping factor gene B (clfB) were determined.
Results: The patient was not colonized by *S. aureus*. The genetic background of the strain isolated from the lesion was ST121, PVL+ and cna+, a genotype frequent among community carriage isolates and also present in 20% (4/20) of the osteomyelitis and bone infection isolates, in this study. Ninety-four percent (34/36) of the ST121 isolates were cna+.
Conclusions: ST121 was identified in carriage and osteomyelitis isolates. Although this genetic background could not be unequivocally traced to the episode of osteomyelitis in this patient's childhood, the clinical diagnosis of the lesion and presence of virulence factors, such as the cna gene, in most isolates indicate that this genotype, which is common in carriage in the community, is also well-equipped to cause osteomyelitis and other diseases.

R1899

A report of *Staphylococcus aureus* isolates presenting CA-ORSA profile in a Brazilian hospital

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Oxacillin-resistant *Staphylococcus aureus* (ORSA) presents the mecA gene that is carried by a staphylococcal chromosomal cassete mec (SCCmec). There are five SCCmec types: I, II and III, normally, present in hospital ORSA isolates (HA-ORSA), that encode resistance to the majority of the antibiotics and, types IV and V that are found in community-acquired ORSA strains (CA-MRSA) and encode resistance only to beta-lactam antibiotics. Moreover, the pathogenicity of CA-ORSA isolates is related to extra cellular virulence factors, like Panton-Valentine leukocidin (PVL) and gama-haemolysin (Hlg).
Objective: To analyze characteristics of ORSA strains isolated from patients in Clementino Fraga Filho Hospital University (CFFHU), Rio de Janeiro, Brazil, that presented resistance only to beta-lactam by the disk diffusion test.
Methods: Eleven *S. aureus* strains were isolated from eight patients, between Nov/2004 and Aug/2005, and were obtained mainly from the ninth floor at hospital (45.5%). Six strains were isolated from bloodstream infections, three from cutaneous abscess and the others from broncheoalveolar liquid and nostril. Strains identification and the disk diffusion susceptibility test were confirmed, according to Bannerman (2003) and CLSI (2005), respectively. Detection of mecA, pvl and hlg genes and SCCmec type was performed by PCR. Genotypic relationship was evaluated by using rep-PCR and PFGE.
Results: Two patients died, one with pneumonia and another with cutaneous abscess, by the acquired infection by ORSA; all isolates were resistant to penicillin, oxacillin and cefoxitin and susceptible to trimethoprim-sulfamethoxazole, rifampin and tetracycline. Besides that, all strains presented the SCCmec type IV. PVL gene was found in five strains, while hlg gene was observed in nine. Clonal relationship was observed among the 11 strains of ORSA isolated.
Conclusion: Reports of ORSA isolation presenting SCCmec type IV profile are important to encourage about the more pathogenicity showed by these strains, mainly in hospitalized patients.

R1900

The prevalence of CDT genes in clinical *Campylobacter jejuni* isolates

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Campylobacter jejuni (*C. jejuni*) the major cause of acute diarrhoea in humans throughout the world, encodes a toxin termed cytolethal distending toxin (CDT) which is considered to be an important virulence factor. In this study, 28 *C. jejuni* strains from epidemiologically unrelated patients with diarrhoea were screened for presence of CDT genes (cdtA, cdtB, cdtC) by PCR. Bacterial DNA was extracted by CTAB (cetyltrimethylammonium bromide) method and PCR was performed by using the previously described primers cdt A, cdt B, cdt C. Following PCR, amplification products were seperated by gel electrophoresis, stained with ethidium bromide and visualized under an UV transilluminator. All isolates were found positive for these three CDT genes. A high prevalence (100%) was found for the toxin genes. Presence of the CDT genes appear to be indispensable for *Campylobacter jejuni* to cause the human

disease. For verification of the presented data, further comperative studies, including both strains isolated from asymptomatic carriers and clinically ill patients are required.

R1901

Bordetella pertossis infection, Isfahan, Iran, 2002–2005

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Objectives: Over the last decade several changes occurred in the field of pertussis. Immunization controlled the diseases in children but did not disrupt circulation of the organism. *Bordetella pertussis* illnesses are common and endemic in adults and these infections are the reservoir for pertussis in susceptible children and infants. But what about pertussis in Isfahan?
Methods: In a case control study 50 patients more than 18 years old with paroxysmal cough lasting more 2 weeks and no history of COPD or Asthma were age and sex matched with 50 controls and without history of cough. For laboratory confirmation of *B. p* infection in cases their nasopharyngeal swabs were cultured in Bordet-Gengou Agar and ≥4-fold increase in IgG antibody titres to *Bordetella* antigens by ELISA methods between the acute and convalescent serum samples were evaluated and compared with antibody titre against *B.p* in controls.
Results: Anti-pertussis antibody was 12% positive, 16% equivocal, 72% negative in control and 48% positive, 6% equivocal and 46% negative in cases and this difference was statistically significant (P value < 0.001). The mean level of IgG antibody against *B.p* in acute sera was higher in cases than controls (18.2 ± 32.4, 28.8 ± 70.6) Also the increase of mean antibody titre between acute and convalescent serum in cases after two weeks was statistically significant (P value: 0.04) and in 12% the serum antibody had been increased ≥4 rise. The level of antibody titre in 28% of cases was >100 IU/ml. *B.p* was not isolated from any of nasopharangeal cultures. No direct relation has been found between age and antibody titre in controls in contrast a weak invert relation in cases.
Conclusion: It is clear that immunity after immunization and even infection is not life long. Sensitivity of organism isolation is less than optimal especially if the specimen is obtained in a vaccinated person, after antibiotic treatment has been started or if taken late in the course of illness. IgG antibody assays provide sufficient sensitivity for diagnosis in paired acute and convalescent specimens. However single specimen analysis is more practical for routine purposes. The frequent finding of asymptomatic antibody boosting in an immune person proves susceptibility for infection, and probably active transmission despite protection from disease. Until pertussis in adults is universally prevented through immunization clinicians must be on the alert of the clinical disease

R1902

Seronegative spondyloarthritis after *Chlamydia trachomatis* disease

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Objectives: Ankylosing Spondilitis, Reiter Syndrome, non-differential arthritis and other arthritis belong to group of seronegative spondiloarthritis. This study was conducted to determine the relation between urogenital diseases from *Chlamydia trachomatis* and seronegative spondiloarthropathies.
Methods: We examined samples of urethral or vaginal secretion and blood serum from 100 patients hospitalized in the Rheumatologic unit of our hospital (14 with Ankylosing Spondilitis, 28 with Reiter syndrome, 24 with non differential arthritis and 38 with other arthritis non seronegative as control group.The urethral and vaginal excretion was examined for antigen *Chlamydia trachomatis* with immunofluoresce (IFA) method, and the blood serum for detection antibodies IgG and IgA with immunoassay (ELISA) method.
Results: Frequency of *Chlamydia trachomatis* in seronegative spondiloarthritis

PATIENTS	NUMBER	CHL.TR(+)	%
ANGYLOSING SPONTILITIS	14	4	28.50%
SYNDROME REITER	28	11	39.20%
NON DIFFERENTIAL ART.	24	10	41.60%
OTHER	34	9	26.47%

Conclusion: Antigen of *Chlamydia trachomatis* or antibodies or both of them have the highest frequency 41% in non differential arthritis and 39% in Reiter syndrome while they are less frequent in ankylosing spondilitis 28.5% and in control group 26.5%.
Results: The results match with the international bibliography and they show the relationship between sexually transmitted diseases and non-differential arthritis and Reiter syndrome.

R1903

The correlation between infection with *Helicobacter pylori* and the levels of hs-CRP and lipoproteins in women patients, which appear dyslipidaemia

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Objective: The purpose of the study was the investigation of the hs-CRP's and the lipoproteins' influence from the presence of systematic infection with *Helicobacter pylori* in feminine population.
Method: In the study 26 women with age 42–68 years old with BMI 23–41 kg/m^2 with known individual case history of dyslipidaemia took part. During the checking the presence of antigen Hp was determined in the stools as well as the presence of antibodies IgG and IgA of *Hp* in the serum, the levels of hs-CRP and the levels of Cholesterol, Triglycerides, HDL-Chol, LDL-Chol and the non-HDL-Chol. From the study patients who had gastro-oesophagus retrogression, small intestine ulcer, infection or inflammation were being excluded.
Results: The statistical consideration of the results was made by the Student t-test Results. From the total of studying persons to 17 from 29 (group A) an infection with *Hp* in development was found in contrast with 9 (group B) where there was not a recent infection. The persons of group A had considerable higher levels of hs-CRP ($p < 0.001$), of cholesterol ($p < 0.001$), of triglycerides ($p < 0.02$) and of LDL-chol ($p < 0.002$) than the patients of group B, although the average level didn't differ.
Conclusion: The conclusion is that the presence of infection with *Hp* is being correlated with the presence of high levels of hs-CRP chol of triglycerides and of LDL-chol, which are the risk factors for the cardiovascular disease.

Animal models incl. experimental treatment

R1904

Moxifloxacin is highly active in a murine granuloma pouch model of complicated skin and skin structure infections

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Objectives: MXF has recently been indicated for the treatment of complicated skin and skin structure infections (cSSSIs), so this study was designed to evaluate the efficacy of moxifloxacin (MXF) vs methicillin-susceptible *Staphylococcus aureus* (MSSA) in a murine model of cSSSI and to compare MXF with levofloxacin (LFX), vancomycin (VAN), linezolid (LIN), amoxicillin-clavulanate (AMOX-CLAV) and ceftriaxone (CEF).
Methods: Granuloma pouches were formed by injecting 5 mL of air + 0.5 mL of 0.1% croton oil in olive oil under the loose connective tissue on the back of mice (5 mice/group). After 72 h, the air was replaced by 1 mL of 0.25% agar in saline. 48 h later, a *S. aureus* DSM 11823 suspension (0.5 mL; grown anaerobically in Columbia broth; logarithmic growth phase) was injected into the pouch. Antibiotics were given intravenously at doses of 1.25 and 5 mg/kg at 0.5 h, 4 h, 24 h and 32 h post-infection. At 48 h post-infection the viable bacterial load in pouch exudates was determined by plating serial tenfold dilutions on sheep blood agar plates. Bacterial colony forming units (CFU) were counted after overnight incubation of the plates at 37°C).
Results: The bacterial loads at 48 h post-infection are shown in the Table. MXF showed similar antibacterial efficacy to LFX and AMOX-CLAV. At doses of 1.25 and 5 mg/kg, VAN, LIN and CEF were less effective than MXF in the reduction of CFUs in the pouch.

	Bacterial load in pouch fluid (CFU/mL); mean of n=5		
	1.25 mg/kg	5 mg/kg	Control
MXF	6.46×10^5	1.0×10^1	1.1×10^8
LFX	7.45×10^4	3.49×10^2	1.1×10^8
VAN	2.85×10^8	7.95×10^3	1.1×10^8
LIN	3.36×10^6	1.8×10^6	1.1×10^8
AMOX-CLAV	8.2×10^3	1.79×10^2	1.1×10^8
CEF	7.87×10^7	2.42×10^7	1.1×10^8

Conclusion: In the granuloma pouch model, MXF has higher antibacterial efficacy than VAN or LIN and is at least as effective as LFX and AMOX-CLAV vs MSSA infection. Therefore, MXF is likely to be an effective treatment for abscesses caused by MSSA.

R1905

Changes in serum C3 complement protein levels during early and late experimental sepsis

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Objective: Complement system comprises an important part of innate immunity. Sepsis leads to profound activation of the complement system and this activation occurs in the early phase of the sepsis. C3 Complement protein acts as acute phase reactant in the presence of sepsis. This study aimed to observe the changes of the serum C3 complement protein levels during early and late experimental sepsis.
Methods: Twenty-four male Wistar Albino rats were randomly divided into three groups. Cecal ligation and puncture (CLP) was induced and serum C3 protein levels were measured six hours after CLP in group II (early sepsis group; n = 8), CLP was induced and serum C3 protein levels were measured 48 hours after CLP in group III (late sepsis group; n = 8) and results were compared with the serum C3 protein levels of group I (control group; n = 8) animals. Results were analysed statistically by using Tukey post hoc test and $p < 0.05$ values were accepted as significant.
Results: Compared with the control group (I) animals, serum C3 protein levels were found significantly ($p < 0.001$) high in late sepsis group (III) animals (0.21 g/l and 0.36 g/l respectively). There were no significant differences in serum C3 protein levels between the control (I) and early sepsis group (II) animals (0.21 g/l and 0.19g/l respectively).
Conclusion: Serum C3 protein levels may be normal during initial phase of experimental sepsis and significantly elevated serum C3 protein levels indicate a late phase of experimental sepsis. Thus significantly elevated serum C3 protein levels may be an indicator for poor prognosis in sepsis.

R1906

In vivo antifungal activity of Akacid® cream for experimental cutaneous candidiasis in guinea pigs

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Objectives: The polymeric guanidine Akacid®, a novel member of the cationic family of antimicrobials, shows broad *in vitro* activity against bacteria and fungi. In the present study the antifungal activity of Akacid® was evaluated against *Candida albicans in vitro* and *in vivo*.
Methods: In vitro susceptibility of Akacid® was tested against *C. albicans* ATCC 90029 and ATCC 10231. The fungicidal activity of Akacid in 15 and 60 minutes contact time was investigated by suspension-neutralization method and the effect of Akacid on the morphogenetic transformation by yeast-phase cells to hyphal forms was studied. The *in vivo* efficacy of Akacid® cream at concentrations of 0.1, 0.5 and 2% was evaluated in a model of cutaneous candidiasis caused by *C. albicans* ATCC 90029 in guinea pigs compared to clotrimazole 1% cream. Each therapy regime was topically applied three times daily for a period of 7 days, starting on the first day after the initial infection. Quantitative cultures of the infective lesions in neutralizing solution were performed one day after end of therapy.
Results: After exposure to Akacid® at a concentration of ≥0.1% *in vitro*, *C. albicans* was eliminated in 60 minutes contact time and the transformation to hyphal forms was inhibited. *In vivo*, Akacid® cream at a concentration of ≥0.5% and clotrimazole 1% cream caused a significant reduction of the viable fungal count. Akacid 2% cream achieved the highest mycological eradication rate with in 75%. Akacid® 0.5% cream with in 62.5% was found to be as effective as clotrimazole 1% cream with in 56% in eradicating *C. albicans*.
Conclusion: These results suggest that Akacid® cream could have clinical usability for superficial infections caused by *C. albicans*.

R1907

Impact of colostomy on intestinal microflora and bacterial translocation in young rats fed heat-killed *Lactobacillus acidophilus*

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Objectives: Neonatal digestive diseases of the gut sometimes lead to surgical treatments. Such treatments, along with concomitant antibiotherapy, are causes of intestinal microbial imbalances. These imbalances favour intestinal disorders and/or infectious conditions through bacterial translocation from the gut. To help regulating the neonatal intestinal flora and avoid infectious complications, products such as Lactéol® (heat-killed *Lactobacillus acidophilus*) have been proposed. An experimental approach of colostomy-induced modifications to the intestinal flora and bacterial translocation was set using young rats fed an oral daily dose of Lactéol®.
Methods: Three-week-old Wistar rats received a daily dose of 5.109 heat-killed *L. acidophilus* (Lactéol®) from Day 0 till Day 28 of the experiment. Six rats served as control (Group C), six underwent laparotomy (Group L = sham-operated) and seven colostomy (Group Op = Operated) on Day 0. On Day 28, all rats were anaesthetised and ileum, caecum, colon removed for intestinal colonisation assessment as well as kidney, spleen, liver and mesenteric lymph nodes for bacterial translocation evaluation.
Results: Colostomy induced a lasting growth retardation in group Op (inferior weight from Day 9 till Day 28, $p < 0.01$). In the ileum, groups L and Op more frequently harboured lactobacilli ($p = 0.04$ and $p = 0.025$, respectively). In the caecum, both bacilli and staphylococci were detected more frequently in group L when compared to group Op ($p = 0.016$ and $p = 0.049$, respectively). Bacillus colonisation frequency was also significantly lower in group C when compared to that of group L ($p = 0.001$). Bifidobacteria were isolated more frequently in the caecum of group C animals compared to group L ($p = 0.03$). Overall bacterial translocation was significantly higher in the spleen of group L compared to groups C and Op (100 vs. 33 and 43% $p = 0.03$ and $p = 0.049$, respectively). Staphylococci in the spleen had a higher translocation rate in group L when compared to the two other groups (83 vs. 0 et 29%, $p = 0.008$ for group C and $p = 0.016$ for group Op, respectively).
Conclusion: Lactéol® did not prevent colostomy-induced changes in the intestinal microflora. However, these changes, as well as bacterial translocation (staphylococci and total bacteria), were less important than in animals undergoing laparotomy.

Biofilm

R1908

Comparison of the capacity to form a biofilm on medical materials as well as sensitivity to disinfectants of multiresistant Gram-negative rods isolated from children

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Objectives: The objective of this paper was to assess the capacity of multiresistant Gram-negative rods (MRGN) rods to form biofilm on the surface of polymers widely used in medicine and to compare the resistance of those rods to antibiotics and cationic disinfectants.
Methods: 72 MRGN were chosen for the research, among them 30 strains of *Enterobacteriaceae* rods (*K. pneumoniae*, *E. coli*, *P. mirabilis*, *Serratia* spp., *Enterobactr* spp., *C. freundii*) and 42 strains of non-fermentative rods (*P. aeruginosa*, *A. baumanii*, *A. lwoffii*, *Stenotrophomonas maltophilia*) which are resistant to karbapenems, fluoroquinolones and aminoglycosides. The strains were isolated from lower respiratory tract, wounds, blood and urine of children. The strains were identified by means of Sceptor (Becton-Dickinson) system. Sensitivity to antibacterial medicines and beta-laktamaz reactivity (ESBL, MBL) was marked according to the NCCLS standard. Bacteria sensitivity to cationic disinfectants was marked for chlorhexidine digluconate (CH) and for benzalconium bromate (BZ). The adhesion of rods to polymers and forming of a bacterial biofilm was examined using TTC method. Ureteral catheters made from polychloride vinyl (PCV), latex (L) and latex covered with silicone (LS) were used.
Results: Statistical analysis of the outcome showed that, MRGN strains from *Enterobacteriaceae* family with reduced sensitivity to CH and BZ were significantly often ($p < 0.05$) resistant to amikacin than strains sensitive to CH and BZ. However, for *P. aeruginosa* strains with reduced sensitivity to CH and BZ the resistance to ciprofloxacin was significantly more frequent. All examined strains of *Enterobacteriaceae* rods adhered and formed a biofilm on the surface of polymers from PV, L, LS, while non-fermentative rods actually adhered more frequently and formed a biofilm on the surface of PCV rather than on latex and silicone latex ($p < 0.01$). No statistical differences ($p > 0.05$) were found in forming a biofilm by non-fermentative rods on the surface of L and LS; furthermore, there have been no differences in the ability of forming a biofilm between *P. aeruginosa* and *Acinetobacter* spp. strains.
Conclusion: The resistance of Gram-negative rods to numerous antibiotics and the ability to form a biofilm on the surface of widely used in medicine polymers in addition to lowered sensitivity to disinfectants make it easier for Gram-negative rods to survive in hospital conditions.

R1909

Role of glycocalyx and bound moisture in formation of *Streptococcus mutans* EK2 8184 and TK18 biofilms

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Objective: To investigate the role of glycocalyx (glycoproteins or exopolysaccharides-protein) and bound moisture in formation of *Streptococcus mutans* EK2 8184 and TK18 Biofilms.
Methods: *Streptococcus mutans* EK2 8184 and TK18 were restituted, identified and incubated anaerobically in Trypticase soy broth (TSB) supplemented with 2 g/L sucrose in a incubator at 37°C for 18 hours. Biofilms were collected from plate, -20 freezed for one night, thawed. Biofilm fluid were collected after centrifuged. Total protein concentration was detected with Lowry method and exopolysaccharide was measured by the phenol-sulphate method. The state of moisture in biofilms was analysed by oven drying.
Results: The percentage of free and bound moisture in biofilm to *S. mutans* EK2 and TK18 were 90.94% and 1.09% vs 91.67% and 0.23%. The corresponding percentage in biofilm fluid values were 93.50% and 0.46% vs 93.17% and 0.25% accordingly. The

ratio of protein to exopolysaccharide in the biofilms to *S. mutans* EK2, 8184 and TK18 were 0.019 0.017 and 0.019. The corresponding ratio in the biofilms fluid values were 0.08, 0.09 and 0.03 accordingly.
Conclusions: The glycocalyx may be form various types framework of structures within a biofilm. The bound water molecules were accumulated around the glycocalyx but not polysaccharide, taking the important role of biofilm formation.

R1910

Microbial quality of water in dental unit waterlines

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Objectives: Dental unit waterlines (DUWL) are ideal environment for developing of bacterial biofilms. Microbial contamination of water in DUWLs is though to be the result of biofilm formation as it could serves as a haven for pathogens. Thus, microbial quality monitoring in a dental unit (DU) is of considerable importance, since patients and dental staff are exposed to water and aerosols generated from the DU. The aim of this study was to assess microbial quality of water in dental unit waterlines of 25 dental units located at the dental school of Isfahan University of Medical Sciences, Isfahan, Iran.
Methods: Water samples were collected from air-water syringe and high-speed handpiece at the beginning of the work day after a 2-min purge, and examined for total viable heterotrophic bacteria, fungi and legionella. The media used in the study were including R2A agar, sabouraud dextrose agar and Buffered charcoal yeast extract agar for bacteria, fungi and legionella respectively.
Results: The heterotrophic plate count (HPC) levels were significantly exceeded the American Dental Association recommendations for DUWL water quality (<200 CFU/ml), in both air-water syringe (84%, CFU/ml: 500–20000) and high-speed handpiece (96%, CFU/ml: 710–36800) samples. However, there was no significant statistical difference between the level of contamination in the air-water syringe and high-speed handpiece. Fungal elements were found in 28% and 36% of air-water syringe and high-speed handpiece samples respectively. All water samples were negative for *legionella* spp.
Conclusion: DUWLs should be subjected to routine microbial monitoring and to a decontamination protocol in order to minimize the risk of exposure to potential pathogens from dental units.

R1911

Evaluation of the ability of five *Candida* species to produce *in vitro* biofilms on the surface of teflon, polyurethane and polyvinyl chloride

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Objectives: To assess the in vitro ability of five *Candida* species to produce adherence and biofilms on the surface of three synthetic material commonly used in Medicine: teflon, polyurethane and polyvinyl chloride (PVC).
Material and methods: We have studied 82 clinically relevant isolates of yeasts from genus *Candida*: 23 *C. albicans*, 23 *C. parapsilosis*, 17 *C. glabrata*, 16 *C. tropicalis* and 5 *C. krusei*. Catheters were carefully cut into 1 cm segments and sterilised with ethylene oxide. A surface of 28 mm^2 was estimated for polyurethane, 36 mm^2 for PVC and 45 mm^2 for teflon. The segments were incubated together with an adjusted inoculum of yeasts (optical density of 1.0 at 540 nm wavelength). The incubation was 35°C for 90 minutes to establish baseline adherence and further 72 hours to assess the biofilm production. Nonadherent organisms were removed by washing three times with 1 ml of PBS. Results are shown as logarithm (log) of viable cells in colony forming units per millilitre (CFU/ml). In both cases results are adjusted according surface size as estimated previously.
Results: For polyurethane *C. albicans* and *C. parapsilosis* showed the lesser adherence (6.88 log and 7.29 log respectively) and biofilms production (9.00 log and 9.31 log respectively) with this material, whereas *C. tropicalis* showed the similar values for adherence and higher values for biofilm production (7.22 log for adherence and 9.43 log respectively). The difference was not significant. For PVC, *C. albicans* and *C. glabrata* had the lower measures for adherence (7.20 log and 7.82 log respectively) and again *C. tropicalis* showed the intermediate value (7.29 log). In the case of biofilm formation on PVC the results were 9.39 log for *C. albicans*, 9.79 log for *C. parapsilosis*, 8.93 log for *C. glabrata*, 8.24 log for *C. krusei* and 9.04 log for *C. tropicalis*. No statistic signification was found. For teflon, we found that *C. albicans* had the lower result in adherence (6.56 log) and *C. parapsilosis* the highest (7.54 log) and it was statistically significant. In the case of biofilm production, *C. parapsilosis* and *C. albicans* had the greater growth (10.37 log and 10.17 log, respectively) and *C. glabrata* had the lower biofilm formation (8.93 log) and these differences were statistically significant.

R1912

In vitro study of interactions between bacteria and prostheses used in vaginal surgery

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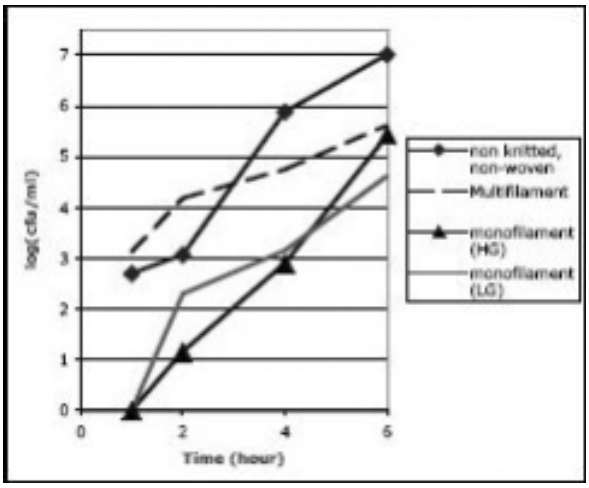
Gynaecological prolapse occurs permanently or upon physical effort through the vagina. The main treatment is surgical repair, and surgeons increasingly use prostheses. Several materials are available, which lead to infectious problems because of the vaginal surgical approach. Several cases of infected prostheses have been reported in the literature. The aim of this study was to compare the adhesion of several bacteria to different types of prosthetic tissue used in vaginal surgery.
Materials and methods: Seven types of prostheses were studied: - five polypropylene prostheses: 1 low- and 1 high-mesh monofilament, 1 multifilament, 1 non-knitted and non-woven and 1 monofilament with collagen- one prosthesis with porcine collagen- one polyester prosthesis with polyurethane.Three adherent bacteria from infected uro-gynaecological prostheses were selected: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*.Four samples, 5 × 5 mm, of each type of prosthesis were infected with 106 CFU/ml of each species and cultured at 37°C. Non-adherent bacteria were eliminated by at least 10 washes in sterile phosphate buffer. Adherent bacterial colonies were counted after vortex and trypsine treatment. Each experiment was done in duplicate after 1, 2, 4 and 6 hrs of incubation at 37°C. Two samples were examined after staining using optical microscopy.
Results (Figure 1): Counting of adherent bacteria: - kinetic studies of adhesion showed differences between prostheses, - *S. aureus* had higher affinity for prostheses containing collagen, - Bacterial affinity varied according to the type of prosthesis.Type of fixation: On all types of multifilament prostheses (polypropylene, polyester), non-woven and porcine collagen, bacteria were attached diffusely along filaments, whereas on monofilament prostheses, fixation appeared only on knots.



Conclusion: Infection rate in vaginal surgery varies greatly according to prostheses. Our *in vitro* results showed that infection is linked to the molecular type, manufacturing type, bacteria properties, and mesh of each prosthesis. Knowledge of interactions between bacteria and prostheses would improve understanding of infection mechanisms in order to optimise the quality of materials and treatment of infection. We suggest that all multifilament prostheses should be removed when infected, whereas conservative treatment may be tried for monofilament prostheses (with partial excision in case of erosion).

R1913

Effect of hydrogen peroxide and N-propanol on biofilms formed by clinical strains of *Staphylococcus epidermidis*

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Objective: *Staphylococcus epidermidis* is the leading organism causing infections of implanted or inserted foreign material or devices generally associated with biofilm formation. We tested the effects of disinfectants commonly used in clinically practice on biofilms of clinical strains of *S. epidermidis*.

Methods: Thirty biofilm-forming strains of *S. epidermidis* isolated from blood of patients with confirmed pacemaker infection, catheter-related bacteremia or from the skin of healthy controls were investigated using the static biofilm microtitre plate model. Biofilms were grown for 24 hours, then incubated with either hydrogen peroxide in 3 concentrations (0.5, 3 and 5 percent) and N-propanol (60 percent) for 1, 5, 15, 30 and 60 minutes. Biofilm formation was ascertained by electrone microscopy. For quantification biofilms were fixed with 2% glutaraldehyde and dyed with 1 percent crystal violet to measure the mean optical density (OD) using a microtitre-plate-reader at 550 nm wavelength. To test the bactericidal activity wells were not fixed and dyed, but the contents were seeded to Columbia agar plates, which were examined for growth after 24 and 38 hours.

Results: Incubation of the biofilms with 0.5 percent hydrogen peroxide for 1 and 5 minutes reduced the OD of the biofilms to 71 ± 26 and 72 ± 35 percent (mean, SD), respectively; incubation with 3 percent hydrogen peroxide for 1 and 5 minutes reduced the OD to 37 ± 22 and 48 ± 28 percent, respectively; and incubation of the biofilms with 5 percent hydrogen peroxide for 1 and 5 minutes reduced the OD to 31 ± 18 and 50 ± 14 percent of the OD of the untreated biofilm, respectively ($p < 0.05$). Incubation for 15, 30 or 60 minutes resulted in no further decrease of the OD. Incubation of the biofilms with 60 percent N-propanol led to an increase of the OD of the biofilms. There was no growth of bacteria recovered from all treated biofilms.

Conclusions: Hydrogen peroxide at a concentration of 3 percent reduces the density of biofilms formed by *S. epidermidis* to the same extent as 5 percent hydrogen peroxide. Sixty percent N-propanol does not reduce the OD at all and even seems to fix the biofilms. Hydrogen peroxide and N-propanol are bactericidal on the biofilms. Thus, hydrogen peroxide can be used topically in foreign body infections.

R1914

The adhesion intensity of *Staphylococcus epidermidis, Salmonella typhimurium* and *Pseudomonas aeruginosa* on the surface of bioactive glass 4N

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Objectives: To estimate the adhesion intensity of different microorganisms on the surface of originally synthesized biomaterial - bioactive glass 4N.

Materials and methods: In this research the bioactive glass 4N, *S. epidermidis* strain ATCC 12228, *S. typhimurium* strain ATCC 14028, *Ps. aeruginosa* strain ATCC 27853 were used. The biomaterial discs were incubated for 1 and 2 hours in 37°C in following bacterial suspensions: 10 CFU/ml, 100 CFU/ml, and 1000 CFU/ml. Several cultivations from every sample were prepared on TSA plate to estimate the total amount of microorganisms and the number of CFU per 1 mm^2 of the surface of biomaterial disc. The Fisher test was used for statistical analysis.

Results: The adhesion intensity of *S. epidermidis*: no bacteria adhered to bioactive glass 4N after 1 h of incubation in concentration 10 CFU/ml, 100 CFU/ml and 1000 CFU/ml, and in concentrations 10 CFU/ml and 100 CFU/ml after 2 h of incubation. The intensity of adhesion after 2 h of incubation in concentration 1000 CFU/ml was 4 10–3 CFU/mm^2 ($p < 0.001$). The adhesion intensity of *S. typhimurium*: 2.5 10–3 CFU/mm^2 after 1 h of incubation. However after 2 h of incubation no adherence was detected in concentration 10 CFU/ml; in concentration 100 CFU/ml accordingly 11.5 10–3 CFU/mm^2 after 1 h of incubation and 18 10–3 CFU/mm^2 after 2 h of incubation were detected; in concentration 1000 CFU/ml after 1 h of incubation 73 10–3 CFU/mm^2 and 207 10–3 CFU/mm^2 after 2 h of incubation were detected ($p < 0.001$). The adhesion intensity of *Ps. aeruginosa*: 2 10–3 CFU/mm^2 after 1 h of incubation and 18 10–3 CFU/mm^2 after 2 h of incubation in concentration 10 CFU/ml were detected; in concentration 100 CFU/ml accordingly 25 10–3 CFU/mm^2 after 1 h of incubation and 91 10–3 CFU/mm^2 after 2 h of incubation; in concentration 1000 CFU/ml - 171 10–3 CFU/mm^2 after 1 h of incubation and 309 10–3 CFU/mm^2 after 2 h of incubation ($p < 0.001$).

Conclusions: *Ps. aeruginosa* and *S. typhimurium* present higher tendency for adhesion on bioactive glass 4N than *S. epidermidis* in this experiment. The minimal infectious dose of *S. epidermidis* on bioactive glass was equal or even higher than 1000 CFU/ml after 1 h of incubation, or 100 CFU/ml after 2 h of incubation. The minimal infectious dose of *S. typhimurium* and *Ps. aeruginosa* on bioactive glass was equal or even higher than 10 CFU/ml after 1 h of incubation. This research was supported by ESF NP ''Support for medical science doctoral studies''.

Antimicrobial pharmacokinetics, pharmacodynamics, general pharmacology

R1915

Potential for improved pharmacokinetics of enrofloxacin in poultry following modified dosage regimens

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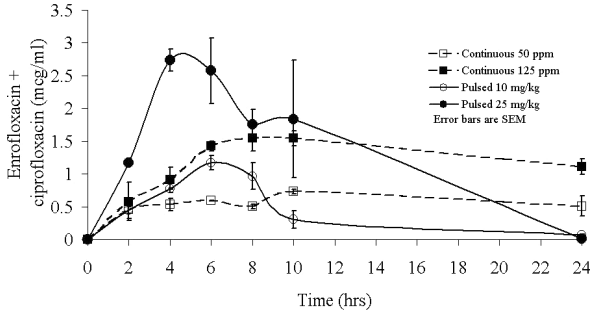
Objectives: Optimal antimicrobial dosage regimens aim to achieve successful clinical outcomes without drug toxicity or emergence of bacterial resistance. For concentration dependant antibiotics, such as the fluoroquinolones, a Cmax:MIC ratio of >10 is considered more important for efficacy and reduced selection of resistance than prolonged antibiotic concentrations just above the MIC. Fluoroquinolone resistance in zoonotic bacteria such as *Campylobacter* and *Salmonella* is a matter of public health concern, and fluoroquinolone treatment of poultry can rapidly select for bacteria with reduced fluoroquinolone susceptibility. In this study we compared basic pharmacokinetic parameters for the recommended dose of Baytril (enrofloxacin) 10% oral solution in poultry to 2.5x this dose for birds dosed by continuous water (standard) treatment compared to pulsed water treatment and dosing by gavage.
Methods: For the pulsed versus continuous water treatments, groups of chickens received Baytril 10% oral solution at 50 (recommended) or 125 ppm continuously in the water or at 10 (recommended) or 25 mg/kg pulsed in the water. For each group, three birds were killed at 0, 2, 4, 6, 8, 10 and 24 hours after start of antibiotic treatment and caecal contents, liver, lung and sera were taken for analysis by fluorescence HPLC for fluoroquinolones. For gavage treatment, dosing was at 10 and 25 mg/kg by crop intubation and four birds were killed in each group at 2, 6 and 24 hours after gavage, caecal contents, liver and sera were taken for analysis. Basic pharmacokinetic parameters were determined using PK solutions software.
Results: Determined mean fluoroquinolone Cmax in caecal contents (and sera) for gavage, pulsed water and continuous water treatments was 78.01 (1.81), 53.19 (1.17) and 24.73 (0.73) mcg/ml (recommended dose) and 115.86 (3.86), 115.63 (2.74) and 68.02 (1.17) mcg/ml (2.5x recommended dose) respectively. Cmax of antibiotic in liver and lung was increased by the modified regimens in similar proportions to above. Both pulsed water and gavage treatment not only resulted in higher Cmax values, but also a faster rate of clearance than continuous water treatment (Figure 1).



Conclusion: Dosing by gavage is not practical for thousands of chickens. However, pulsed dosing at 2.5x the recommended dose can increase the Cmax about fourfold and should improve efficacy and reduce selection of resistance, compared to the 50 ppm continuous water treatment regime (recommended).

R1916

Comparative bactericidal activities of daptomycin, glycopeptides, linezolid, and tigecycline against blood isolates of Gram-positive bacteria, Taiwan

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Objectives: To determine the in vitro bactericidal activities of daptomycin, linezolid, tigecycline, vancomycin, and teicoplanin against resistant gram-positive bacteria.
Methods: MICs and MBCs of the above agents were determined for ten each of blood isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant .*S. aureus* (MRSA) including two vancomycin heteroresistant *S. aureus* (MIC, 6 μg/ml), and vancomycin-resistant *Enterococcus faecium* and *E. faecalis* (VRE).
Results: Vancomycin had better bactericidal activities against MRSA and MSSA than that of teicoplanin. Forty percent of MRSA, 90% of MSSA and vancomycin-resistant *E. faecium*, and 100% of vancomycin-resistant *E. faecalis* exhibited tolerance (MIC/MBC ratio more than 16) to tigecycline. Linezolid had poorest bactericidal activities against isolates tested and tolerance of this agent was found 100% for MSSA and VRE isolates and 90% for MRSA. Daptomycin had most potent bactericidal activities against all isolates tested without tolerance.
Conclusion: The excellent in vitro bactericidal activity of daptomycin presents the potential role of treatment of severe infections due to these resistant bacteria.

R1917

Comparative study of fluconazole and clotrimazole for the treatment of vulvovaginal candidiasis

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About 75% of women in the world are involved by Vulvo Vaginal Candidiasis atleast once in their whole life. *Candida albicans*, opportunistic yeast, causes 85-90% of vaginal mycotic infections. Volve and vaginal itching and cottage cheese like discharge, are most common signs of VVC. The best treatments for this infection are topical Azoles; Clotrimazole and Miconazole or systemic Azoles for example; Fluconazole. The oral administration of Fluconazole has advantages about easily using and high effect on recurrent infections caused by hyper colonization of *Candida albicans* in intestinal tract. In this study Clotrimazole cream for application to the vulvo vaginal area mycosis in a single-blind clinical study. A randomized controlled trial was conducted at Kowsar Uromia Hospital. There were 60 women in the group treated with fluconazole and 60 in the group treated with clotrimazole, there was no significant difference between the two groups regarding age and length of follow-up period. Mycological cure rates approximately 1 week after treatment were 79.3% in the Clotrimazole group and 90% in the Fluconazole group. The side effects were minimal and results were not statistically significant. As a conclusion we recommend that a single oral dose of 150 mg of Fluconazole be used as an alternative method of

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treating VulvoVaginal Candidiasis because of short time and easily using of oral, single dose capsule.

R1918

Evaluation of antibacterial activity of ethanol extracts of some medicinal plants against *Listeria monocytogenes*

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Objective: *Listeria monocytogenes* is a foodborne pathogen capable of causing outbreak or sporadic episodes in human. Most of the outbreak or sporadic episodes were in the last two decades and due to high rate of mortality, caused a great concern in the food industry. There are various medicinal plants with the high performance of antibacterial activity in Iran. The aim of present study was to determine the anti-listerial effects of *Thymus vulgaris* L., *Eucalyptus globules* Labill., *Matricaria recutita* L., *Rosmarinus officinalis* L., and *Saliva officinalis* L., extracts.
Method: The plants were selected and identified by Department of pharmacognosy of Isfahan Faculty of Pharmacy. Extracts of the selected plants were prepared. Then antibacterial activity of extracts was determined by disc diffusion method. The Minimal Inhibitory Concentration (MIC) was also determined by tube dilution method.
Results: Results showed that ethanolic extract of Eucalyptus poses antibacterial effects against *L. monocytogenes* in both disc diffusion and tube dilution method. MIC and MBC of this extract were 31.25 μg/mL and 500 μg/mL, respectively.
Conclusion: The results of present study indicate that only the extract of Eucalyptus showed antibacterial effects on both serotypes of *L. monocytogenes*. Extracts of other plants did not show anti-listerial effects against *L. monocytogenes*.

R1919

Mutant prevention concentrations of ciprofloxacin for *Brucella melitensis*

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Objectives: We determined mutant prevention concentration (MPC) of ciprofloxacin against 28 *Brucella melitensis* clinical isolates.
Methods: MICs of ciprofloxacin were determined using broth microdilution method with brucella broth while MPCs were determined using agar dilution method with brucella agar. The MPC was recorded as the lowest concentration of ciprofloxacin to inhibit growth from an inoculum of 1010 cfu.
Results: MIC of 12 isolates were 0.5 μg/ml and MIC of 16 were 1 μg/ml. Ranges of MPCs were 2–8 μg/ml. MIC-MPC of 9, 12, 3, 2, 1 and 1 strains were 0.5–8 μg/ml, 1–8 μg/ml, 1–4 μg/ml, 0.5–4 μg/ml, 1–2 μg/ml and 0.5–2 μg/ml, respectively.
Conclusion: According to our results, MPC/MIC ratios of ciprofloxacin against *B. melitensis* were reported as highly. MPC values will be useful for dosing strategies of *B. melitensis*.

R1920

Fluoroquinolones concentrations in infected human bone: preliminary results

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Objectives: The management of post-traumatic bone infections relies on prolonged antibiotic therapy and surgical debridement. However, a main determinant of clinical response is drug concentration at the infected site. Antibiotic penetration into bone depends primarily on pharmacological characteristics but can be influenced by degree of vascularisation and presence of necrotic sequestra. Aim of our study was to assess ciprofloxacin and levofloxacin concentrations in infected human bone.
Methods: Patients with a post-traumatic septic pseudoarthrosis undergoing debridément of infected tissue, who received antibiotic therapy for more than 1 week, were studied. Plasma and bone specimens were collected intraoperatively for pharmacokinetic and microbiologic assays at a mean of 4.2 hours after antibiotic administration. Bone samples were crushed and eluted into sterile phosphate buffer and antibiotic concentrations were measured by a validated HPLC method. Literature data on floroquinolones penetration in non-infected bone were used as control.
Results: Two patients were studied. The first patient received iv ciprofloxacin 400 mg bid and *E. coli* was cultured from the bone (MIC = 0.5 mg/L). Plasma concentration of ciprofloxacin at the time of osteotomy was 3.6 mcg/mL. Bone concentrations were 0.7 mcg/mL and 30.2 mcg/mL, respectively in cortical and newly formed bone, with respective bone/plasma ratios of 0.2 and 8.4. The second patient was administered iv levofloxacin 500 mg qd and *Enterobacter cancerogenus* was isolated (MIC = 1 mg/L). Plasma concentration at the time of surgery was 2.5 mcg/mL. Bone concentrations were 0.3 mcg/mL and 26.9 mcg/mL in cortical and cancellous bone, respectively (bone/plasma ratios: 0.12 and 10.8 respectively).
Conclusion: At steady state, only ciprofloxacin provided cortical bone concentrations higher than the susceptibility breakpoint of the infecting agent, and similar to those reported in non-infected bone. However, levofloxacin concentrations ten times higher than plasma levels were measured in cancellous bone and ciprofloxacin concentration in bony callus were eight times higher than those detected in plasma. This original observation may be related to an augmented vascularisation and/or selective accumulation of fluoroquinolones into regenerating bone, as observed in children's cartilage growth plate. These preliminary results suggest that ciprofloxacin may be preferred to levofloxacin for the treatment of post-traumatic bone infections.

R1921

X-ray diffraction evidence for antifungal action of *Cassia fistula* linn. fruit pulp extract

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Objectives: *Cassia fistula* Linn is implicated in Indian ethno medicinal literature as having antifungal properties. Hence a study was undertaken to evaluate the inhibitory effect of fruit pulp of this plant against *Aspergillus fumigatus*. Crude alcohol extract and petroleum ether, benzene, alcohol, chloroform and water fractions of *Cassia fistula* Linn. fruit pulp were assayed for antifungal activity. X-Ray diffraction studies of all fractions and the treated fungal biomass were done to study the inhibitory effect of the extract on the fungus.
Method: *Cassia fistula* fruit pulp was scraped out and dried at 45°C. Crude alcohol, extract was prepared according to the modified method of Shadomy and Ingroff (1974). Partially purified fractions in petroleum ether, benzene, chloroform, alcohol and water were prepared by successive extraction by reflux method of extraction (Harborne 1984). Antifungal activity was assayed by agar dilution method (Joan Stokes 1975) against

Aspergillus fumigatus.15 ml of sterile medium and 1 ml of respective extracts were dispensed in borosilicate glass tubes and autoclaved. Tubes were then inoculated with pure cultures of the test fungus and incubated at 37°C. growth of fungus was observed on 3rd, 6th and 9th day. X-ray diffraction studies were done by exposing the extract/ treated as well as untreated fungal biomass to X-rays from a copper source at an angle of 2 theta for 20 minutes.

Result: Crude as well as partially purified fractions of *Cassia fistula* Linn. fruit pulp inhibited the growth of fungus as inhibition of fungal growth was observed from 3rd day onwards. The magnitude of inhibition differed and maximum inhibition was observed with chloroform fraction. Crystalline nature of extracts was established by presence of sharp peaks in the X-ray diffraction spectra of the various fractions of the extract. Peak identification suggests that these peaks correspond to several primary and secondary metabolites. Several peaks present in the control spectra were absent in the treated spectra suggesting a change in crystalline nature of fungal biomass after treatment.

Conclusion: *Cassia fistula* Linn. fruit pulp possesses antifungal property. Secondary metabolites present in the extract are possibly responsible for the inhibition of fungus. Absence of well-defined peaks in the spectra of treated biomass suggests degradation of functional as well as structural metabolites.

R1922

Serum and ascitic fluid concentrations following a single IV dose of 400 mg moxifloxacin

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Objectives: Ascitic fluid is a favourable milieu for bacterial proliferation. Consequently, spontaneous bacterial peritonitis is not an uncommon complication in patients with ascites. A variety of pathogens have been implicated, the most common being *Escherichia coli* and *Streptococci*. So we estimated the concentrations of moxifloxacin, a new quinolone with activity against gram-negative and gram-positive (especially streptococci), into serum and ascitic fluid in patients with ascites and cirrhosis.

Methods: Six patients with decompecated cirrhosis and ascites were enrolled to the study. The Ethics committee of the hospital approved the study and all patients gave written consent. Patients were given a single 400 mg of moxifloxacin as a 60 min IV infusion and 2 hours after the end of infusion blood and ascetic fluid samples were collected. Samples were assayed for the presence of moxifloxacin by a high-performance liquid chromatography (HPLC) method.

Results: Following a single 400 mg infusion of moxifloxacin, mean serum concentration for the six patients was 4.29 mg/L (range 3.9–5.9 mg/L) 2 hours after the end of infusion. The concentration of moxifloxacin into ascetic fluid at the same time was 3.05 mg/L (range 2.6–4 mg/L). The penetration of moxifloxacin into ascetic fluid was 71.1%.

Conclusion: The levels that were achieved in the ascites are well above the MICs for *E. coli* (0.016 mg/L) and other members of the family *Enterobacteriaceae*, such as *Proteus mirabilis* and *Klebsiella pneumoniae* (0.03 mg/L). On the basis of the present findings of high levels of moxifloxacin in ascitic fluid and the excellent in vitro activity against members of the family *Enterobacteriaceae*, this fluroquinolone appears to be very promising for the treatment of spontaneous bacterial peritonitis.

R1923

Pharmacokinetic/pharmacodynamic analysis and evaluation of clinical effectiveness of nomogram based dosing regimen in critically ill patients

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Objectives: There is limited data on the association of vancomycin serum concentrations, pharmacokinetic/pharmacodynamic (pk/pd) parameters and clinical efficacy. In the present study we aimed to analyze the pk/pd profile and parallel evaluate the clinical effectiveness of our routinely used vancomycin dosing regimen in critically ill patients.

Methods: A prospective, open-label, descriptive pilot study in a 6-bed, surgical, adult intensive care unit (ICU) has taken place. Nine critically ill patients with normal renal function were enrolled in whom vancomycin therapy for documented Staphylococci infection had to be started. Patients were dosed according to a previously developed nomogram [Moise-Broder et al. Clin Pharmacokinet 2004]. As all eligible patients belonged to the same dosing group, vancomycin 1 g twice daily was infused intravenously over 60 minutes. Timed blood samples were taken prior to and during two dosing interval at steady state. Vancomycin serum concentrations were determined by the Abbot TDx system. MIC values were determined for the causative organisms. WinNonLin 5.0 package was used for pharmacokinetic analysis. Clinical outcome (recovery or death) and nephrotoxicity (increased serum creatinine by 40 μmol/l or by 50% on consecutive measurements) was also evaluated.

Results: Our main results are summarized as follows.

Conclusion: Our nomogram based dosing proved to be successful in 88% of cases despite the relatively low AUC/MIC ratios in some cases. No toxic effects were detected. The current findings support the justification of this simplified dosing nomogram in stable critically ill patients with normal renal function. However AUC/MIC probably drives outcome further investigations are needed to clarify the optimal target value.

No	Sex	Age (years)	LOS (days)	Th (days)	Cure	Nephro toxicity	Cbac	MIC	Apache 1	Apache 2	Clcr (ml/min)	Clva (ml/min)	t ½ (h)	AUC (µg*h/ml)	V (l)	Cltb (µg/ml)	AUC/MIC
1	F	65	33	12	YES	NO	MRSE	1.5	19	8	115.8	38.0	6.7	436.3	27.4	14.6	282.2
2	F	67	6	3.5	NO	NO	MRSA	2	20	25	140.5	43.7	8.0	381.4	28.1	12.4	190.7
3	M	48	16	5.5	YES	NO	MRSE	1.5	16	11	271.4	117.8	4.5	141.5	38.8	3.2	94.3
4	M	73	18	9	YES	NO	MRSE	1	16	14	149.7	118.5	5.1	140.7	46.8	4.2	140.7
5	F	53	13	7.5	YES	NO	MRSE	3	17	13	227.2	139.5	2.7	119.4	28.2	2.5	39.8
6	F	49	11	5	YES	NO	MRSE	1.5	12	6	98.7	73.0	4.1	228.5	23.2	6.6	152.3
7	M	22	18	9.5	YES	NO	MRSE	2	17	13	195.2	114.7	2.9	146.3	25.6	2.8	72.7
8	M	54	26	7.5	YES	NO	MSSA	1.5	9	7	82.4	45.1	9.0	369.7	33.2	11.7	246.5
9	F	16	22	9.5	YES	NO	MRSE	1.5	12	8	129.5	73.5	4.1	226.8	22.9	5.9	151.2

No: number of patient, LOS: length of ICU stay, Th: length of vancomycin therapy, Cbac: causative bacteria, Apache: acute phisiology and chronic health evaluation II score on study entry (1) and at vancomycin discontinuation (2), Clcr: Estimated Creatitine-clearence based on Cockroft-Gault equotation, Clva: vancomycin clearance, AUC: area under the curve extrapolated to infinity, t1/2: elimination half-life, V : volume of distribution, C_{12h}: trough concentration

R1924

Continuous infusion versus intermittent administration of meropenem in critically ill patients

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Background: Meropenem is a broad-spectrum carbapenem antibiotic, which is usually given intravenously as a short infusion over 30 min, three times a day (TID). This intermittent application, however, is likely to be suboptimal for therapy since the time of antibiotic concentrations above the minimal inhibitory concentrations (MICs) of relevant bacterial pathogens may be too short. A continuous infusion of the drug may provide constant adequate concentrations over 24 h and be the better choice.
Objective: To determine meropenem serum concentrations during continuous infusion of 2 g/day versus intermittent dosing of 1 g TID in critically ill patients.
Methods: Serum meropenem levels were determined in two groups of critically ill intensive care unit (ICU) patients who received either intermittent short time infusion therapy of 1g over 30 min TID or 1 g loading dose followed by a 2 g continuous infusion over 24 h. Meropenem serum concentrations were determined by HPLC. Antibiotic serum concentrations were analysed in correlation to creatinine levels.
Results: In both patient groups, antimicrobial serum levels were suboptimal. In patients who received intravenous infusion over 30 min, serum concentrations did not surpass the MIC90 of *Pseudomonas aeruginosa* (8 μg/ml) at any time. Similarly, serum drug levels in patients with normal renal function who received continuous infusion were persistently lower than 8μg/ml; only patients with impaired renal function (creatinine level >1 mg/dl) showed meropenem serum concentrations >8 μg/ml.
Conclusions: Continuous infusion of meropenem may have the potential of improving antimicrobial therapy in critically ill patients, but should be investigated in further studies. In patients with impaired renal function receiving continuous infusion therapy, creatinin levels are the most important factor for predicting effective serum concentrations of the drug.

Mechanisms of action, resistance and resistance surveillance

R1925

A new mandatory enhanced methicillin-resistant *Staphylococcus aureus* surveillance system for England. An explanation of the new web-based surveillance system

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(London, UK)

Summary Sentence: On the 1st of October 2005 the Health Protection Agency (HPA) introduced a new enhanced MRSA mandatory surveillance scheme, designed to capture a more detailed data set to inform a national intervention target.
Objectives: The enhanced system captures information on each MRSA bacteraemia episode, in order to give Trusts a more accurate picture of their situation and to contribute to building a better evidence base for intervention. The system enables reports to be entered in 'real time' as they occur. As well as recording the age and sex of each patient, the system allows Trusts to specify the hospital, speciality and sub-specialty where the patient was located when the infection was identified, and whether the patient was on dialysis. The date the specimen was taken and the date of admission of the patient are both recorded which allows for the separation of MRSA bacteraemia infections identified within 2 days of admission. The source or provenance of the patient, such as home or nursing home can also be recorded.
Methods: There will ultimately be two different methods available to Trusts by which they can report the enhanced dataset to the HPA; either via the electronic reporting system CoSurv, or via an MRSA Enhanced Surveillance System website. Details of the MESS website will be given, and a summary of the results from the pilot study described below.
Results: The website was piloted in 21 acute Trusts between May and July 2005. 246 records were entered, of which 60% had a specialty of general medicine, geriatric medicine or general surgery. The majority of patients with MRSA positive bacteraemia were listed as inpatients or emergency assessment patients; 27% of these inpatients had been admitted for less than 2 days, and 70% admitted directly from home.
Conclusion: Feedback from the pilot demonstrated that the collection of the proposed dataset was achievable by the majority of Trusts and the time taken to collect and enter the data was acceptable. All 173 acute Trusts in England have access to the website, and are required to enter this mandatory national data set on MRSA bacteraemia. The key finding from the pilot study was that 27% of the MRSA positive bacteraemias were present on admission.

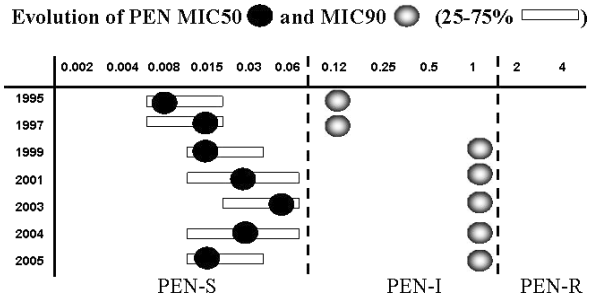
R1926

Evolution of antibiotic resistance in non-invasive clinical isolates of *Streptococcus pneumoniae* collected in Belgium from 1995 to 2005

R. Vanhoof, J. Van Eldere, J. Verhaegen *and the Belgian SP Study Group*

A total of 1971 non-invasive clinical isolates of *Streptococcus pneumoniae* collected during 7 surveys (1995, 1997, 1999, 2001, 2003, 2004 and 2005) by 15 laboratories evenly spread throughout Belgium, were included. Susceptibility to Penicillin (PEN), Amoxicillin (AMX), Cefuroxime (CRX), Cefotaxime (CTX), Ciprofloxacin (CIP), Moxifloxacin (MOX), Erythromycin (ERY) and Tetracycline (TET) was determined using the NCCLS standardised microdilution test. Overall, 26.1% (18.5%–31.0%) of the isolates were from children (0–15 y) and the mean age for the children and adults varied from 1.6 to 3.4 y and 62.5 to 66.4 y respectively. The insusceptibility rates (% I + R following NCCLS) in the various surveys were as follows: PEN (12.1; 12.2, 14.9; 19.8; 15.0; 14.7; 15.2), AMX (0; 0; 1.3; 2.0; 2.6; 1.2; 0.9), CRX (7.9; 9.8; 14.8; 17.0; 13.6; 12.7; 11.9), CTX (0, 0.6; 7.7; 4.0; 4.9; 6.2; 3.1), CIP (Not tested = NT; NT; 16.1; 12.9; 13.8; 9.0; 7.3), MOX (NT; NT; NT; NT; 0.6; 0.2; 0.2), ERY (20.0; 29.2; 32.9; 28.3; 26.1; 24.7; 29.9) and TET (26.4; 29.2; 21.9; 37.2; 32.3; 22.1; 26.4). In every survey we found a bimodal distribution for the beta-lactams. The MIC50 for PEN shifted with 3 dilutions to the right side of the curve from 1995 (0.008 μg/ml) to 2003 (0.06 μg/ml) and returned to 0.015 μg/ml in 2005. The same phenomenon was found for AMX (2 dilutions, return 1 dilution, from 0.008 to

0.015), CRX (2 dilutions, return 1 dilution, from 0.015 to 0.03) and CTX (3 dilutions, return 2 dilutions, from 0.008 to 0.015). CIP and MOX had a mono-modal distribution and their MIC50 remained between 0.5–1.0 (CIP) and 0.06 μg/ml (MOX). The MIC50 of ERY and TET shifted with 3 and 2 dilutions (0.008/0.06 and 0.12/0.25 μg/ml) respectively. Resistance rates for CIP were systematically higher in lower respiratory tract (LRT) isolates while TET rates were always higher in upper respiratory tract (URT) isolates. However, significant differences were only found for PEN (1995, 2005/URT) and ERY (1999, 2004/URT). Overall, the resistance rates for ERY and TET were always higher in children than in adults but significant differences were only found in 1997, 2004 (ERY), 2004 (TET) and 1995 (PEN). Throughout the years, capsular types 6, 9, 14, 19 and 23 were the most frequent in the PEN-R isolates. Type 23 evolved from 61% in 1995 to 20.6% in 2005. Type 14 appeared in 2001 (22.7%) and became the most important type (2004; 41.7%/2005; 28%).



R1927

Investigation of inducible clindamycin resistance among hospital and community-acquired *Staphylococcus aureus* isolates in the Aegean region of Turkey

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Objective: Clindamycin is a good alternative for the empirical treatment of *Staphylococcus aureus* infections. But clindamycin-susceptible and erythromycin resistant *S. aureus* may develop inducible clindamycin resistance; this resistance is detected by the disk diffusion induction test (D-test) and varies according to geographic location and bacterial susceptibility profile. The aim of this study was to investigate some antibiotic susceptibilities and inducible clindamycin resistance of *S. aureus* in our region.
Methods: In this study, 152 *S. aureus* strains were investigated. Of these strains, 89 were isolated from inpatients, 63 from outpatients attending to two distinct university hospitals. The antibiotic susceptibilities were determined by Kirby Bauer disk diffusion method using Mueller-Hinton agar (OXOID) and inducible clindamycin resistance by disk diffusion induction test (D-test) according to the recommendations of CLSI. The susceptibilities to oxacillin (OX), cefoxitin (FOX), clindamycin (DA), rifampin (R), erythromycin (E), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE), gentamicin (CN), ciprofloxacin (CIP) and vancomycin were examined. Quality control was performed with *S. aureus* ATCC 25923 and ATCC 43300 (MRSA).
Results: Susceptibility rates of *S. aureus* strains isolated from hospital and community to oxacillin were 13.4%, and 100 %, respectively. Community acquired MRSA was not detected. Of 152 strains, 12 (13.4 %) were D-test positive. Of these D-test positive strains, 10 (83.2 %) were nosocomial MRSA, 1 (8.4 %) was nosocomial MSSA and 1 (8.4 %) was community acquired MSSA. All of E resistant and DA susceptible isolates were positive by D-test. Among D-test positive strains, only one (8.4 %) strain was found as resistant to SXT whereas R, CN and CIP resistance rates were 9 (75%), 10 (83.3%) and 11 (91.6%), respectively. All strains included in the study were susceptible to vancomycin. Other antibiotic susceptibility rates of isolates are shown in Table.

Antibiotics	Nosocomial n (%)		Community n (%)		Total n (%)
	S	R	S	R	
OX	12	77	63	0	152
FOX	12	77	63	0	152
TE	15	74	60	3	152
SXT	88	1	63	0	152
R	17	72	61	2	152
CIP	15	74	61	2	152
CN	13	76	60	3	152
DA	38	51	59	4	152
E	29	60	59	4	152

Conclusion: The ratio of inducible clindamycin resistance is important in cases treated with clindamycin empirically and it varies between hospitals. Therefore, it must be known inducible clindamycin resistance in every region. According to our data, clindamycin still can be used empirically in MSSA infections but especially D-test should be evaluated for E resistance strains in each individual laboratory.

R1928

Factors of pathogenicity of *Pseudomonas aeruginosa* treated with amikacin, ciprofloxacin and meropenem

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Objectives: Gram-negative nonfermenting bacteria *P. aeruginosa* are one of the bacterial species very often responsible for nosocomial infections as well as for chronic lung infections including cystic fibrosis. Effect of subinhibitory concentrations (sub-MICs) (1/4, 1/8, 1/16 of the MICs) of amikacin, ciprofloxacin and meropenem on some potential factors of pathogenicity of two clinical *P. aeruginosa* strains (1010, 2643) was tested.
Methods: Potential factors of pathogenicity were evaluated *in vitro* using test for hydrophobicity (adherence to xylene), motility (0.35 % agar), production of biofilm (microtitre plate assay), HSLs (biosensors: *Chromobacterium violaceum* 026 and *Agrobacterium tumefaciens* NTL4) and response to oxidative stress evoked by hydrogen peroxide.
Results: Amikacin at 1/4 of the MICs, ciprofloxacin and meropenem at two or at all sub-MICs concentrations decreased surface hydrophobicity both of strains to 90.1–99.6% of the control values. Swimming motility of strain 2643 treated by all antibiotics ranged between 85.0% and 98.8% of the control values. Decrease of this activity in strain 1010 was found only after incubation with ciprofloxacin. The tested antibiotics at all concentrations (with the exception of 1/16 of the MIC of amikacin in strain 2643) reduced production of biofilm (to 90.6–99.4% of the control values). On the other hand, increase of resistance to oxidative stress was observed after effect of ciprofloxacin /two or three concentrations/ for both strains, after amikacin and meropenem /two concentrations/ only for strain 2643. Production of N-acylhomoserine lactone signal molecules (C4-HSL and 3-oxo-C12-HSL) by antibiotic treated bacteria was not changed.
Conclusion: The tested antibiotics at sublethal concentrations that do not kill bacteria in the majority of cases affected potential factors of *P. aeruginosa* in a dependence on strain, antibiotic and concentration.

R1929

Relationship between the presence of pathogenicity islands and serotypes, antimicrobial resistance and geographical origin in *Salmonella* spp.

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Objective: A potential relationship between acquisition of antimicrobial resistance and the presence of Pathogenicity Islands (PAIs) has previously been described in other microorganisms. Our main objective was to analyse the relationship between the presence of PAIs and antimicrobial resistance, serotypes and geographical origin in a collection of *Salmonella* spp. strains.
Methods: A total of 29 *Salmonella* strains causing traveller's diarrhoea and 26 *Salmonella* strains causing acute gastroenteritis in Cuba were analysed. The susceptibility to antimicrobial agents was determined by the Kirby-Bauer method. The different PAIs were detected by PCR with specific primers for each.
Results: All the *Salmonella* strains studied presented resistance to at least one antimicrobial agent. The PAIs studied were present in all the strains analysed. In some serotypes the PAIs were detected for the first time, corresponding to: Haifa, Goldcoast, Risseu, Paratyphi, kiambu and Wangata. These serotypes were isolated from the patients with traveller's diarrhoea. No relationship was found between antimicrobial resistance and the presence of PAIs.
Conclusions: The presence of PAIs was independent of the serotype and antimicrobial resistance. Moreover, these PAIs were detected for the first time in many different serotypes.

R1930

Trends in the antimicrobial resistance of some worldwide monitored pathogens during 2002–2004: data from BulSTAR

A. Bachvarova, M. Petrov, T. Velinov, T. Kantardjiev *(Sofia, BG)*

We present data on the antimicrobial resistance of some important bacteria collected during the 2002–2004 period in the Bulgarian Surveillance Tracking Antimicrobial Resistance – BulSTAR. The sources monitored in BulSTAR are blood cultures, cerebrospinal fluid, upper and lower respiratory tract, urine and wound samples. All participating laboratories use the Clinical and Laboratory Standards Institute methodology. Presented results concern some worldwide monitored pathogens – *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and their resistance to indicator antimicrobial agents. In most of the cases the resistant levels are rising during the period as in the case of Methicillin Resistant *Staphylococcus aureus* – from 6.0% to 13.9% and the Extended-spectrum beta-lactamases *Escherichia coli* producers – from 1.0% to 4.8%. An interesting exception is the observed decrease in macrolide resistance among *Streptococcus pyogenes* isolates – from 10.4% to 6.6%.

R1931

Staphylococcus aureus and coagulase negative *Staphylococcus* bacteraemia: epidemiology and antimicrobial resistance between 2001 and 2004 in the Ile de France microbiologists network

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Objectives: To survey epidemiology and antimicrobial resistance of community acquired (CAB) and hospital acquired (HAB) Staphylococci bacteraemia
Methods: Since 1st January 2001 to 31st December 2004, all bacteraemia (7222) occurring in surgical, medical obstetrical and re-adaptation wards, were included in the prospective survey study. CA and HA bacteraemia were separated according to the clinical history. Incidence rates were calculated for 1000 days of hospitalisation (HInc) and for 100 hospital admission (AInc). Antimicrobial susceptibility and resistance surveillance were studied according to the french recommendations: CA-SFM (www.sfm.asso.fr) and ONERBA (www.onerba.org). Statistical analysis were performed using Chi^2 tests.
Results: For 4 years, 1144 *S. aureus* and 335 coagulase negative staphylococci (CNS) bacteremia have been included. HAB *S. aureus* HInc (2001–2004 respectively : 0.18, 0.20, 0.22, 0.20) was twice CAB *S. aureus* HInc and both were stable. Almost all CNS bacteraemia were HAB with a lower stable incidence (2001–2004 : 0.08, 0.09, 0.10, 0.08). Oxacillin resistant *S. aureus* significantly decreased, reaching 29% in 2004, followed by tobramycin resistance (25% in 2004), but no decrease of fluoroquinolones resistance was observed; Oxacillin susceptible and fluoroquinolones resistant strains seemed to increase (10% in 2004). Only one strain in 2003 was not fully susceptible to vancomycin. For CNS, increasing resistance was observed for oxacillin, gentamycin, erythromycin and fusidic acid (respectively in 2004: 60%, 46%, 60%, 47%). The increase of CNS resistance to fluoroquinolones (54%) and rifampicin (21%) was not significant. No vancomycin resistant CNS was isolated.
Conclusion: For severe infection like bacteraemia, routine surveillance (epidemiology and antimicrobial resistance) allow to update recommendations for initial antimicrobial therapy and participate to the impact evaluation of infection control and drug resistance monitoring measures.

R1932

Antibiotic resistance of staphylococci isolated from bloodstream infections

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Objective: The aim of the present study was to examine the resistance of *Staphylococcus aureus* and coagulase negative Staphylococci isolated from blood samples to vancomycin, teicoplanin, and quinupristin/dalfopristin during a 2 year period (October 2003–September 2005).
Methods: 492 non-repetitive isolates of Staphylococci, derived from blood cultures (Bactec, Becton Dickinson) of patients hospitalized in different wards of our hospital, were examined. The identification and the susceptibility testing were performed by the automated system VITEK-2 (Biomerieux). The MICs of the examined antibiotics were confirmed by E-test (AB Biodisk, Sweden), according to NCCLS guidelines.
Results: Identification resulted in 60 strains of *Staphylococcus aureus* and 432 strains of coagulase negative Staphylococci

CoNS. High levels of oxacillin-resistance were observed among both *S. aureus* and CoNS (65% and 73% respectively). Resistance rates didn't vary between the 2 years of the study for the rest antimicrobials. Resistance to fusidic acid was at 72% for *S. aureus* and 90% for CoNS, to gentamicin rates reached 69% for *S. aureus* and 84% for CoNS. Erythromycin-resistance rates were 69% for *S. aureus* and 84% for CoNS. Ciprofloxacin resistance rates were 33% for *S. aureus* and 70% for CoNS and as for trimethoprim/sulfamethoxazole resistance rates were 13% for *S. aureus* and 42% for CoNS. The first year (October 2003–September 2004) 1 resistant and 14 intermediately resistant to teicoplanin CoNS strains were isolated. The latter year (October 2004–September 2005) 1 intermediately resistant to teicoplanin strain of *S. aureus* was isolated. Also, 1 resistant to teicoplanin, 19 intermediately resistant to teicoplanin, 1 intermediately resistant to vancomycin and 2 resistant to quinupristin/dalfopristin strains of CoNS were isolated.

Conclusions: The coagulase negative Staphylococci resistance to antimicrobial agents was higher than that of *S. aureus*. The emergence of resistance to glycopeptides and streptogramins is being documented and shows an increasing tendency during the last two years. It is essential to survey for antimicrobial resistance in blood stream infections as new resistant strains appear to agents that were previously active against all Staphylococci.

R1933

Extended-spectrum beta-lactamases producing isolates of *Enterobacteriaceae* among hospitalised patients in a university hospital, Zagreb, Croatia

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Objectives: The aim was to determine the prevalence of ESBLs in *Enterobacteriaceae* isolates from hospitalized patients, their antibiotic susceptibilities and the transferability of resistance to oxymino-cephalosporins and aztreonam.

Methods: In total 76 ESBL producers were investigated (27 *E. coli*, 37 *K. pneumoniae*, 6 *P. mirabilis*, 4 *K. oxytoca* and 2 *E. cloacae*). Antibiotic susceptibility of ESBL producing isolates and their *E. coli* transconjugants was determined by disk-diffusion and broth microdilution method according to NCCLS. MICs were tested for: amoxycilline/clavulanic acid, piperacillin/tazobactam, ceftazidime alone and in combination with clavulanic acid, ceftriaksone, cefotaxime, cefepime, aztreonam, imipenem, meropenem, gentamicin, and ciprofloxacin. Antibiotic susceptibilities to chloramphenicol, tetracycline and cotrimoxazole were determined by disk-diffusion method. ESBLs were detected by double-disc synergy method, and by >8-fold reduction in ceftazidime MIC in the presence of clavulanate. Transferability of R plasmids was determined by conjugation (broth mating method) employing an *E. coli* A15 R – strain resistant to rifampicin as recipient. Frequency of conjugation was expressed relatively to the number of donor cells. Cotransfer of resistance to non beta-lactam antibiotics was tested as well.

Results: The percentage of ESBL producers was as follows: 3% of *E. coli*, 17% of *K. pneumoniae*, 2% of *P. mirabilis*, 1% of *K. oxytoca*. There was no resistance detected to imipenem, meropenem, cefoxitin and ciprofloxacin among *E. coli* isolates. 67% of these strains were resistant to amoxycillin/clavulonate and ceftazidime, 70% to aztreonam and 78% to gentamicin. All *K. pneumoniae* strains were susceptible to imipenem, meropenem and cefoxitin, but 78% of the strains were resistant to ciprofloxacin, 86% to amoxycillin/clavulanic acid and 92% to aztreonam. All *K. pneumoniae* isolates were resistant to ceftazidime. 38% of *K. pneumoniae* strains transferred the plasmid encoding ESBL to *E. coli* recipient with the frequency ranging from 10^{-5} to 10^{-10}. Transfer of plasmid was successful in 96% of *E. coli* isolates with the frequency of 10^{-5} to 10^{-10}. Transconjugants had similar resistance patterns to beta-lactam antibiotics as their respective donors.

Conclusions: No resistance to carbapenems among *Enterobacteriaceae* was observed so far. The prevalence of ESBL isolates is in concordance with the prevalence in Croatia. Further analysis of the type of beta-lactamase is necessary.

R1934

Detection of production of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* spp. isolated from hospitalised patients as part of the MYSTIC Program Brazil 2004

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Objective: To determine the ESBL production in clinical isolates of *E. coli* and *Klebsiella* spp from the MYSTIC Program Brazil 2004, identifying possible differences among confirmatory methods.

Methods: One hundred and thirty (130) clinically relevant isolates of *E. coli* (29), *K. pneumoniae* (94) and *K. oxytoca* (7) collected as part of the MYSTIC program Brazil 2004 were randomly selected for the study if their minimal inhibitory concentration (MIC) for cefepime (CPM), ceftazidime (CTZ) or cefotaxime (CTX) by E-test® methodology was ≥ 2mcg/mL (NCCLS, 2004). ESBLs were confirmed via CTZ/clavulanate (CTZ/TZ) and CTX/clavulanate (CTX/TX) E-Test® for all isolates. A difference of more than three dilutions in the results was considered positive for ESBL, according to manufacturer's instructions. Disc approximation tests were applied in all isolates, but a positive reading was only considered confirmatory when at least one of the E-test® ESBL results was either positive or indeterminate for the same isolate.

Results: All isolates as a group (*E. coli* and *Klebsiella* spp) were 88.5% positive when both ESBL E-test® were used, CTZ/TZ and CTX/TX, with 11.5% indeterminate. For *E. coli*, the consolidated E-test results were 93.1% positive, with no negative results. For *E. coli*, no negative results were observed with either ESBL E-test® separately. For *Klebsiella* spp, the consolidated E-tests were 89.1% positive, with no negative results. For *Klebsiella* spp, CTZ/TZ ESBL test presented five negative results and 16 indeterminate, while CTX/TX had only one negative result and 15 indeterminate. Disc approximation tests were positive for all indeterminate isolates.

Conclusions: The quantitative method used for confirmation of ESBLs showed the presence of these enzymes in the majority of the isolates, although with technical limits of detection. Consolidated results were guided by the CTX/TX test, since positive results were more frequent and negative results occurred less with this E-test®. This seems to confirm that cefotaxime was a better substrate for the detection of ESBLs in the environment analyzed.

R1935

Nosocomial strain of *Serratia marcescens* producing new type of extended-spectrum beta-lactamase transfers the multidrug-resistance

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Objectives: To monitor the production of extended-spectrum beta-lactamases and transferability of antibiotic resistance from clinical isolates of *Serratia marcescens*.

Methods: The strain No. 37/364 was isolated from the sputum of a patient hospitalized in the Dept. of Anesthesiology of the Regional Hospital Pribram, Czech Republic. Antimicrobial susceptibility testing was performed by the disc diffusion test. ESBLs profile of strain was performed using double-disc diffusion test. Transfer of genes for antibiotic resistance was performed in mixed liquid cultures. As recipient strains were used *Escherichia coli* K-12 No. 3110 rif+ and *Proteus mirabilis* P-38 rif+.
Results: The double disc diffusion test demonstrated an unusual broad spectrum of the activity of ESBLs hydrolyzing cephalosporins of higher generations (cefotaxime, ceftazidime, cefepime, cefoperazone) and aztreonam. The multidrug resistant strain of *Serratia marcescens* transferred directly to *E. coli* K-12 No. 3110 rif+ determinants of resistance to kanamycin, ticarcillin, cephalosporins of all generations (cephalothin, cefotaxime, ceftazidime, cefoperazon) as well as to aztreonam. Indirect selection procedure confirmed co-transfer of total spectrum of antibiotic resistance: kanamycin, ticarcillin, cephalosporins of all generations and aztreonam.
Conclusion: Production of ESBL(s) hydrolyzing cephalosporins of higher generations and transferability of resistance showed the possibility of spreading of antibiotic resistance in clinically important strains in hospital environment.

R1936

Results from the meropenem yearly susceptibility test information collection (MYSTIC) programme: report from two Croatian hospitals

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Objectives: Meropenem Yearly Susceptibility test Information Collection (Mystic) Programme is a global, longitudinal resistance surveillance network that monitors the activity of meropenem, only in selected centres that are prescribing meropenem. We now report the four years period results (2002-2005) for the antimicrobial potency of meropenem compared to other agents, from the two Croatian Hospitals.
Materials and methods: The 2 hospitals in Croatia (Clinical Hospital Center Zagreb and University Hospital Split) participate in the MYSTIC Programme. The minimum-inhibitory concentrations (MICs) were determined by broth microdilution method according to NCCLS.
Results: There was no resistance to either imipenem or meropenem observed for *E. coli*, *K. pneumoniae* and *Proteus mirabilis* in both medical centres. *E. coli* strains from Split were more resistant to ceftazidime (8%) and cefepime (4%) than those from Zagreb (1%, and 0.7%, 2% respectively) whereas isolates from Zagreb were more resistant to ciprofloxacin (7%) and gentamicin (13%) compared to those from Split (2% and 12% respectively). There was a higher percentage of resistance among *K. pneumoniae* isolates in comparison to *E. coli* from both centers to ceftazidime (28% from Zagreb and 44% from Split) and to cefepime (14% from Zagreb and 20% from Split). Genamicin and ciprofloxacin were also less active against *K. pneumoniae* from both centres (28% strains resistant to gentamicin from Zagreb and 37% from Split, 10% of the strains resistant to ciprofloxacin from Zagreb and 8% from Split). Meropenem was the most potent agent against *A. baumannii* and *P. aeruginosa* strains from both centres. There was a high percentage of *A. baumanii* strains from Split resistant to all beta-lactam agents (above 80%). More *P. aeruginosa* strains from Zagreb were resistant to gentamicin and ciprofloxacin compared to those from Split. Imipenem showed best activity against Gram-positive cocci from both centres.
Conclusions: According to our results meropenem remains the antibiotic of choice for the treatment of severe infections caused by Gram-negative bacteria. Resistance to carbapenems observed in some *P. aeruginosa* strains could be due to production of metallo-beta-lactamases, which are already detected in some of our strains (unpublished data), alterations in outer membrane proteins and efflux. Carbapenem resistance found in *A. baumannii* isolates from Split could be explained by the production of oxacillinases (unpublished data).

R1937

Subinhibitory concentrations of clarithromycin in vitro readily select for resistance in clinical isolates of *Streptococcus pneumoniae*

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Objectives: Subinhibitory concentrations were described to have an influence on resistance development to antibiotics.
Methods: 20 clinical isolates of *S. pneumoniae*, 14 clarithromycin susceptible, 2 clarithromycin intermediate and 4 clarithromycin resistant (MICs: 0.031 to 16 mg/L), were exposed to subinhibitory concentrations of clarithromycin. Multistep resistance selection was performed by subculturing the strains in 1 ml medium with antibiotic concentrations varying from 3 doubling dilutions above to 3 doubling dilutions below the MIC of each strain. Daily passages were performed with the strain growing at the highest concentration. Serotypes, macrolide resistance genotypes and phenotypes and presence of the ermB and the mefE gene were determined.
Results: 10 of 20 isolates showed a 4 to 2048 times increase in MIC after 15 to 20 days of subculturing under subinhibitory selection pressure. The increase was not steady. Most strains needed a long 'lag phase' before the MIC started to increase. In 7 out of 20 cases the strains 'fell back' to a lower MIC (or even full sensitivity) but then continued their increase to higher MICs. The highest increase in MIC was 2048 fold (0.125 to 64 mg/L). The highest obtained MIC was 256 mg/L for a strain with a starting MIC of 16 mg/L. The reference strain *S. pneumoniae* ATCC 49629 (CLSI MIC: 0.03-0.125 mg/L) showed no difference in its MIC after 20 days under antibiotic pressure at subinhibitory levels. None of the strains had obtained either an ermB or a mefE gene. Therefore other resistance mechanism must be responsible for resistance development.
Conclusions: Subinhibitory macrolide concentrations readily lead to the selection of resistant strains even within a relatively short period of 20 days. This implies that non or poor compliance in antibiotic treatments leads to accelerated resistance development.

R1938

Macrolides resistance evolution and telithromycin utility in *Streptococcus pyogenes* strains from faringeal origin. Five-year study

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Streptococcus pyogenes is the most common cause of bacterial pharyngitis, penicillin continues to be the drug of choice for treatment of streptococcal infections and macrolides are its

alternative in patients who cannot take penicillin. However, the utility of this group of antibiotics is being limited by the acquisition of different resistance mechanisms.

Objectives: 1 To know the rate of macrolides resistance and its phenotypes distribution among pharyngeal strains as well as its evolution during 5 years. 2 To see the utility of telithromycin as therapeutic alternative in macrolides-resistant strains.

Methods: During the period, 2000–2004 a total of 853 SPY strains were studied in our laboratory. They were isolated from throat swabs cultured in SSA plates (BD Diagnostics) and incubated at 35°C in anaerobic atmosphere 24–48 hours. The identification was done by commercial antigenic extraction (Bio-Merieux). Strains were studied for susceptibility to penicillin, erythromycin, clindamycin, and tetracycline. Telithromycin was only tested last year (2004). We used the disk diffusion method in Muller-Hinton agar supplemented with 5% sheep blood according to NCCLS standards. In this way we were also able to detect the inducible MLSB phenotype by the double diffusion disk test.

Results: Overall, the number of erythromycin resistant strains during the five years was 195 (23%), 145 (74%) of them expressed M phenotype, 41 (21%) constitutive MLSB phenotype and 9 (5%) inducible MLSB phenotype. The global resistance, the annual rates, the different phenotypes and the resistance to telithromycin are shown in the next table.

Year	Strains tested	Erithromycin Resistance n %	M phenotype n %	Constitutive MLSB phenotype n %	Inducible MLSB phenotype n %	Telythromycin Resistance n %
2000	127	20 15.7	19 95	1 5	0 0	-
2001	147	21 14.3	20 95.2	1 4.8	0 0	-
2002	168	24 14.3	22 91.7	1 4.2	1 4.2	-
2003	210	65 31	39 60	22 33.8	4 6.1	-
2004	201	65 32.3	45 69	16 24.6	4 6.1	13 6.5
TOTAL	**853**	**195 23**	**145 74**	**41 21**	**9 5**	

Conclusions: 1 The frequency of resistance to erythromycin in SPY from pharyngeal exudates increased twice during the study period. Both the medium resistance (23%) and the range (14.3%–32.3%) remain within the published limits for Spain.2 The resistance mechanism also suffered a substantial change during the 5 years: M phenotype decreased (95%–74%) while the constitutive MLSB phenotype increased (5%–21%). This implies resistance not only to erythromycin but to all macrolides and clindamycin. Therefore it would leave penicillin allergic patients without any therapeutic alternative.3 Telithromicyn displayed a good activity *in vitro* against erythromycin resistant SPY. According to these results, telithromicyn offers a wide coverage against SPY in patients where we cannot use penicillin, macrolides or clindamycin.

R1939

Surveillance of methicillin-resistant staphylococci among small animal isolates from otitis externa in Portugal

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Objectives: To characterize resistance to methicillin among staphylococci causing otitis externa in cats and dogs, in order to evaluate the impact of antibacterial mechanisms resistance in small animal clinical strains.

Methods: The samples were collected at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine and at Veterinary private practices in the Lisbon area. From January 1999 until October 2005, a total of 217 staphylococci were isolated from dogs and cats with otitis externa. Cytology confirmed otitis externa and the presence of Gram-positive cocci. BBL Crystal Gram Positive ID System (Becton Dickinson) was used for identification at the species level. All isolates were screened for methicillin resistance by disc diffusion with a 1 μg oxacillin disc, according to NCCLS (1). Methicillin resistance was confirmed by the presence of the mecA gene using PCR.

Results: Three coagulase-negative isolates (1.4%) (2 *Staphylococcus haemolyticus* and 1 *S. epidermidis*) were phenotypically resistant to methicillin and carried mecA. Regarding coagulase-positive isolates, none of the 122 (56%) *S. intermedius* and 8 (36%) *S. aureus* isolates were methicillin resistant. Resistance to methicillin was also not detected among the remaining staphylococci isolated in the study: 30 (13.8%) *S. schleiferi*, 10 (46%) *S. simulans*, 4 (18%) both *S. haemolyticus* and *S. felis*, 1 (0.5%) both *S. lentus* and *S. caprae*, and 35 (16%) *Staphylococcus* spp.

Conclusions: Staphylococcal methicillin-resistance surveillance is a major concern worldwide. Our results are different from recent data (2), in the fact that we only found methicillin resistance among otitis externa coagulase negative staphylococci, which points out to the importance of local surveillance studies. Our findings are of critical relevance, as they may have a direct impact in therapeutic decision in the management of companion animal's infections. Furthermore, transfer of resistance markers and resistance strains between animals and owners/caretakers is a strong possibility either by infection or direct contact.

References:

(1) NCCLS (2002) Performance standards for antimicrobial disk and dilution susceptibility for bacteria isolated from animals; approved standards – second edition – M31 – A2.

(2) Duquette R. A. and Nutall T. J. (2004) Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *Journal of Small Animal Practice* 45, 591–7.

R1940

Beta-lactam resistance in *Haemophilus parasuis*

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Objectives: *Haemophilus parasuis* is a commensal organism of the upper respiratory tract of conventional pigs that, under appropriate conditions, can cause the Glasser disease, characterized by severe systemic infection, fibrinous polyserositis, arthritis and meningitis. This is an emerging infection with increasing importance in swine production. In our laboratory, we are detecting an increase in the prevalence of resistance to beta-lactam antibiotics in *H. parasuis*, being this antimicrobial family the first clinical election against respiratory tract infections in swine. Resistance to beta-lactam antimicrobials has yet not been described nor characterized in *H. parasuis*.

Methods: Antimicrobial susceptibility test and MICs were performed folowing NCCLS standard procedures. Identification by PCR was performed with modified procedure of Oliveira, S. et al, 2001. Nitrocefine test was done using OXOID beta-lactamase identification sticks. CAMP test was performed following standard procedures. DNA digestions and cloning manipulation were performed following standard procedures.

Results: Phenotypic characteristics and specific tests were used for presumptive identification of *H. parasuis*. We have developed a PCR based on specific amplification of 821 pb from the coding gene of the 16S subunit rRNA. We confirmed 67 *H. parasuis* isolates and tested them for antimicrobial susceptibility, 10 were highly resistant to ampicillin (MIC ≥ 32 microg/ml). All resistant isolates showed positive reaction to the nitrocefine test, indicating that beta-lactamases enzymes are involved in this phenomenon. This kind of resistance mechanism is usually harboured in plasmid in other swine respiratory pathogens. The plasmid profile analysis of these strains in agarose gel electrophoresis, after digestion with PstI, was indifferenciable in 8 of the 10 strains. We attempted to transform plasmid DNA directly into *E. coli* altuogh results were negative, indicating that replication origin is specific to *Haemophilus* spp. Currently we are cloning the gene responsible for this phenotype in *H. parasuis*.
Conclusion: We have identified beta-lactam resistance in *H. parasuis*. Resistance is due to beta-lactamases. We are characterizating the genetic determinants responsible for this emerging phenotype.

R1941

In vitro susceptibility to antibiotics of anaerobic bacteria isolates from periodontal pockets

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Introduction: The studies having as objective the sampling from periodontal pockets have confirmed constant implication of Gram-negative anaerobic bacteria belonging to *Bacteroides*, *Fusobacterium*, *Porphyromonas*, *Prevotella* and *Treponema* genera. Facing a microbial mixture with synergic action over marginal periodontium, the antimicrobial therapy should take account of the susceptibility of the germ complex, as a whole.
Method: The sampling – made by means of sterile paper points was followed by direct microscopy of smears and then microbial isolation on culture media (Schaedler Agar supplemented with 5% sheep blood) incubated in anaerobiosis. Identification of the isolates was made by biochemical tests performed using commercially available kits – Rapid ID 32 A (Biomerieux, France) and antibiotic sensibility testing by means of Etest at 5 agents: Ampicillin/ Sulbactam; Azithromicine; Doxiciclyne; Erithromicyne and Tetraciclyne.
Results and conclusions: In our studies, the most frequent bacteria isolated from periodontal pockets belonged to Prevotella, with *Prevotella oralis* on first position, followed by *Fusobacterium*, *Bacteroides* and *Porphyromonas*, and a few isolates of *Peptostreptococcus*. Sensibility of isolates was 100 % at Ampicillin/ Sulbactam; Doxiciclyne and Erithromicyne (91.7 %); Azithromicine (83.3%); Tetraciclyne (66.6 %). The resistant isolates belonging to Peptostreptococci and Fusobacteria and shown resistance to Tetraciclyne and Azithromicine. According to our findings, for the treatment of periodontitis it is recommended either:- The use of a commercially available association a betalactamic agent with a betalactamase inhibitor or- Doxicicline.

R1942

Community-acquired urinary tract infections in 2004: results from BulSTAR

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Bulgarian Surveillance Tracking Antimicrobial Resistance – BulSTAR monitors the isolation and antimicrobial susceptibility of clinically significant microorganisms from blood cultures, cerebrospinal fluid, upper and lower respiratory tract, urine and wound samples in the participating Bulgarian microbiology laboratories since 1997. In 2004 the number of participants was 103 – 23 public, 53 hospital and 27 private microbiology laboratories. Clinical and Laboratory Standards Institute methodology was used in all participating centres. This presentation provides an update on the current state of etiological structure and antimicrobial resistance of community-acquired urine pathogens in Bulgaria based on BulSTAR data for 2004. The number of positive samples was 21 202–14 298 from female outpatients and 6904 from male outpatients. The etiological structure in both sexes is similar with the leading pathogen *Escherichia coli* – 65.2% and 51.8% respectively, followed by *Proteus mirabilis* – 8.3% and 12.0%. The difference is in the third position taken by *Enterococcus faecalis* – 4.6% among women and by *Pseudomonas aeruginosa* – 8.1% among men. The main therapeutic problem is *Pseudomonas aeruginosa* with it's resistance to ceftazidime – 21.7%, to gentamicin – 31.5 and to ciprofloxacin – 32.1%. High-levels of resistance are also found against the two important antibacterial agents for ambulatory treatment of *Escherichia coli* – ampicillin and trimethoprim/ sulfamethoxazole.

R1943

Surveillance of Antimicrobial Susceptibility of the *Bacteroides fragilis* group at a teaching hospital in Maribor, Slovenia, between 2002 and 2004

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Objectives: Antibiotic resistance among anaerobic organisms started to increase in recent years. The aim of the present study was to investigate the susceptibility of the *Bacteroides fragilis* group isolates to metronidazole, ampicillin – sulbactam (AM-SB) or amoxicillin-clavulanate (AM-CL), clindamycin and penicillin.
Methods: A total of 153 isolates of anaerobic bacteria were collected at the Department of Gynecology from clinical samples (wounds, fistulae, abscesses, endometrium, peritoneal cavity, blood) of 116 patients during a 3-year period (2002–2004). The isolates included: *B. fragilis* group 32 (21%), other *Bacteroides* spp. 9 (5.9%), *Fusobacterium* spp.16 (10.5%), *Prevotella* spp. 7 (4.6%), other anaerobic gram-negative bacilli 13 (8.5%), *Propionibacterium* spp. 18 (19.8%), *Eubacterium* spp. 5 (3.3%), *Clostridium* spp. 3 (2%), *Actinomyces* spp. 3 (2%), other gram-positive bacilli 8 (5.2%), *Peptostreptococcus* spp. 36 (23.5%), *Peptococcus* spp. 1 (1.3%) and anaerobic gram-negative cocci 1 (1.3%). We investigated the susceptibility of the predominant gram-negative isolate B. fragilis group. Antimicrobials tested were metronidazole, AM-SB or AM-CL, clindamycin and penicillin. Routine in vitro susceptibility testing was performed using the EtestR (AB Biodisk, Solna, Sweden) according to recommendations of the producer.
Results: Metronidazole (32) and AM-SB or AM-CL (32) were all highly active (resistance rates 0%), whereas 8 (25%) isolates were resistant to clindamycin. All isolates (32) were resistant to penicillin.
Conclusion: The present data show an emergence of resistance of the *B. fragilis* group against clindamycin. Consequently the importance of routine in vitro susceptibility testing of anaerobic bacteria is growing, especially as clindamycin is used in empirical therapy to treat anaerobes at the Department of Gynecology. With the local susceptibility data it is possible to choose the most active antibiotics for treatment of anaerobes.

R1944

Determine variation of antimicrobial susceptibility patterns according to five years in intensive care unit of a university hospital

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Background: Antimicrobial resistance is a significant problem in the intensive care unit (ICU). There are many factors that contribute to the high rate of antibiotic resistance in the ICU, but one factor having major impact is the ecological pressure exerted by high usage of antibiotics in critically ill patients.
Methods: We conducted a five-years prospective study of the hospital-acquired infections and colonization causative microorganisms and antimicrobial resistance in a 11-bed medical and surgical ICU of a Karadeniz Technical University Hospital, Trabzon, Türkiye. From 2000 to 2004 all patients staying in ICU more than 48 h were consecutively enrolled and followed prospectively until discharge or death. From every patient, common specimens were sent for bacteriologic culture and sensitivity analysis.
Results: A total of 1400 bacterial pathogens were obtained from 728 patients in ICU during the study period. Of these, 1306 (93.3%) were single isolates and 94 (6.7%) were polymicrobial isolates. The isolates consisted of 797 (56.9%) Gram-negative strains, 495 (35.4%) Gram-positive strains and 108 (7.7%) *Candida* spp. The most common isolates were *Pseudomonas aeruginosa* (277; 19.8%), methicillin resistant *Staphylococcus aureus* (MRSA) (174; 12.4%), *Acinetobacter* spp (166; 11.9%), methicillin resistant coagulase-negative staphylococci (MRCNS) (122; 8.7%), *Enterobacter* spp (92; 6.6%). The most active agents were ticarcillin/clavulanate and amikacin against *P. aeruginosa*, imipenem against *Acinetobacter* spp, imipenem-cefoperazone/sulbactam-ciprofloxacin and amikacin against *Enterobacter* spp, *Klebsiella* spp and *E. coli*, trimetoprim/sulfamethoxazole against *S. maltophilia*, vancomycin against *S. aureus* , CNS and *Enterococcus* spp.
Conclusions: In determine variation of microorganisms according to five years, *P. aeruginosa* was the most prevalant microorganism, while MRSA and *Acinetobacter* spp remained to be less frequent. An increasing rathe of Gr(+) microorganisms has been overemphasised in recent years. We observed the presence of multiple antimicrobial resistant microorganisms, which play an important roll in massive colonization of patients and in the aetiology of nosocomial infections. In approach to empirical treatment, such microorganisms and their susceptibilities were primarily considered. This fact is very worrisome, exception a few therapeutic options are failure to treat infections caused by this multidrug-resistant microorganisms.

R1945

Antibiotic resistance for *Streptococcus pneumoniae* isolated from otitis media and invasive infections in children between 2001 and 2004 in Iasi, Romania

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Background: *S. pneumoniae* belongs to community species which developed multiple antibiotic resistance. Resistance profiles and ratio recognize regional differences.
Objectives: To follow-up the sensitivity against different antibiotic classes for *S. pneumoniae* strains isolated from two infection types: otitis media (characterized by frequency and motivation of antibiotic prescribing) and invasive infections (rare, but extremely severe).
Material and method: Our study comprised a number of 109 *S. pneumoniae* strains isolated from children aged between 2 months and 14 years old (68 children less than 2 years old), admitted in the ''Sf. Maria" Emmergency Pediatric Hospital from Iasi, Romania during 2001–2004, 26 strains being from invasive infections (pleural effusion, peritonitis, meningitis, arthritis, bacteraemia). Sensitivity testing was performed by standard disk diffusion method against following agents: Erythromycin (E), Clindamycin (DA), Tetracycline (TE), Chloramphenicol (C), Trimethoprim-sulfamethoxazole (SXT) and Linezolid (LNZ). Oxacillin (OXA)(1 microgram), Norfloxacin (NOR) (5 microgram)or Ciprofloxacin (CIP) (5 microgram) were used as ''indicator antibiotics" to detect Penicillin (P) and fluoroquinolone resistance, respectively. MICs for P, Cefotaxime (CTX), Clarithromycin (CLR), Levofloxacin (LVX) and Gatifloxacin for resistant isolates were detected by E-test (AB Biodisk, Solna, Sweden).
Results: Global P resistance was extremely high, 76.1%, the number of high level resistance (HLR) strains being approx. equal with that of low level resistance (LRR) strains: 43 and 40 strains, respectively. In strains isolated from invasive infections , the resistance ratio was 63.4%. In 24.8% of cases, P resistance was associated with CTX resistance. We found that the number of HLR strains was approx. equal with that of LRR-13 and 14 strains, respectively. Only two HLR strains were isolated from invasive infections (bacteremia). We also found high resistance ratio for antibiotics belonging to other classes: E, DA, TE, SXT, 57.8%, 56.9%, 56.9% and 82.6% respectively. With only one exception, macrolide resistance was associated with DA resistance, CLR MIC for these strains being ≥256 micrograms/mL. The lowest resistance ratios were found for C and fluoroquinolones, 4.6% and 0.92%, respectively. No LNZ resistant strain was found.
Conclusion: In our region, Vancomycin, LNZ, C or new fluoroquinolones are alternatives for multiresistant *S. pneumoniae* strains.

R1946

Multiresistant *Pseudomonas aeruginosa* isolations in a critical care unit

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Objective: To describe the incidence of multiresistant *Pseudomonas aeruginosa* isolations in blood cultures, bronchoaspirates, urinary specimens and surgical wounds from January 2004 to December 2004 in critical patients from a teaching hospital in Madrid.
Methods: A 1-year, retrospective, observational study was conducted. We studied a total of 1370 specimens from 393 patients. All blood cultures (n = 487), bronchoaspirates (n = 582), urinary specimens (n = 170) and surgical wounds (n = 131) from critical patients were cultured. Identification and susceptibility testing was performed in all positive samples using a standard microdilution broth method (MicroScan®, Dade Behring). Susceptibility breakpoints were based on Clinical and Laboratory Standards Institute (formerly NCCLS). No universal criteria of multiresistant strains have been accepted. Resistance to two or more of the following groups of antibiotics has been the criterium to define a multiresistant strain: carbapenems, monobactams, antipseudomonal cephalosporins, fluoroquinolones, aminoglycosides and aminopenicillins. Colonization and infection were not discriminated.
Results: *Pseudomonas aeruginosa* was isolated from 35 samples (2.6%) corresponding to 22 patients (5.6%). The distribution was

as follows: 22 (62.9%) from bronchoaspirates, 5 (14.3%) from blood cultures, 1 (2.9%) from urine culture and 7 (20.0%) from surgical wounds. Amikacin, imipenem, tobramycin, ciprofloxacin and cefepime were the most effective antibiotics. Five strains (14.3%) showed resistance or reduced susceptibility to imipenem; 19 strains (54.3%) were susceptible to all tested betalactams and 4 (11.4%) were resistant to all of them. Only 3 strains (8.8%) were amikacin resistant, and none was resistant to all aminoglycosides (table 1). Finally, 20 strains (57.1%) could be considered multiresistant.

Antibiotics	Susceptible		Intermediate		Resistant	
	n	%	n	%	n	%
Piperacillin	25	73,5%			9	26,5%
Ticarcillin	23	67,6%			11	32,4%
Ceftazidime	27	77,1%	5	14,3%	3	8,6%
Cefepime	26	76,5%	6	17,6%	2	5,9%
Imipenem	30	85,7%	3	8,6%	2	5,7%
Ciprofloxacin	25	78,1%	1	3,1%	6	18,8%
Ofloxacin	22	62,9%	7	20,0%	6	17,1%
Gentamycin	26	74,3%			9	25,7%
Tobramycin	27	84,4%			5	15,6%
Amikacin	31	91,2%			3	8,8%
Aztreonam	21	61,8%	6	17,6%	7	20,6%

Conclusions: *Pseudomonas aeruginosa* can be considered one of the most important resistant pathogen in critical care units. Although susceptibility to some antipseudomonal antibiotics remains acceptable, multiresistant strains are an increasing problem reducing the number of available effective drugs. In addition, *Pseudomonas aeruginosa* easily acquires multiple resistance mechanisms and therapeutic choices decrease.

R1947

Staphylococcus aureus susceptibility to three antibiotics isolated from hospitalised vs. outpatients in a local area of Madrid

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Objective: To describe the differences between susceptibility patterns to cloxacillin, erythromycin and ciprofloxacin of *Staphylococcus aureus* (SA) strains isolated from skin lesions and wound exudates from hospitalized and outpatients attended in a local health care area of Madrid.
Methods: A total of 2108 skin lesions and wound exudates were analysed, 1123 corresponded to hospitalized patients and 985 to outpatients from January 2003 to December 2004. Cloxacillin, erythromycin and ciprofloxacin susceptibility patterns using a commercial microdilution broth method (MicroScan®, Dade Behring laboratories) were performed. Susceptibility breakpoints were based on Clinical and Laboratory Standards Institute (formerly NCCLS). Resistance rates are described as percentage and have been analysed using Chi^2-test. Statistical significance has be considered in all cases with $p < 0.05$ values.
Results: *Staphylococcus aureus* was isolated from 153 (13.6%) inpatient specimens and 226 (22.9%) from ambulatory samples ($p < 0.001$). Cloxacillin resistance was observed in 41 (26.8%) inpatient samples and in 52 (23%) ambulatory samples ($p = 0.47$). Erythromycin resistance was observed in 48 (31.3%) inpatients and in 71 (31.4%) outpatients ($p = 0.92$). Finally, 49 (32%) inpatient samples and 57 (25.2%) outpatient samples were resistant to ciprofloxacin ($p = 0.18$) (table 1).

	Cloxacillin		Erythromycin		Ciprofloxacin	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Inpatients	41 (26,8%)	112 (73.2%)	48 (31.3%)	105 (68.7%)	49 (32.0%)	104 (68.0%)
Outpatients	52 (23,0%)	174 (77.0%)	71 (31,4%)	155 (68.6%)	57 (25.2%)	169 (74.8%)
		p=0.47		p=0.92		p=0.18

Conclusions: *Staphylococcus aureus* isolations were more frequent in outpatients specimens than in hospitalized samples. Erythromycin susceptibility patterns were similar between hospitalized and ambulatory patients. Although cloxacillin and ciprofloxacin resistance differences have been found, there was not statistical significance.

R1948

Two-year surveillance of antimicrobial susceptibility in *Pseudomonas aeruginosa* isolates from a hospital centre, Coimbra, Portugal

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Objective: *Pseudomonas aeruginosa* (PA) is one of the leading causes of nosocomial infections. Severe infections, such pneumonia or bacteraemia are associated with high mortality rates, often difficult to treat, because useful anti-pseudomonal agents are limited. Surveillance of antimicrobial susceptibility of PA clinical isolates has been monitored in this study, since the determination of guidelines for empirical regimens and prompt enforcement of infection control measurers are of great importance.
Methods: Isolates (N = 787) were collected during April 2003 to April 2005. They were identified with VITEK (BioMérieux) and MicroScan WalkAway (Dadebehring) and susceptibility patterns were determined with these panels. Susceptibilities to Piperacillin (PIP), Piperacillin plus Tazobactam (TZP), Aztreonam (AZT), Ceftazidime (CAZ), Imipenem (IP), Meropenem (MP), Amikacin (AMK), Tobramicin (NN), Gentamicin (GN), and Ciprofloxacin (CIP) were guideline by NCCLS.
Results: In the set of 787 isolates, 522 were obtained from nosocomial infections and 265 from community acquired infections of different clinical specimens, including urine (15.5%, 36.6%, respectively), sputum (49.2%, 32.1%), exudates (14.6%, 14.3%), blood (4.4%, 4.5%), and others sources (16.3%, 12.5%). Among all isolates MP was most potent antibiotic (89.8% susceptible), GN and CIP were the worst agents (63.8% and 69.0% of susceptibility, respectively). Susceptibilities rates varied significantly between nosocomial and ambulatory infections. Among the nosocomial, TZP (83.5%), CAZ (80.3%), and PIP (79.5%) demonstrated better activity than IP (76.4%), but MP had better result (85.2%). Amikacin had improved susceptibility (82.0%), but GN is the worst (58.4%). CIP inhibited 64.6% of these isolates. Ambulatory isolates were more susceptible, and susceptibilities were above ninety per cent, except to CIP (77.7%) and GN (74.3%). Carbapenems resistance had increased in second year relatively to first year (7.9% for IP and 6.4% for MP) and equal to CAZ (5.3%). Amikacin improved activity (7.5%).
Conclusions: Considering the results and the high prevalence of multidrug resistant strains (10.2%) (resistance to three or four of these agents: PIP, CAZ, IP and CIP) in this hospital environment, it is necessary to detect and follow sources of infection, in order to prevent the spreading and transmission of

these strains. Susceptibility rates should be determined and effective antibiotics usage policy should be performed.

R1949

Antibiotic resistance in the community environment: a regional epidemic survey in the Pays de la Loire region by MedQual

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Objectives: MedQual was created in January 2004 in the Pays de la Loire Region, supported by public health insurance funds (FAQSV), as an answer to the circular DHOS/E2-DGS/SD5A n° 272, May 2nd 2002 ''concerning the good use of antibiotics in health care centres and the setting up of experimental centres to give advice to physicians on the good use of antibiotics''. This resource network for the community and hospital health workers of the Region is in accordance with the ''plan to preserve the effectiveness of antibiotics'' of the Health Ministry. MedQual is doing an epidemiologic survey on *E. coli* in the Region to see the evolution of some antibiotics' susceptibility for *E. coli* community samples. We survey the resistance to antibiotics currently used for these infections.
Methods: A regional prospective epidemiologic survey of *E. coli* has been carried out with 18 microbiology laboratories in 2004 of the 5 departments of the Pays de la Loire region. They send their results monthly to MedQual. They use data collection sheets developed and validated by MedQual. The resistance of *E. coli* to aminopenicillin, amoxicillin + clavulanic acid, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, and pefloxacin is supervised. The results of this survey are given on the MedQual website (http://www.sante.univ-nantes.fr/medqual) monthly, for the physicians to adapt their antibiotics prescriptions to the local microbial ecology.
Results: After one year of survey, we have obtained 16367 samples for *E. coli*; 95.94% are urine samples. The rates of *E. coli* resistance in the Region are 39.01% for aminopenicillin, 22.54% for amoxicillin + clavulanic acid, 11.15% for nalidixic acid, 10.11% for ofloxacin, 8.29% for norfloxacin, 6.56% for pefloxacin and 5.49% for ciprofloxacin.
Conclusion: This regional survey of *E. coli* continued with 30 microbiology laboratories in 2005. The variabilily amongst different departments confirms the need for local resistance prevalence data to be available to the practitioners who treat urinary infection. Ongoing surveillance studies are required to assess the evolving resistance patterns in community environment. MedQual shows an efficient collaboration with the private medicine network (URML) for a joint action aiming at the good use of antibiotics and at the survey of the evolution of the bacterial resistances of *E. coli*.

R1950

Antibiotic susceptibility pattern of nosocomial Gram-negative pathogens: results from MYSTIC study in a university hospital, Ankara, Turkey (2000–2004)

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Objectives: The aim of this study was to present the susceptibility pattern of 559 Gram negative nosocomial isolates involved in the MYSTIC programme in Hacettepe University Adult Hospital between 2000 and 2004.
Methods: Only 45% of the isolates were from patients in intensive care units. The susceptibility testing was performed by Etest (AB BIODISK, Solna, Sweden) using CLSI citeria and percentage susceptibilities of *Escherichia coli* (n = 144), *Klebsiella pneumoniae* (n = 133), *Pseudomonas aeruginosa* (n = 144) and *Acinetobacter baumannii* (n = 138) against different antimicrobials were determined. Extended-spectrum ß-lactamase (ESBL) production of *E. coli* and *K. pneumoniae* were also obtained.
Results: The ESBL production rate of *E. coli* and *K. pneumoniae* isolates were 28.0% and 46.6%, respectively. All *E. coli* and *K. pneumoniae* isolates were susceptible to carbapenems. The susceptibility of ESBL (+) *E. coli* isolates to ceftazidim, cefepime, piperacillin + tazobactam and tobramicin were in between 60% and 75%. The susceptibility of ESBL (-) *E. coli* isolates to ceftazidim, cefepime, piperacillin + tazobactam and tobramicin were in between 84 and 95%, 57.8% to ciprofloxacin. The susceptibility rate of ESBL (+) *K. pneumoniae* isolates to piperacillin + tazobactam and cefepime was 51–55%, 42–49% to ceftazidim, tobramicin and ciprofloxacin. The susceptibility rate of ESBL (-) *K. pneumoniae* isolates to seftazidim, piperacillin + tazobactam and ciprofloxacin was 60–70%, 87–92% to cefepim and tobramicin. Of the *P. aeruginosa* isolates, 77% were multi-drug resistant. The susceptibility of *P. aeruginosa* isolates to piperacillin + tazobactam, carbapenems and ciprofloxacin was 50%. Of the *A. baumannii* isolates, 67% were multi-drug resistant with the higher susceptibility rates to meropenem and tobramicin 53% and 44%, respectively.
Conclusions: The ESBL production of *E. coli* and *K. pneumoniae* are high and carbapenems are reliable choices for the treatment of their infections. However, most of the infections caused by *P. aeruginosa* and *A. baumannii* have limited options.

R1951

Susceptibility patterns of *Klebsiella pneumoniae* to quinolones and beta-lactams

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Objectives: *Klebsiella pneumoniae* is has been recognized as major human pathogen and an important source of transferable antibiotic resistance. During the last decade an increasing resistance to commonly used antibiotics has been noticed. This fact is extremely worrying and limits the selection of antibiotics for the effective treatment of severe *K. pneumoniae* infections. The aim of the present study was to evaluate the resistance rates of *K. pneumoniae* strains to quinolones and b-lactams throughout a three year period and to identify trends, if possible, in the susceptibility patterns.
Methods: *K. pneumoniae* stains isolated from clinical specimens were reviewed from January 2002 to December 2004. The identification of microorganisms and susceptibility testing were performed using the Vitek 2 automated system (bioMerieux, France).
Results: A total of 824 non duplicate *K. pneumoniae* isolates were recorded. The sources of these *K. pneumoniae* strains were: blood cultures:(39.5%), wounds:(31.5%), urine:(19%), respiratory tract secretions:(9%) and cerebrospinal fluid:(1%).The resistance rates

Antimicrobial agent	Resistance rates (%) of K.pneumoniae stains in:			
	2002	2003	2004	p
ciprofloxacin	10.33	10.42	24	<0.0001
Piperacillin/ tazobactam	11.57	15.62	20.79	<0.01
Ticarcillin/ clavulanic acid	43.8	61.46	54.46	<0.05
ceftazidime	56.61	64.46	70.64	<0.001
imipenem	0	0	1.3	

of *K. pneumoniae* isolates are shown in the table: According to the statistical analysis performed using the z-test, an increasing resistance trend towards b-lactams and ciprofloxacin was observed during the study period.
Conclusions: The resistance rates of *K. pneumoniae* to ciprofloxacin, piperacillin/tazobactam, ticarcillin/clavulanic acid, and ceftazidime during the 3 year period of the study has increased significantly. What is really alarming is the emergence in 2004 of *K. pneumoniae* strains resistant to imipenem, which renders the selection of antimicrobial therapy difficult. More effective infection control measures, rational antibiotics policies and rapid laboratory detection of resistance are required to prevent the spread of resistant isolates in the hospital.

R1952

Antimicrobial susceptibility of six members of the family *Enterobacteriaceae* collected from 28 centres in a Central European area, 2004

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Objectives: Members of the family *Enterobacteriaceae* frequently cause nosocomial infections. The publication of current antimicrobial susceptibility data is a prerequisite for the optimal management of patients with hospitalized infections. The objective of this study was to evaluate the *in vitro* susceptibilities of clinical isolates of *Enterobacter cloacae* (ECL), *Escherichia coli* (ECO), *Klebsiella pneumoniae* (KPN), *Klebsiella oxytoca* (KOX), *Proteus mirabilis* (PMI), and *Serratia marcescens* (SMA) to various frequently used broad-spectrum antibacterial agents.
Methods: In November 2004, a total of 1786 isolates (ECL, n = 267; ECO, n = 745; KPN, n = 288; KOX, n = 169; PMI, n = 208; SMA, n = 109) were prospectively collected from 28 microbiology laboratories distributed throughout three Central European countries (Austria, Germany, and Switzerland). Minimal inhibitory concentrations of ceftriaxone, ceftazidime, cefepime, ertapenem, meropenem, piperacillin-tazobactam, ciprofloxacin, and tobramycin were determined by the broth microdilution method according to the standard of the German DIN.
Results: Of the ECO, PMI, KPN, and KOX isolates, 5.1%, 1.9%, 7.3%, and 12.4% produced extended-spectrum beta-lactamases. Ertapenem (100%) and meropenem (99.9%) were the most active compounds against ECO. The susceptibility of ECO to cefepime, ceftazidime, and ceftriaxone ranged from 94.5 to 96.1%. In contrast, susceptibility of ECO to ciprofloxacin was 77.7%. For PMI, susceptibility was highest for ertapenem and meropenem (each 99.0%) and lowest for tobramycin (88.9%). Among KPN and KOX isolates, susceptibility to cefepime, ceftazidime, and ceftriaxone ranged from 93.4 to 95.8% and 78.7 to 97.0%, respectively. Susceptibility to piperacillin-tazobactam was 85.1% and 75.1% for KPN and KOX, respectively. The carbapenems were the most active drugs against KPN and KOX, with susceptibilities of >98% each for ertapenem and meropenem. For SMA, susceptibilities varied from 77.1% (piperacillin-tazobactam) to 97.2% (ertapenem). Susceptibility patterns of ECL showed the highest susceptibility to meropenem (99.6%) followed by ertapenem (98.9%), ciprofloxacin (95.1%) and tobramycin (95.1%), whereas susceptibilities to ceftazidime, ceftriaxone and piperacillin-tazobactam were each below 70%.
Conclusion: The carbapenems seem to be the most active antimicrobial agents against isolates of ECL, ECO, KPN, KOX, PMI, and SMA recovered from patients in hospitals located in Germany, Austria, and Switzerland.

R1953

Four patients with a *Pseudomonas aeruginosa* BEL-1 clavulanic acid-inhibited extended-spectrum beta-lactamase: what's the connection?

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Objective: A 72-year-old male patient was admitted to our hospital with a dissecting aneurism of the left arteria renalis. A scrotal swab yielded a *Pseudomonas aeruginosa* strain producing a novel Extended-Spectrum Beta-Lactamase (ESBL), coded by a BEL-1 gene. The relatively low-level MICs of extended-spectrum betalactams in the BEL-1 carrying *P. aeruginosa* strains may hinder accurate detection in routine laboratory practice. Nevertheless we discovered 3 other patients with the same BEL-1 producing *P. aeruginosa*.
Methods: Screening for ESBL genes was performed using double-disk synergy test and PCR techniques. Sequence analysis identified the Ambler class beta-lactamase BEL-1 in all strains. Consecutively PFGE was performed.
Results: The 4 samples strongly suggest to be closely related if not identical. To the best of our knowledge all patients had no contact with each other. Admissions were separated in time and place. One of the patients wasn't even hospitalized in our hospital. This patient underwent a Bricker deviation and continues to shed the strain until now.
Conclusion: We believe that BEL-1 carrying *P. aeruginosa* is highly underestimated especially when using VITEK-2 or Phoenix sensitivity testing systems, since they hardly detect synergy in an accurate way. There is a consensus that most *P. aeruginosa* infections are acquired from the environment, but no environmental sources have ever been proved to be high risk for certain populations. Only a very small part of the population carries *P. aeruginosa* in faeces; and although this accounts for a large number of people in absolute numbers, the bacterial counts are usually low while a high inoculum seems to be required in order to transmit strains. Beside tomatoes, which are notorious for yielding the highest numbers of *P. aeruginosa* among vegetables, possible other sources are mushrooms and bean sprouts. The use of antimicrobials such as streptomycin and oxytetracycline in agriculture could be responsible for transferring resistance genes. Although there is no evidence for a correlation between the agricultural use of azoles as fungicides and fungal resistance in humans, such concerns have been expressed. The same concerns are justified when using antibacterial agents. More research on this issue is warranted.

R1954

Antimicrobial resistance patterns of *Escherichia coli* isolates

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Objectives: To determine and compare antimicrobial susceptibility patterns of *Escherichia coli* isolated from both hospitalized and outpatients in a tertiary-care hospital in Greece.
Methods: *E. coli* strains isolated from clinical specimens were reviewed. Identification of microorganisms and susceptibility testing was performed using the Vitek 2 automated system (bioMerieux, France).
Results: A total of 3023 *E. coli* strains were recorded during a three year period (2002–2004). 74.2% were isolated from inpatients and 25.8% from outpatients. The most common source of *E. coli* strains was the urine, followed by pus, wounds, blood and other sources. Susceptibility rates to commonly used

antimicrobial agents like ampicillin, amoxicillin/clavulanate, ceftazidime, amikacin, ciprofloxacin, piperacillin/tazobactam, cotrimoxazole were 46%, 82%, 88%, 95%, 88%, 98% and 75% for inpatients and 55%, 90%, 95%, 98%, 91%, 99% and 77% for outpatients, respectively. All isolates were sensitive to imipenem. According to statistical analysis *E. coli* strains isolated from outpatients were significantly more susceptible to all drugs tested ($p < 0.05$), except for cotrimoxazole and piperacillin/tazobactam. The incidence of extended spectrum b-lactamase (ESBL) producing strains among *E. coli* isolated from inpatients and outpatients was 8.2% (185/2244) and 3.7% (29/779), respectively.

Conclusion: Outpatient's strains showed higher susceptibility to all drugs tested. 8.2% and 3.7% of *E. coli* strains isolated from inpatients and outpatients, respectively, were found to be ESBL-producers demonstrating the dissemination of those strains in the community.

Surveys molecular epidemiology of resistance and resistance genes, strains or serotypes

R1955

Characterisation of *Campylobacter jejuni* strains isolated in the United Arab Emirates

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Objectives: Although the Middle East has been known as a high-risk area for campylobacteriosis, little is known about the features and variety of the local *Campylobacter jejuni* isolates. The aim of the study was to investigate the type distribution and antibiotic susceptibility of strains isolated in Al Ain, United Arab Emirates.

Methods: Forty one *C. jejuni* strains isolated from different patients during a two year-long period (2002 September–2004 September) in Tawam Hospital, Al Ain, Eastern Region of the United Arab Emirates, were studied. The isolates were speciated by biochemical tests, their heat stable antigens were determined by passive haemagglutination and the strains were further typed by molecular techniques, i.e. by restriction fragment length polymorphism (RFLP) of the flaA gene and by pulse-field gel electrophoresis (PFGE) following SmaI digestion of the genome. The susceptibility of the isolates to erythromycin, nalidixic acid and ciprofloxacin were determined by agar dilution. The presence of the Thr-86-to-Ile mutation of in the gyrA gene of quinolone and/or fluroquinolone resistant isolates was investigated by mismatch amplification mutation assay PCR.

Results: 75.6% of the isolates were serotypable representing 10 different serogroups. Beyond the non-typable strains, those expressing heat-stable antigen HS 4, 13, 16, 43, 50 (21.9%) and HS 2 (14.6%) were the most frequently encountered ones. All isolates were sensitive to erythromycin (MIC 0.5–4 mg/L), but 85.4% of them showed nalidixic acid and ciprofloxacin resistance with MIC values between 128–512 mg/L and 8–64 mg/L, respectively. All resistant isolates carried the Thr-86-to-Ile mutation. The 35 fluoroquinolone resistant isolates belonged to 17 different RFLP and 20 different PFGE groups. Among them a cluster of 8 isolates (22.8% of all resistant ones) with indistinguishable RFLP and PFGE patterns was identified with isolation dates spanning a one year-long period of time.

Conclusions: Our data show that fluoroquinolone resistance among *C. jejuni* is remarkably high in this region of the Middle East. While most isolates seem to be independent strains, a resistant clone present in the region for an extended period of time could be identified.

R1956

Antibiotic susceptibility of *Campylobacter* species isolated in West-Tallinn Central hospital in 2000–2004

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Objectives: *Campylobacter* remains one of the most important intestinal pathogens in Estonia. Of all bacterial diarrhoeal diseases diagnosed in West-Tallinn Central Hospital, infections due to *Campylobacter* showed a steady increase of the prevalence from 14.7% in 2000 to 30.5% in 2004. The objective of the study was to determine susceptibility patterns of isolated strains against commonly used antibiotics.

Methods: During the study period (2000–2004) *Campylobacter* cultures were isolated from stool samples of patients admitted to the hospital and identified to species level using conventional laboratory methods. Antimicrobial susceptibility tests were performed by disk diffusion method according to the recommendations of the NCCLS. A total of 219 strains were studied, 211(96.3%) belonged to *Campylobacter jejuni* and 8 (3.7%) to *Campylobacter coli*. The antibiotics tested were ciprofloxacin (CIP), erythromycin (ERY), tetracycline (TET), nalidixic acid (NAL).

Results: Susceptibility rates of all 219 strains tested were as follows: CIP – 94.5%, TET – 87.7%, ERY – 86.3%, NAL – 62.6%. The percentage of *Campylobacter* isolates reported as resistant to NAL significantly increased from 25 in 2000 to 40.4 in 2004. Resistance to NAL was detected in both *Campylobacter jejuni* and *Campylobacter coli* with the same prevalence – 37.4% and 37.5% respectively. Most of the isolates were susceptible to TET and ERY. However there was a little increase in the occurence of ERY-resistant strains over time. Resistance to CIP increased from 3.1% in 2000 to 10.6% in 2004. 12.3% of isolates were found to be simultaneously resistant to 2 and 4.6% to 3 antibiotics.

Conclusions: Resistant rates of *Campylobacter* species in Estonia are increasing. Co-resistance to several drugs has been detected. The high incidence of NAL-resistant isolates indicates that susceptibility to NAL can no longer be considered a reliable test for differentiating between *Campylobacter* species. ERY remains relatevily effective for the treatment of campylobacteriosis. Although the resistance of *Campylobacter* to CIP is increasing it has not yet emerged as a significant problem in Estonia.

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R1957

Genotypic evaluation of high-level resistant enterococci to glycopeptides isolated from blood cultures in a tertiary hospital

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Objective: The purpose of the present study was to analyse enterococci recovered from blood stream infections during a 3-year period (from 2003 to 2005) with regard to their resistance to glycopeptide antimicrobials and to characterise the mechanism of resistance to vancomycin and teicoplanin.
Methods: Enterococci were isolated from blood cultures (Bactec system, Becton Dickinson) of patients hospitalized in different wards of our hospital. Identification and antibiotic susceptibility testing of enterococci were performed by the Vitek II automated system (Biomerieux). The MICs for vancomycin and teicoplanin were confirmed by E-test (AB Biodisk, Solna, Sweden), according to NCCLS guidelines. Further analysis of the vancomycin resistance genes (vanA, and vanB) was performed by sandwich hybridization method (EVIGENE VRE detection kit, Statens Serum Institut, Denmark).
Results: Identification resulted in 42 vancomycin resistant enterococci (VRE) that consisted of 29 *Enterococcus faecalis*, 10 *Enterococcus faecium*, and 3 *Enterococcus gallinarum*. MIC values were higher than 64 μg/ml for vancomycin and higher or equal to 32 μg/ml for teicoplanin. Genotypic analysis of the isolates yielded that all glycopeptide resistant enterococci possessed the vanA gene.
Conclusions: The resistance of enterococci to glycopeptides consists a major problem, as these antimicrobials are the last therapeutic resource especially when isolated from patients with bloodstream infections and in the hospital environment. The possession of vanA gene, conferring high-level resistance to both vancomycin and teicoplanin, is limiting the therapeutic options for VRE infections.

R1958

Detection of VIM-2 metallo-beta-lactamase in *Pseudomonas aeruginosa* isolates from a cystic fibrosis patient

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Objective: *Pseudomonas aeruginosa* (PA) is a major pathogen in cystic fibrosis (CF) patients and leading cause of morbidity and mortality. Once chronic infection is established with PA, it is virtually impossible to eradicate. The development of resistance to antibiotics frequently occurs in CF due to intensive selective pressure provided by large amount of antibiotics used in these patients. The aim of this study was to identify metallo-beta-lactamases (MBLs) in a multi-resistant PA isolate from a CF patient.
Methods: Paediatric Hospital of Coimbra has collected from April 2003 to December 2004, eight samples of sputum harboured a multi-resistant PA that colonize a fourteen years old girl with CF. Identification and susceptibility was performed by MicroScan WalkAway (Dadebehering) system. Double-disk synergy test was used for screening MBLs. For research of blaVIM, blaIMP, and class 1 integron, PCR was done. PCR products obtained were sequencing and analysed. Samples were typed by Random Amplified Polymorphic DNA (RAPD) analysis using sequences of 10-mer RAPD primers 272 and 208. RAPD patterns were visually compared.
Results: These strains were resistant to all antimicrobials used in this system, except to colistin. The double disk synergy test was positive for all samples. The presence of blaVIM was positive but blaIMP was negative, and DNA sequencing showed the presence of blaVIM-2 gene. This genetic determinant was inserted in a class 1 integron about 3000 bp. The RAPD typing demonstrated that banding patterns were very similar, indicating a single RAPD type. This genotype has been conserved during the chronic infection.
Conclusions: The presence of a MBL in a strain of CF is of great concern because these patients are outpatients, but are in close proximity to other CF patients. Capacity of this gene cassette to excise from the integron and insert into another integron within another bacterium of the same or different species is of particular significance when the new host is pathogenic. Therefore it is eminent a regular screening/monitoring system to prevent the wider spread of this worrisome resistance determinant, especially among these CF isolates.

R1959

Detection of vancomycin-resistance genes and virulence factors in vancomycin-resistant enterococci isolated from patients hospitalised in a large university clinical hospital during a two-year period

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Objectives: Our aim was to analyze group of 98 vancomycin-resistant enterococci (VRE) strains isolated from patients hospitalized in large university clinical hospital in Warsaw, Poland during two-year period (2000–2002). Strains were isolated from blood, wound, peritoneal cavity, bile and feces. We tested VRE strains for their ampicillin and glycopeptides susceptibility, type of van genes carried and presence of enterococcal virulence factors genes.
Methods: Strains were identified with commercial API identification system (bioMerieux) and subsequently checked for species-specific *Enterococcus faecium* and *Enterococcus faecalis* ddl genes by PCR. MIC for ampicillin, vancomycin and teicoplanin was determined according to CLSI standards. PCR was used for van genes detection using primers for vanA, vanB, vanC and vanD genes. Virulence factors genes were detected using PCR method with primers specific for five genes from cytolysin complex, gelatinase (gelE), aggregation substance (agg), enterococcal protein A (AefaAfs, efaAfm) and enterococcal surface protein (esp) genes.
Results: Among 98 strains of VRE, the most prevalent was *E. faecium* (82.7%), according to biochemical identification results. Other VRE species embraced *E. faecium*, *E. durans*, *E gallinarum* and two strains considered as *Enterococcus* spp. There was discrepancy between identification results using phenotypic and genetic methods in case of 11.2% of strains. Resistance to teicoplanin was detected in 96.9% of strains. Three strains of VRE were ampicillin susceptible. The most prevalent glycopeptide resistance gene was vanA (92.9% of VRE strains). Other types of glycopeptide resistance genes were vanB (three strains) and vanD (one strain). In two strains of VRE, we were unable to detect any van gene. The most prevalent virulence factor gene was efaA (88.8% of strains). Other virulence factors genes were also present: esp in 72.4 % of strains, gelE (12.2%) and agg (10.2%). Various genes of cytolysin complex were found in 11.2% of strains, but there was no strain carrying complete set of cyl genes.
Conclusions: Basing on obtained results, we can assume, that there was multiple insertion of different VRE strains into hospital environment during analyzed two-years period. We also characterized two groups of epidemic strains, and 7 groups

of VRE, which were not responsible for epidemic infections. Only in case of esp gene we were able to find significant correlation between site of infection (blood) and carried virulence factor (U-test, $p < 0.01$).

R1960

Evolution of antibiotic resistance and genetic characteristics of *Pseudomonas aeruginosa* isolates resistant to imipenem

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Objectives: The aim of this study was to analyse the evolution of the antibiotic resistance and genetic relatedness among clinical isolates of *P. aeruginosa* resistant to imipenem during the years 1999 and 2002.
Methods: The study included all imipenem resistant isolates obtained at a Hospital from Bilbao (Northern Spain) during 1999 and 2002 (19 and 33 isolates respectively, both representing the 14% of total isolates). Susceptibility to antimicrobial agents was determined by the disk diffusion method following the NCCLS recommendations. The antibiotics tested were imipenem, meropenem, ticarcillin, cefotaxime, ceftazidime, cefepime, amikacin, gentamicin and ciprofloxacin. Total DNA was used as target for PCR-fingerprinting experiments with primers RD1 and ERIC2. To detect class 1 integrons, primers 3́CS and 5́CS were used in amplification experiments.
Results: Resistance to antibiotics tested was as follows (1999/2002): meropenem (63%/46%), ticarcillin (21%/60%), cefotaxime (63%/75%), ceftazidime (41%/33%), cefepime (32%/36%) amikacin (0%/0%), gentamicin (37%/76%) and ciprofloxacin (47%/64%). Using the RAPD technique, 10 distinct genotypes were identified in 1999 and 23 in 2002. Patients with several isolates maintained the same clone along time, being each clone specific for each patient. Class 1 integrons of 1700, 1400 and 800 bp. were detected in 50% of isolates from 1999. 82% of isolates from 2002 showed integrons ranging in size from 1700 to 600 bp (10 isolates bored combinations of two or three structures).
Conclusions: Amikacin showed the best activity against imipenem resistant *P. aeruginosa* isolates but the results obtained showed an increasing level of resistance to ticarcillin, cefotaxime, cefepime, gentamicin and ciprofloxacin. These findings could be related to the increasing percentage of isolates boring class 1 integrons. Despite the high genetic diversity found by PCR-fingerprinting, it is remarkable that the same integron structures were present in different clones.

R1961

Multiresistant strains of *Mannheimia haemolytica* isolated from lambs

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Objectives: To analyse the antimicrobial susceptibility of *Mannheimia haemolytica* isolates from ill and healthy lambs.
Methods: Thirteen strains of *M. haemolytica* were isolated from 13 lambs. Nine isolates were obtained from the lungs of 9 lambs affected with fatal pneumonia (mean age of 3 months) while 4 isolates were obtained from the nasal swabs of healthy lambs (mean age of 6 weeks). All the strains were tested for susceptibility to 20 antimicrobial drugs of different classes by the agar diffusion method. The strains were also analysed by a PCR specific for the sul2, strA and catA3 genes that encode for resistances to sulfonamides, streptomycin and chloramphenicol.
Results: All of the 13 M. haemolytica strains (100%) displayed a multiresistant pattern, i.e. they were resistant to drugs of four or more different classes. In detail, 100% of the strains were resistant to ampicillin, sulfamethoxazole, streptomycin and fusidic acid. Seven out of 9 M. haemolytica strains (77.7%) isolated from ill lambs were resistant to kanamicin; 5 (55.5%) to tetracyclines, 4 (44.4%) to erythromycin, 3 (33.3%) to tilmicosina and 3 (33.3%) to co-trimoxazole. None of the nasal isolates displayed resistance to those molecules. None of the 13 strains displayed resistance to ampicillin combined with sulbactam, augmentin, cephalosporins, norfloxacin, chloramphenicol and florfenicol. By PCR, the sul2 and strA genes were detected in all the isolates, while the catA3-type gene was not detected.
Conclusion: Analysis of *M. haemolytica* strains of ovine origin revealed resistance to old-generation and low-cost molecules, that are used commonly for the control of respiratory bacterial infections, such as ampicillin, sulfonamides, streptomycin and tetracyclines. No drug resistance was observed to high- cost molecules such as beta-lactam combined with beta-lactamase inhibitor or cephalosporins that are used rarely in ovine breedings. The high rate of resistance observed in the isolates from ill lambs suggests the need for surveillance of antibiotic resistance in *M. haemolytica* strains of ovine origin, and the need for adoption of therapeutic protocols based on the profiles of sensivity/resistance to antimicrobials exhibited by the various *M. haemolytica* isolates.

R1962

Screening for the presence of class 1 and 2 integrons in non-fermenting isolates from a tertiary hospital: association with antibiotic resistance phenotypes

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Objectives: We assessed the association between multiresistant (MDR) non-fermenting clinical isolates and the presence of class 1 & 2 integrons in a tertiary hospital.
Methods: A sample of 110 *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus baumannii* from various infections were collected between 2002 and 2004 and tested for patterns of resistance to beta-lactams (piperacillin, piperacillin/tazobactame, ceftazidime, cefepime, imipenem), aminoglycosides (amikacin, gentamicin, netilmicin, tobramycin) and fluoroquinolones. The isolates were screened for the presence of class 1 and class 2 integrons using sets of primers specific for 5 and 3 CS of the integrase 1 and 2 genes respectively.
Results: Of the 110 non-fermenting isolates tested (70 *P. aeruginosa* and 40 *Ac. calco. baumannii*) 30 (43%) isolates of *P. aeruginosa* and 32 (80%) of *Ac. calco. baumannii* were multiresistant (resistant in ≥2 of the following: beta-lactams, aminoglycosides or quinolones). Screening for class 1 and class 2 integrons showed 29 (41.4%) isolates of *P. aeruginosa* and 28 (70%) isolates of *Ac. calco.baumannii* containing class 1 integron, while no isolate contained integron class 2. 96.6% of the MDR Pseudomonas and 87.5% of the MDR Acinetobacter were class 1 integron positive (92% of total MDR isolates), while no integron class 1 positive isolates were detected among the non-MDR groups.
Conclusions: No class 2 integrons were detected in a two-year period collection of non-fermenting clinical isolates in a tertiary hospital covering a large Athens area. Yet, the presence of class 1 integrons in 92% of the MDR isolates show the importance of these elements in multidrug resistance epidemiology.

In vitro antibacterial susceptibility & drug interaction studies

R1963

Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of Pistacia vera

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Objective: Antibacterial, antifungal, and antiviral properties of fifteen lipophylic extracts obtained from different parts (leaf, branch, stem, kernel, shell skins, seeds) of *Pistacia vera* were screened against both standard and the isolated strains for antimicrobial activity.
Material and methods: Strains of *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* and *C. parapsilosis* were investigated by microdilution method. Both Herpes simplex (DNA) and Parainfluenza viruses (RNA) were used for the determination of antiviral activity of the *P. vera* extracts by using Vero cell line. Ampicilline, ofloxacin, ketoconazole, fluconazole, acyclovir and oseltamivir were used as the control agents.
Results: The extracts showed little antibacterial activity between the range of 128–256 μg/ml concentrations whereas they had noticeable antifungal activity at the same concentrations. Kernel and seed extracts showed significant antiviral activity compared to the rest of the extracts as well as the controls.

R1964

Antimicrobial resistance and ESBLs production among *Salmonella enterica* non-typhi clinical isolates in Plovdiv region, Southern Bulgaria

M. Murdjeva, M. Stoycheva-Vartigova *(Plovdiv, BG)*

Objectives: Adequate antibacterial therapy in salmonellosis when necessary requires constant monitoring of the antibiotic susceptibility. This together with careful consideration of the phramacodynamics, pharmacokinetics and the in vitro distribution of antibiotics is also a prerequisite for the successful therapy. The aim of this study was focussed on antimicrobial resistance of *Salmonella enterica* non-typhi isolates, the frequency of multiresistant strains and the ESBLs producers in Plovdiv region, Southern Bulgaria for 3 years period (2002-2004).
Methods: The study included 274 S. enterica non-typhi clinical fecal isolates tested for in vitro antibacterial susceptibility. The Kirby-Bauer testing was applied to a number of antibacterial agents most of which in current use in medical practice when certain indications are present. The double-disk synergism test was performed for ESBLs production.
Results: The frequency of resistance among Salmonella isolates was highest to ampicillin (94%), then to nalidixic acid (43%) and chloramphenicol (26%) and lowest to ciprofloxacin and ceftriaxone. The dominant Salmonella serogroup – OD, showed significantly higher resistance to nalidixic acid (55%) than OB, which was more resistant (71%) to chloramphenicol. The development of multiresistant strains (10%, mainly to ampicillin, nalidixic acid, chloramphenicol and trimethorpim/sulfamethoxazole) marked an anxious trend. ESBLs producers were limited – 7.5% among all tested strains.
Conclusion: We confirm the highest worldwide in vitro susceptibility of *S. enterica* non-typhi serotypes to 3d generation cephalosporins and fluoro-quinolones in Plovdiv region, Bulgaria. Resistance to chloramphenicol among the commonest isolate – *S. enterica* serotype Typhimurium, which can potentially give extraintestinal complications, may exclude application of chloramphenicol in these forms of salmonellosis. ESBLs producing strains although in low rate, indicate the necessity of regular phenotypic screening for enzyme production. This may lead to better treatment and strategies for prevention and limitation of Salmonella spread in Southern Bulgaria.

R1965

Streptococcus agalactiae and neonatal infections: place of pristinamycin in their prevention?

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Objectives: The objective of this study was to evaluate the *in vitro* activity of pristinamycin (PRI), belonging to the synergistin group of antibiotics, against Streptococcus agalactiae (group B streptococci, SGB), the main cause of neonatal infections.
Methods: In total, 100 strains of SGB recently isolated from the vagina of pregnant women and from newborns were tested. MICs of PRI, penicillin (PEN), amoxicillin (AMX), erythromycin A (ERY) and clindamycin (CL) were determined by agar dilution according to the recommendations of the Comité de l'Antibiogramme de la société Française de Microbiologie.
Results: 100% of isolates were susceptible to PEN (MICs: 0.03–0.06 mg/L), AMX (MICs: 0.03–0.125 mg/L), PRI (MICs: 0.125–0.25 mg/L), 77% were susceptible to ERY (MICs: 0.03–0.25 mg/L) and 88% to CL (MICs: 0.03–0.5 mg/L). Twelve per cent of isolates were highly resistant (MICs: 4 ≥ 16 mg/L) to ERY and CL (ermB methylase), 9% were resistant (MICs: 1–2 mg/L) and 2% highly resistant (MICs > 16 mg/L) to ERY only.
Conclusions: Penicillin G or amoxicillin are recommended as prophylaxis by French guidelines in women colonized with SGB either during delivery or during the last month of pregnancy. Macrolides are alternative, in penicillin-allergic patients. However, resistance to macrolides has risen restricting therefore their use. Based on these in vitro study results, pristinamycin may be an alternate treatment of SGB infections in penicillin-allergic patients.

R1966

Evaluation of the antibiotic susceptibility of *Brucella* strains by MIC method

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Objectives: Brucellosis is a zoonosis caused by several species of the genus *Brucella*. It is a worldwide common infection disease. The disease, which is generally seen in the country side, is endemic for our region. The aim of this study is to evaluate using the MIC50 and MIC90 values of various antibiotics against clinical isolates of *Brucella* spp. from a brucellosis endemic region.
Methods: Thirty-four *Brucella* isolates were collected between 1999-2005 from blood cultures of adult patients with brucellosis at Suleyman Demirel University department of Clinical Bacteriology and Infection Diseases. *Brucella* species were identified on the basis of typical Gram staining, carbon dioxide requirements for growth, production of hydrogen sulphide and urease, dye sensitivity (20–40 μg/ml basic fuchsin) and growth in thionine. Sensitivity of the 34 strains was tested by using Broth microdilution method. The lowest concentration that

completely inhibited visual growth was recorded and interpreted as the MIC.
Results: All of the isolates were determined as *B. melitensis*. In vitro activity of doxycycline, tetracycline, rifampin, streptomycin, ciprofloxacin, levofloxacin, ceftriaxone, amoxicillin against clinical isolates of *Brucella melitensis* is shown in Table 1.

Antibiotic	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Doxycycline	0.16-0.64	0.32	0.64
Tetracycline	0.08-0.64	0.16	0.32
Rifampin	0.5-4	1	2
Ciprofloxacin	0.25-1	0.5	1
Levofloxacin	0.25-1	0.5	0.5
Streptomycin	1-8	4	8
Ceftriaxone	0.125-2	0.5	1
Amoxicillin	0.5-4	1	2

Conclusions: In conclusion, there is no important resistance problem for antibiotics targeted to *Brucella melitensis* strains in Isparta region. Thus, routine antibiotic susceptibility tests for *B. melitensis* strains are not necessary. However, antimicrobial resistance must be examined periodically.

R1967

An antibiotic sensitivity of *Escherichia coli* strains in nosocomial surgical site infections

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Objective: To study antibacterial sensitivity of *Escherichia coli* (*E. coli*) strains in nosocomial surgical site infections (NSSI).
Significance: We've established that *E. coli* is one of the most problem pathogen of NSSI because of high resistant to antibiotics. Its sensitivity was determined.
Study design: Prospective case series study.
Setting: Eight surgical departments and 4 surgical intensive care units in a central region's hospital, city hospitals #4 and #6.
Study population: All patients with clinic and criteria of NSSI.
Methods: Thanks to nosocomial infections control programs in 3 Dnipropetrovsk's hospitals 118 strains of pathogens in NSSI were isolated, 22 of which were are *E. coli*. Their sensitivity to the antibacterial medicines (ampicillin, amoxycillin/clavulanate, ciprofloxacin, peflofloxacin, moxifloxacin, gentamicin, netilmicin, amikacin, cefazolin, cefuroxime, ceftriaxone, cefoperazone, ceftazidime, cefepime, imipenem, meropenem) was studied. The microbiological research was accomplished using the ''BioMerieux'' growth mediums and antibiotic disks.
Results: It was established that *E. coli* strains' sensitivity was minimal to ampicillin (0%), to cefazolin (18%), to ciprofloxacin, cefuroxime, ceftriaxone, cefoperazone (27%), to peflofloxacin and ceftazidime (36.4 %) was intermediate to amoxycillin/clavulanate, cefepime (41%), to gentamicin, amikacin (50%) and to netilmicin (59%), was maximal to imipenem, meropenem (100%) and to moxifloxacin (77%).
Conclusions: It was established that *E. coli* are high resistant pathogen of NSSI. Pathogens had the highest sensitivity only to imipenem, meropenem, and moxifloxacin. The seriousness of problem is resistance to 2nd generation fluoroquinolones.

R1968

In vitro susceptibility of *Brucella melitensis* to antibiotics

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Objectives: In this study, the in vitro susceptibilities of 50 clinical isolates of *Brucella melitensis* obtained from blood cultures were determined to tetracycline, gentamycine, streptomycin, ceftriaxone, ciprofloxacin, levofloxacin, ofloxacin and rifampin.
Methods: The isolates were identified on the basis of colony morphology, CO_2 requirement, H_2S production, dye sensitivity (basic fucsin and thionin), slide agglutination with monospecific A and M antiserum and susceptibility to Tbilisi bacteriophage. The microdilution method was performed in 96-well trays by using Brucella broth. Two-fold dilutions of each drug were prepared. All drugs were tested at 11 different concentrations with one well used as a bacterial growth control. Final drug concentrations were 32–0.03 μg/ml for all drugs. The MICs of the antimicrobial agents were determined and according to it MIC50 and MIC90 values were calculated.
Results: In this study, *Brucella* species were all isolated from blood cultures and were identified as *B. melitensis* biotype-3. In our study, while MIC90 value (0.25 μg/ml) of tetracyclin was observed the lowest, MIC90 values of streptomycin and ceftriaxone were obtained as 8 μg/ml.
Conclusion: In our study, MICs of ceftriaxone and streptomycin were reported highly, so susceptibility of strains to these agents should be followed during therapy.

R1969

Antimicrobial resistance and serotype distribution of *Streptococcus pneumoniae* isolated from patients with community-acquired pneumonia in Crete, Greece

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Objective: To determine the antimicrobial susceptibilities and serotypes of *Streptococcus pneumoniae* isolated from clinical specimens of patients with community-acquired pneumonia in the University Hospital of Crete, Greece.
Material and methods: One hundred and one *Streptococcus pneumoniae* isolates were studied. Seventy-one (70.3%) isolates were recovered from sputum, 24 (23.7%) from bronchoalveolar lavage, 3 (3%) from blood and 3 (3%) from pleural fluid. Susceptibility tests were done by the E-test method according to manufacturer's reccomendations and were interpreted according to the NCCLS guidelines. The following antibiotics were tested: penicillin G, cefuroxime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, clarithromycin, clindamycin, roxithromycin, azithromycin, ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin. Serotyping was performed by the capsular swelling method with specific antisera.
Results: Nineteen isolates (18.8%) showed intermediate resistance and 14 (13.9%) high-level resistance to penicillin. Erythromycin, clarithromycin, clindamycin, roxithromycin, azithromycin, chloramphenicol, tetracycline, and trimethoprim/sulfamethoxazole resistance rates were 21.8, 21.8, 6.9, 21.8, 21.8, 7.9, 16.8 and 14.8%, respectively. Multiple resistance was observed in 11 strains. All isolates were sensitive to

vancomycin and to all 4 quinolones tested. The most prevalent serotype was 19, followed by 9, 3 and 14.
Conclusion: Our results emphasize the importance of continued surveillance of antimicrobial resistance profiles of *S. pneumoniae* in our region for determining appropriate empiric therapeutical regimens.

R1970

Resistance patterns in *Pseudomona aeruginosa*

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Objective: To study the sensibility and the resistance patterns of *P. aeruginosa* isolated in different clinic samples in outpatients.
Methods: 180 *P. aeruginosa* strains were studied between January 2004 and September 2005. The identification and sensibility were performed by the MicroScan Walkaway System (DADE-Behring) according to NCCLS criteria. The antibiotics studied were: Amikacin (Ak), Aztreonam (AZT), Cefepime (Cpe), Cefotaxime (Cft),Ceftazidime (Caz), Ciprofloxacin (Cp), Gentamicin (Gm), Imipenem (Im), Meropenem (Me), Ofloxacin (Ofl), Piperacillin/Tazobactam (P/T), Piperacillin (Pi), Ticarcillin (Ti) and Tobramicin (To).
Results: *P. aeruginosa* were isolated in: urine 37 (20.56%), wounds 61 (33.89%), otics exudates (ex.) 45 (25.0%), sputum 10 (5.56%), conjunctival ex. 8 (4.44%), nasopharingeal ex. 6 (3.33%), vaginal ex. 3 (1.67%), urhetral ex.2 (1.11%), nasal ex. 1 (0.56%) and other 7 (3.89%).-The sensibility to antibiotics studied were: Ak 93.9%, Azt 86.1%, Cpe 95.6%, Cft 19.4%, Caz 97.8%, Cp 87.8%, Gm 85.6%, Im 93.9%, Me 99.5%, Ofl 74.6%, P/T 99.6%, Pi 97.8%, Ti 95.0% and To 95.6%.-The resistance patterns more frequent were: Sensitive to all antibiotics studied 17 (9.44%); resistance to Cft: 95 (52.78%); resistance to Cp + Ofl + Cft: 12 (6.67%); Cft + Gm: 4 (2.22%); Cp + Ofl + Cft + Azt: 7 (3.89%); Cp + Ofl: 4 (2.22%); Cft + Azt: 6 (3.33%); Cft + Im: 3 (1.67%); Gm: 3 (1.67%); Cp + Ofl + Im: 3 (1.67%); Gm + To + Ak + Cp + Ofl + Cft + Caz + Cpe + Azt: 3(1.67%) and other 23 (12.78%).
Conclusions: Wounds were the samples where *P. aeruginosa* was isolated most frequent.-The antibiotics studied have a good activity against *P. aeruginosa*, excep Cefotaxime (only 19.4%).-There is a low percentage of *P. aeruginosa* sensitive to all antibiotics studied.-The resistance patterns most frequent were: resistance to Cefotaxime and Cefotaxime with quinolones (Cp + Ofl).

R1971

In vitro activities of four new antibiotics against blood isolate VGS collected in Southwest Finland, 1993 - 2004

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Objectives: The aim of our study was to evaluate the variation of the *in vitro* activities of the new antibiotics telithromycin, linezolid, quinupristin/dalfopristin and levofloxacin against 263 viridans group streptococcae, collected in Turku, Finland.
Methods: 263 viridans group streptococci (VGS) isolated from blood samples in Turku University Central Hospital from 1993 to 2004 were tested. The isolates were identified with an API 20 Strep test and a Vitek 2 GPC-card. Antibiotic susceptibility testing was done with agar dilution method on Müller-Hinton II medium, according to CLSI. MIC values were tested against levofloxacin, linezolid, quinupristin/dalfopristin and telithromycin. Linezolid susceptibility was confirmed with Disk diffusion method and levofloxacin susceptibility with Etest.
Results: Based on the minimum inhibitory concentrations all the VGS isolates were susceptible to telithromycin. Rank order of the antibiotic susceptibility was: Telithromycin (100%) > linezolid (98.9%) > levofloxacin (94.6%) > quinupristin/dalfopristin (57.0%). Based on the agar dilution method, 3 isolates were non-susceptible to linezolid (MIC 4 mcg/ml). However, the disk diffusion method revealed that those isolates were susceptible to linezolid (zone range 26–33 mm). Levofloxacin non-susceptibility rate was 5.4%, and high-level resistance was noted in five (1.9%) isolates. Results from the levofloxacin Etests confirmed that those 5 isolates were resistant to levofloxacin. Only 57% of the VGS isolates were susceptible to quinupristin/dalfopristin (Q/D) whereas the amount of Q/D resistant isolates was 11%.
Conclusion: In our material, telithromycin was the most active antimicrobial agent towards VGS. Linezolid was the second effective antibiotic tested. The reason for higher linezolid MIC values with an agar dilution method was probably due to the method's inferior accuracy and sensitivity compared to the disk diffusion method. Also levofloxacin showed good in vitro activity against VGS, although the susceptibility rate was lower than has been reported earlier. Contrary to the previous studies, quinupristin/dalfopristin was the least effective of the new antimicrobial agents.

R1972

Tigecycline *in vitro* activity against *Acinetobacter* spp. resistant to other drugs from Europe – T.E.S.T. Program 2006

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Background: Tigecycline is a glycylcycline, a new generation of tetracyclines significantly different to be classified as a separate antimicrobial class. Glycylcyclines are being developed to overcome the problem of bacterial resistance to tetracyclines and other antimicrobials. Tigecycline is better tolerated and is more active than tetracyclines against a wide variety of Gram-positive and Gram-negative bacteria including Acinetobacter spp. The T.E.S.T. program determined the in vitro activity of TIG against Acinetobacter resistant to two or more of piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), and imipenem (IMP). Study strains were collected from 33 hospitals in Europe throughout 2004-2006.
Methods: A total of 435 clinical isolates were identified to species level in participating sites and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines, with TIG susceptible breakpoint defined as < 2 mcg/ml.
Results: Resistance rates for comparator drugs were CAX 35%, CAZ 35%, LVX 29%, PT 28%, CPE 23%, AK 18%, IMP 12%, and MIN 3.2%. TIG inhibited 100% of all strains of Acinetobacter resistant to 2 or more drugs. There were no TIG-resistant (>8 mcg/mL) strains found. TIG MIC50/90 was 0.5/1 mcg/ml, respectively, for strains resistant to CAX, AK, IMP, CAZ and 1/2 mcg/ml for strains resistant to LVX, PT, CPE and MIN. The modal TIG MIC for resistant strains was 1 mcg/ml compared to 0.12 mcg/ml for strains without resistant parameters, indicating an 8-fold diminishment of activity.
Conclusions: TIG inhibited most *Acinetobacter* strains resistant to one or more other drugs in this study, although the higher TIG MICs seen for these strains suggests some linkage to

resistance mechanisms for other drugs. TIG remained effective in inhibiting multi-drug resistant *Acinetobacter* spp., further broadening its wide spectrum of activity vs. drug-resistant bacteria.

R1973

Penetration and activity of linezolid and vancomycin in *S. epidermidis* biofilms

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Objectives: The aim of this study was to study the activity of linezolid and vancomycin against *S. epidermidis* biofilms on silicone catheters and to evaluate the penetration of both compounds in the biofilms.
Methods: *S. epidermidis* ATCC 12228 (slime producer, ica+) and *S. epidermidis* RP62A (non slime producer, ica-) were used. The activity of the drugs at different concentrations against 6 and 24 hour-old biofilms was evaluated by colony counting. The penetration of both antibiotics in biofilms was also analized. For biofilms preparation, 50 μl of diluted overnight planktonic cultures were inoculated on individual sterile, black, polycarbonate membrane filters resting on agar culture medium and incubated at 35°C for 24 h. To measure antibiotic penetration, black polycarbonate membrane filters were placed on top of 24-h old biofilms. A concentration disk (30 μg) of linezolid or vancomycin was placed on top of this structure. Disk was removed after specified exposure times. The drug concentrations in disk were measured by a bioassay method.
Results: The MICs values of linezolid for both strains were 1 mg/L. The MICs value of vancomycin for slime and non-slime producing *S. epidermidis* strains were 1 and 2 mg/L, respectively. None of the concentrations evaluated of linezolid or vancomycin were able to completely sterilize the catheter surface. At low concentrations (1 mg/L), linezolid was significantly more active than vancomycin against sessile *S. epidermidis*. For the slime producing strain, bacterial survival in biofilms incubated with 1 mg/L of either linezolid or vancomycin were 0.25% and 27% respectively respect to the controls without antimicrobial. At high concentrations (16 mg/L), the number of surviving bacteria of biofilms incubated with linezolid was 58 times lower than that observed with vancomycin for the slime producing strain. For both strains, more than 60% of the initial concentration of linezolid in the disk was released through the biofilm in the first 60 minutes. These percentages increased until 75% after 3 or 6 hour incubations. No differences were observed between slime producing and non-producin strains. At 3 hours, only 20% of the initial load of vancomycin in the disk penetrates into the biofilms of both *S. epidermidis* strains.
Conclusion: Linezolid showed high bactericidal activity against *S. epidermidis* biofilm on silicon elastomer. This activity is partially owed to its high penetration and concentration in biofilms.

R1974

Oral streptococcal strains isolated from odontogenic infections and their susceptibility to antibiotics

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Objectives: Oral streptococci are the main facultatively anaerobic bacteria involved in odontogenic infections. The aim of this study was to identify at species level and to investigate the antibiotic susceptibility of oral streptococcal strains isolated in pure culture or in association with other bacteria from 100 pus samples collected from Romanian patients with different odontogenic infections.
Methods: The isolates were identified at species level using the Rapid ID 32 STREP system (Bio-Merieux, France). Susceptibility testing of the isolates to: penicillin G (PG), ampicillin (AM), erythromycin (EM), clindamycin (CM) and tetracycline (TC) was performed by the Etest (AB Biodisk, Sweden). In addition, the phenotype of EM resistance was identified using a double disk diffusion test.
Results: The isolates belonged to the following species: Streptococcus anginosus, Streptococcus constellatus, Streptococcus intermedius, Streptococcus mitis, Streptococcus oralis, Streptococcus sanguinis (formerly known as S. sanguis) and Streptococcus gordonii. The MICs (mg/l) ranges were: PG 0.008 to 0.75 (85.3% Susceptible), AM 0.016 to 0.5 (97.9% Susceptible), EM 0.016 to 4 (90.5% Susceptible), CM 0.016 to 0.047 (100% Susceptible) and 0.047 to 256 TC (50.5% Susceptible). The isolates with intermediate susceptibility to beta-lactam antibiotics belonged to: S. sanguinis, S. mitis and S. oralis species. Resistance to EM was detected among all species, except for: S. constellatus, S. intermedius and S. gordonii, and M phenotype was established. Resistance to TC was found within all species but S. gordonii.
Conclusions: Reduced susceptibility to PG or to both PG and AM was found among *S. oralis*, *S. mitis* and *S. sanguinis* strains, while resistance to EM has also been detected in some *S. anginosus* strains. In contrast, CM was fully active and therefore might be an alternative for antimicrobial therapy of odontogenic infections in Romanian patients. As these are mostly mixed infections, often involving strictly anaerobic bacteria too, which are frequently beta-lactamase producers, the association of a penicillin and a beta-lactamase inhibitor, like Amoksiklav, is recommended.

R1975

Telithromycin resistance in clinical isolates of beta-haemolytic streptococci

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Objective: This study evaluated the current status of antimicrobial resistance in clinical isolates of beta-haemolytic streptococci.
Methods: From January 2004 to September 2005, 180 different isolates of beta-haemolytic streptococci (54% Group A, 7% Group B, 27% Group C, 4% Group F and 14% Group G) were collected from clinical samples of in and out patients. Susceptibility testing was assayed by disk-diffusion method according to CLSI (previously NCCLS) guidelines.
Results: All of the isolates were susceptibles to penicillin, cefotaxime, imipenem and vancomycin. There were 3% of strains intermediate or resistant to levofloxacin. Not susceptibility to erythromicin was detected in 26% of the isolates (8 Group A, 2 Group B and 1 Group C), and 14% to clyndamicin. Eleven strains (6%) were not susceptibles to telithromycin. All of these were resistant to erythromicyn and belonged to Group A (n = 8), Group B (n = 2) and Group C (n = 1) beta-haemolytic streptococci.
Conclusion: The low prevalence of resistance to telithromycin suggests the potential utility of this drug to treat infections produced by beta-haemolytic streptococci in our Health Care Area.

R1976

The susceptibility of *Escherichia coli* strains isolated from urinary tract infections in an infectious diseases clinic in Romania

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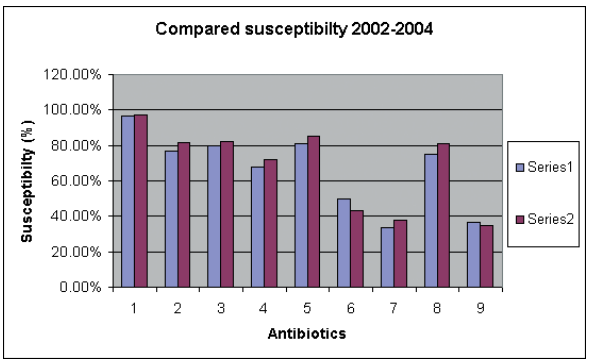
Objectives: Establishing the compared susceptibility of *E. Coli* strains isolated from patients with uncomplicated UTI hospitalized in 2002 compared to year 2004 in the ''Prof. Dr. Matei Bals'' Institute for Infectious Diseases, Bucharest.
Methode: Retrospective study regarding the in vitro testing susceptibility of 1496 strains of *E. Coli* isolated from urine cultures. The standard disk diffusion method was used for susceptibility testing, and the results were interpreted according to NCCLS standards. The antibiotics used for testing were: nalidixic acid (An), Nitrofurantoin (Fd), Ciprofloxacin (Cp), Amoxicillin/Clavulanate (Aug), Cefoperazone (Cpr), Piperacillin (Pi), Ticarcillin (Ti), Ticarcillin/Clavulanate (Ti/C), Ampicillin (Am).
Results: The cumulative results of the susceptibility are showed bellow. The presence of ESBL producing strains has been determined in 7 cases (5.78%) from 121 analyzed.
Compared susceptibility 2002–2004: Antibiotics 20022004 Fd 96.65%97.05%An 76.54%81.81%Cp 80.01%82.01%Aug 68.04%71.99%Cpr 80.81%85.02%Pi 50%42.98%Ti 33.33%37.67%TI/C 75%80.76%Am 36.89%35.02%



Conclusions: The results suggest a constant susceptibility of *E. coli* strains for the tested antibiotics during 2002–2004. A high susceptibility for Nf, An, Cp, Cpr has been maintained, also a moderate one for Aug and Ti/C and a very low susceptibility for Pi, Ti, Am. ESBL producing strains were isolated in 5.78% of the cases.

R1977

The importance of occurrence of the *Enterococcus faecalis* multiresistant isolates to antibacterial drugs

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Objectives: of our paper were to establish the frequency of the resistance of the *Enterococcus faecalis* isolates to penicillin, ampicilin, vancomycin, gentamycin (high level of resistance, RHL), streptomycin (RHL), tetracycline, chloramphenicol, amoksicilyn with clavulanic acid, ciprofloxacyn, norphloxacin, ryphampin, by the application of the standard methods. The study included 112 enterococcus isolates of different sample types taken within the Institute for Public Health Niš.
Methods: The sensitivity to antibiotics was tested by the disc-diffusive method of Kirby-Bauer. The isolates that expressed resistance to these antibiotics in the disc-diffusive test were submitted to the agar-diluting test or E- test.
Results: Among the types of *E. faecalis* there were 29 isolates resistant to penicillin (MIK ≥ 64 μg/mL) and at the same time were sensitive to vancomycin. There is a statistically significant difference in the resistance to penicillin and ampicillin ($\chi^2 = 17.83$ for $p < 0.01$). Resistance of *E. faecalis* to chloramphenicole (26.6%), tetracycline (48.5%) and rifampin has been noticed. The testing results of 17 enterococcus isolates to gentamicin and streptomycin showed resistance of a high level. The differences in resistance between gentamicin and streptomycin were statistically insignificant for $p = 0.005$. As compared to penicillin, vancomycin performed a significantly greater efficiency ($\chi^2 = 33.16$ for $p < 0.01$), while the efficiency related to ampicillin was on the border of statistical significance ($\chi^2 = 6.14$ for $p < 0.01$). Ciprofloxacin showed significantly higher efficiency as compared to norfloxacin ($\chi^2 = 17.59$ for $p < 0.01$). We registered the resistance of E. faecalis to chloramphenicol, tetracycline and rifampin. As compared to penicillin, vancomycin performed a significantly greater efficiency ($\chi^2 = 33.16$ for $p < 0.01$), while the efficiency related to ampicillin was on the border of statistical significance ($\chi^2 = 6.14$ for $p < 0.01$). Ciprofloxacin showed significantly higher efficiency as compared to norfloxacin ($\chi^2 = 17.59$ for $p < 0.01$). RVN frequency to aminoglycozides is high.
Conclusion: Levee and found mechanisms of the enterococcal isolate resistance are seriously limiting abilities of antibiotic therapy and indicate the necessity of the usage, especially of the wide spectrum cephalosporine and vancomycine.

R1978

Antibiotic susceptibilities of group A beta-haemolytic streptococci clinical isolates

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Objectives: The aim of this study was to determine penicillin G (PG) and erythromycin (E) MIC values and antibiotic resistance in Group A beta-haemolytic streptococci (GABHS) isolated from various clinical samples in our hospital between February 2004-October 2005 prospectively.
Methods: Total 49 GABHS strains isolated from throat (n: 34), and wound (n: 15) specimens were included. Identification based on characteristic haemolysis on sheep blood agar; sensitivity to bacitracin, and resistance to trimethoprim-sulfamethoxazole; a commercial latex agglutination test for grouping streptococcal strains (AVIPATH STREP, Omega Diagnostics, Scotland, UK). E and PG MIC values were determined by the E-test method. Antimicrobial susceptibility testings of wound isolates were performed for clindamicyne

(DA), levofloxacine (LEV), and tetracycline (TE) by disc diffusion method.
Results: All of the isolates were found susceptible to PG and 6 (12.2%) strains resistant to E by E-test method. All of the wound isolates were susceptible to LEV, 2 resistant to DA and 4 resistant to TE by disc diffusion method.
Conclusion: Penicillin is still the first choice of the treatment of

Table1: Penicillin G and Erythromycine MIC ranges, MIC50, MIC90 values of the Group A Beta-haemolytic streptococci

Antibiotic	MIC range (µg/ml)		MIC50 (µg/ml)		MIC90 (µg/ml)	
	Throat	Wound	Throat	Wound	Throat	Wound
Penicillin G	0.003-0.047	0.003-0.064	0.006	0.008	0.012	0.012
Erythromycine	0.016-8	0.023-3	0.047	0.094	0.094	2

GABHS infections. Although erythromycin is an alternative in patients who have penicillin hypersensitivity, there is an increasing resistance against it. Levofloxacin and clindamycin could be the other therapy options. Therefore antibiotic susceptibility testing and resistance surveillance of GABHS should be done.

R1979

In vitro activity of cefepime against clinical isolates from patients in Tehran, Iran

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Objective: Infections caused by resistant bacterial pathogens, including Gram positive and Gram-negative bacteria, have become an increasing problem with respect to therapy in many of the Irananian medical center. Cefepime has been recently introduced in Iran, which is highly effective against these organisms. The purpose of this study was to determine in vitro activity of this antibiotic against isolated organism from patients, before introducing to the market, in Iran.
Methods: In vitro activity of Cefepime were tested against 304 clinical isolates samples including *Escherichia coli* (n = 167), *Klebsiella* (n = 49), *Staphylococcus* (n = 52), *Pseudomonas* (n = 18) and other isolates (n = 18), obtained during January–May 2005 from patients of Hazrat Rasoul hospital in Tehran, Iran. Minimum Inhibitory Concentration (MIC) of antibiotics was adjusted using an agar dilution method, described by NCCLS.
Results: The overall susceptibility rate for Cefepime against all isolates was 75%. Against *Pseudomonas aeroginosa* and *Staphylococcus* spp strains Cefepime was less active (40% resistance) and against *Enterobacteriaceae* Cefepime was excellent activity (more than 80% susceptibility).
Conclusion: Cefepime could be a valuable alternative for the treatment of infections due to multiply resistant organisms in Iran. Hence, it seems this drug could be suitable for empiric coverage of serious nosocomial infections.

R1980

Inhibitory activity and killing activity of extracts from the gall of *Quercus infectoria* against methicillin-resistant *Staphylococcus aureus*

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Objectives: Methicillin-resistant *S. aureus* has been well-documented as a major cause of hospital-acquired infection. Medicinal plants have been increasingly used to reduce the problem of antibiotic-resistant bacteria. *Quercus infectoria* was previously reported from this laboratory to produce high antibacterial activity. The aims of this study were to closely investigate the antibacterial activities of the extracts from *Q. infectoria* galls and to determine the effects of these extracts on the growth of clinical MRSA strains.
Methods: Fifty-one clinical isolates of MRSA were collected from Hat-Yai hospital. All isolates were multidrug-resistant. Galls of *Q. infectoria* were extracted with acetone, ethyl acetate, 95% ethanol, and water. Paper disc agar diffusion method was used to determine the antibacterial activity of these extracts. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were evaluated by modified broth microdilution method according to National Committee for Clinical Laboratory Standards. Growth curves demonstrating bacteriostatic and bactericidal activities of *Q. infectoria* against MRSA strains were documented for 24 h.
Results: All extracts of *Q. infectoria* show antibacterial activities against all strains of MRSA. The inhibition zones ranged from 11–23 mm. The ethanolic extract demonstrated the largest inhibition zone. Most of the MRSA strains treated by the ethanolic extract of *Q. infectoria* exhibited the MIC and MBC values at 0.4 and at 1.6 mg/ml, respectively. At the MIC concentration, the growth of a representative was inhibited and gradually decreased after 16 h incubation. The survival cells of the MRSA were not detected within 2 h after treated with the extract at its MBC concentration. *Staphylococcus aureus* ATCC 25923, a reference strain showed similar results.
Conclusion: The ethanolic extract of the galls of *Q. infectoria* have a high potential as antibacterial agent against MRSA. More detailed studies on the extract may provide an alternative way to treat infections caused by MRSA. Use of the active compounds could be developed as antibacterial agent in order to reduce problems with antibiotic-resistant bacteria in the hospitals.
Acknowledgement: This work was supported by Thailand Research Fund, Fiscal year 2005–2008.

New antimicrobials

R1981

Plant extracts as new anti-tuberculous agents, evaluation by MRA

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Objectives: Tuberculosis (TB) is a disease of antiquity, which is thought to have evolved sometime between the seventh and sixth millennia BC. Current estimates suggest that one third of world's population are infected resulting in some 2 million deaths per year. Pulmonary TB, the most common type of the disease, is usually acquired by inhalation of the bacillus and causes irreversible lung destruction, although other organs are sometimes involved. 50 years ago the introduction of the first drugs for TB treatment (streptomycin, para-aminosalicylic acid, isoniazid) led to optimism that the disease could be controlled if not eradicated. However, since the late 1980s the disease has

been undergoing a resurgence driven by variety of changes in social, medical and economic factors. Concomitant with the resurgence of TB has been the occurrence of multidrug-resistant mycobacterium strains MDR, which has exposed the weakness of the current drug armamentarium. Given that in many regions of the world, effective, indigenous therapeutic antimycobacterial agents may be crucial to improving overall public health, and therefore enhancing economic well-being, we must make every effort to develop anti-tuberculous agents which are based on renewable, relatively inexpensive materials, to get novel therapy starting from natural products

Methods: Plant extracts were obtained by distillation or purchased from manufacturers. The antimycobacterial activity of crude or purified plant extracts and essential oils was evaluated in vitro against Mycobacterium tuberculosis reference and clinical strains, including MDR isolates. MRA, microdilution resazurin assay, was employed as a short-term test that has been already evaluated to measure antibiotic susceptibility of tubercular clinical isolates (Banfi E et al JAC 2003 52: 796-800; Scialino G et al *Chemotherapy* 2006 in press)

Results: Interesting inhibiting activity comparable to standard anti-TB drugs were obtained for Euphorbia cerebrosides and Cetraria usnic acid; Rhododendron, Melaleuca and Leptospermum essential oils had minimal inhibiting concentrations in a range of 0.25–0.025% (v/v). Most plant extracts had no toxic effect against human cells in vitro

Conclusion: Novel therapy can start from natural products, developing anti-tuberculous agents based on renewable, relatively inexpensive materials like plant extracts; MRA proved to be a rapid assay suitable to evaluate the antitubercular activity of different preparations

R1982

Antimicrobial activity of novel N1-benzylidene-pyridine-4-carboxamidrazones against *Clostridium difficile*

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Objectives: *Clostridium difficile* is the leading cause of hospital acquired antibiotic-associated diarrhoea and colitis. Although the incidence of *C. difficile* infection has risen significantly over the past decade, there have been very few developments in the treatment and prevention of infection. Recent studies have demonstrated that a novel N1-benzylidene-pyridine-4-carboxamidrazone (compound LW/1/1) and several analogues developed at Aston University have significant antimicrobial activity against many Gram-positive organisms associated with hospital-acquired infections, including *Staphylococcus aureus* and coagulase-negative staphylococci. This study was undertaken to investigate the antimicrobial efficacy of compound LW/1/1 and analogues against clinical isolates of *C. difficile*.

Methods: Antimicrobial efficacy of 8 novel N1-benzylidene-pyridine-4-carboxamidrazones was assessed by agar diffusion against thirty-two clinical isolates of *C. difficile* and *C. difficile* NCTC 11204. Minimum inhibitory and bactericidal concentrations were determined in line with NCCLS guidelines on those compounds demonstrating antimicrobial activity by agar diffusion. Data were analysed by the two-tailed Mann–Whitney *U*-test.

Results: The novel N1-benzylidene-pyridine-4-carboxamidrazone compound LW/1/1 and an analogue, designated LW/1/16, demonstrated activity against all 33 strains of *C. difficile* tested. The mean minimum inhibitory concentration for compounds LW/1/1 and LW/1/16 was 6.43 ug/ml (range 4 to 8) and 29.21 ug/ml (range 2 to 256) ($p = 0.1958$) respectively. The minimum bactericidal concentration for compounds LW/1/1 and LW/1/16 was 7.27 ug/ml (range 4 to 16) and 37.76 ug/ml (range 2 to 256) ($p = 0.0004$) respectively.

Conclusion: Compound LW/1/1 and L/W1/16 are novel N1-benzylidene-pyridine-4-carboxamidrazones demonstrating excellent in vitro antimicrobial activity against clinically relevant strains of *C. difficile*.

R1983

A pilot study on the efficacy of ertapenem for early-onset ventilator-associated pneumonia

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Background: Early ventilator-associated pneumonia (VAP) is a frequent complication of unconscious patients with central nervous system disorders. Ertapenem might be useful for this indication, which has not been considered for licensing in the drug approval process.

Objective: To evaluate the usefulness and safety of ertapenem in the treatment of VAP.

Methods: From July 2004 to July 2005, we administered ertapenem (1 gr iv/24 h) to 15 adult patients hospitalized for less than 72 hours and diagnosed of early-onset VAP. Pts with prior antibiotics or recent hospitalization were not considered candidates. Administration of other antibiotics was not allowed. Assessment of efficacy and safety was done at the end of therapy and a month thereafter. MICs of ertapenem were determined by E-test.

Results: Nine men and 6 women were included, with a mean age of 43 years, and a mean APACHE II score on admission of 19.8, who received a mean of 7.4 days of ertapenem therapy. Underlying conditions were: cranial trauma (4), intracranial haemorrhage (4), stroke (1), drug intoxication (3), polytraumatism (2), Guillain-Barré syndrome (1). Causative organisms were: *H. influenzae* (alone in 5 cases, with *S. aureus* in 3, and with *S. pneumoniae* in 1), MS – *S. aureus* (2), *S. pneumoniae* (2), *E. coli* (1) and unknown (1). One patient was withdrawn at day 7due to thrombocytopenia and was considered a failure, although pneumonia was improving. The remaining 14 pts were considered clinically cured at all assessments. We did not observe emergence of resistance nor respiratory superinfections, but 2 pts had catheter-related bacteraemia due to MR-*S. epidermidis*. The drug was well-tolerated; 3 pts presented a transient increase in aminotransferase levels. Two pts died of unrelated causes (progressive brain damage) lately during hospitalization.

Conclusions: Ertapenem appears to be suitable for early-VAP therapy. This pilot study opens the door for subsequent controlled trials considering this indication

Epidemiology of MRSA, VRE & other Gram-positives

R1984

A mortal case of methicillin-resistant *Staphylococcus aureus* prosthetic aortic valve endocarditis with a paravalvular abscess and mediastinitis

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Objectives: To present an aortic valve endocarditis with paravalvular aortic abscess and mediastinitis that required extensive surgical debridement in addition to broad-spectrum antimicrobial chemotherapy.
Methods: The record of 53-year-old man with prosthetic aortic valve admitted to Cardiovascular Surgery Department with fever and purulent drainage after 3 weeks postoperatively was evaluated in detail.
Results: A 53-year-old male with a history of aortic valve replacement and coronary artery bypass (CABG) surgery 3 weeks ago was readmitted to our institution on the 5th day of discharge with fever greater than 38°C and purulent drainage from sternum. On admission the following sings were noted: blood pressure 110/70 mmHg, pulse rate 124/min, body temperature of 39.7°C, a systolic murmur, C-reactive protein 350 mg/L, erythrocyte sedimentation rate 98 mm/hour, haemoglobin 11.4 g/dl, white blood cell 17.9×109/L, platelet count 379×109/L, creatinine 1 mg/dl. Because of mediastinitis and history of cardiovascular surgery vancomycin plus gentamicin plus rifampin began empirically due to risk of endocarditis. On the 3rd day of the therapy MRSA was isolated from 2 blood and surgical wound cultures. Transesophageal echocardiographic examination revealed a huge (3.5×1.5 cm) prosthetic aortic valvular vegetation, mild aort and mitral valve regurgitation. An ill-defined hypoechoic area located between prosthetic aortic and native mitral valves, which was considered as an abscess. Colour Doppler showed features of blood entry into this space. On the same day the diagnosis of aortic valve endocarditis and paravalvular abscess was made on the basis of physical examination, laboratory findings and transoesophageal echocardiography. Urgent surgical intervention by means of complete debridement of all infected tissue was made on under antimicrobial chemotherapy, because of haemodynamic deterioration and risk of embolization. Despite intensive surgical and appropriate medical intervention, patient was died because of sepsis and biventricular failure on the same day of operation.
Conclusion: The complicated prosthetic aortic valve endocarditis with an abscess formation requires extensive surgical debridement plus appropriate antibiotic therapy. However, treatment will fail to control infection and as a result of sepsis, mortality will occur.

R1985

Outbreak of PVL-producing MRSA among newborns in a Greek tertiary hospital

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Objectives: To describe an outbreak of MRSA PVL-producing strains caused impetigo among newborns in a Labour Ward of a tertiary Greek Hospital.
Methods: From June to September 2005, cultures from skin lesions were collected from 24 newborns in the Labour Ward of the University Hospital of Larissa that suffering from impetigo. Gram stain of pus showed many polymorphonuclear leucocytes with intracellular or extracellular gram-positive cocci, while the cultures yielded pure growth of staphylococci on blood agar plates. Strains were identified as *S. aureus* by Gram stain, catalase and coagulase activity. Susceptibility tests to various antimicrobial agents (penicillin, co-trimoxazole, ofloxacin, clindamycin, erythromycin, gentamicin, tobramycin, kanamycin, amikacin, rifampicin, tetracycline, fusidic acid, vancomycin and teicoplanin) were performed by disk diffusion method and by E-test. The presence of PVL-gene was assessed by PCR. The clonality of the isolates was examined by pulse-field gel electrophoresis analysis. Nasal screening for carriage of *S. aureus* was performed first in the personnel of the Labour Ward (25 persons) and next in the mothers of the infected children. Isolates identified as *S. aureus* were further analyzed for antibiotic susceptibilities, genetic relatedness by PFGE, and PCR for the detection of the PVL-gene.
Results: All isolates were resistant to oxacillin (MRSA), and surprisingly, exhibited the same resistant phenotype, comprising resistance to kanamycin, tetracycline and fusidic acid. The presence of PVL-gene was detected in all MRSA. PFGE analysis revealed that all MRSA were genetically identical, and belonged to clone C, that predominates in the Greek community. Nasal screening revealed that five nurses were colonized by *S. aureus* isolates, and two of them were carriers of PVL(+) MRSA, that was the causative agent for the outbreak.
Conclusions: Outbreak was controlled when the two nurses were excluded from the Labour Ward.

R1986

Prevalence of *Streptococcus pneumonia* isolated with reduced susceptibility to penicillin in northeast of Iran

T. Rashed, K. Ghazvini, A. Heidari, M. Faramarzi *(Mashhad, IR)*

Objectives: *Streptococcus pneumonia* is common cause of pneumonia, meningitis, acute otitis media and many other infections. The introduction of antibiotics heralded a new era in the chemotherapy of infectious diseases, but over the ensuing years bacteria have developed resistance to virtually every known antibiotic. Historically, penicillin was highly active against *S. pneumoniae*, but with the reports of decline in the susceptibility to penicillin the importance of susceptibility testing of *S. pneumoniae* is apparent. The intent of the present surveillance was to evaluated the susceptibilities of *S. pneumoniae* strains to penicillin in northeast of Iran.
Methods: During 2002 and 2003, 105 isolates of *S. pneumoniae* were subjected to quantitative MIC testing against penicillin by using antimicrobial gradient strips (E-test) in two major laboratories of Mashhad University of Medical Sciences, Iran. In this study, breakpoints used to confirm the susceptibility of *S. pneumoniae* to penicillin was those which recommended by the NCCLS. (<0.06 mg/L: Sensitive, 0.12–1.0 mg/L: Intermediate, >2.0 mg/L: Resistant).
Results: Among 105 *S. pneumoniae* strains tested, 69 (65.71%) were isolated from male patients and 36 (34.28%) were isolated from female patients. Overall, 27.61% of the isolates were sensitive to penicillin, 59.04% of the isolates showed intermediate to penicillin and 13.33% of the isolates were resistant to penicillin.
Conclusion: The prevalence of penicillin-resistant strains varies geographically. Some studies have shown very high rates of

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penicillin-resistant *S. pneumoniae* in certain areas (e.g. Spain, Eastern Europe, some Asian countries and…), ranging from 25 to 86%. By contrast, penicillin-resistant strains remain uncommon (<5%) in some other countries. Once resistant strains are present in a geographic locale, subsequent spread may escalate rapidly by selection pressure. The dramatic rises in resistance have major implications for the appropriate selection of antibiotics and brought empiric use of penicillin into question.

R1987

Heterogeneity of methicilin-sensitive *Staphylococcus aureus* at a German university hospital: a potential source of new emerging MRSA clones

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Objectives: Recently we could demonstrate rapid dissemination of different methicillin-resistant *Staphylococcus aureus* (MRSA) clones at the University Hospital. The majority of them harboured the readily transmissible mec cassette type IV. Thus, theoretically MSSA might capture the mecA gene from circulating MRSA or MRSA strains may catch mobile toxin genes from MSSA. The aim of the present study was to characterize the MSSA strains at the University Hospital in Magdeburg and the nearby chronic care facility (RCCF) and to compare these with the simultaneously circulating MRSA clones.
Methods: To serve this purpose, we determined their antibiotic resistance phenotypes and used a combination of different molecular typing methods including MLST, spa typing, agr specificity, and analysis of their pathogenicity profiles.
Results: Among a total of 84 MSSA strains under study about 40% possessed the tst gene and up to four additional enterotoxin genes. Tst-positive MSSA strains belonged to all known agr groups (I-IV), to 14 different spa types (t008/t012/t015/t019/t024/t056/t065/t127/t133/t162/t271/t287/t399/t400) and were classified by MLST as ST1/ST8/ST30/ST39/ST45/ST101/ST121/ST395 and ST426. In contrast, simultaneously circulating MRSA (n = 24) harboured in general two or three genes of the enterotoxin gene cluster and the tst-positive MRSA belonged to the well known epidemic strains ST22, ST45, ST228 and were classified as spa types t001, t028 and t032.
Conclusion: From our results one may conclude that the pool of circulating MSSA is an important parameter with regard to the epidemiology of hospital- and community aquired MRSA and their potential virulence.

R1988

Streptococcus pneumoniae in adults: serotype distribution and antimicrobial resistance

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Objectives: The aim of this study was to investigate the serotypes and the antimicrobial resistance of *Streptococcus pneumoniae* among adults of northwestern Greece.
Methods: A total of 106 strains of *Streptococcus pneumoniae* were isolated from various sites: 40 strains from patients with invasive pneumococcal diseases (IPD) and 66 with non-IPD. Serotype was performed by Quellung reaction (Statens Serum Institute). Susceptibility testing to 8 antibiotics was performed by E-test (AB Biodisk). According to our results, the most common serotype found among the isolates from IPD and non-IPD was 19F (45% and 27.3%, respectively). The 7-valent pneumococcal conjugate vaccine (7vPCV) and the 23-valent pneumococcal capsular polysaccharides (23vPPV) were able to cover 65% and 95% of IPD isolates. Overall, 22 isolates (20.75%) were resistant to at least one antibiotic tested. 6 (15%) IPD isolates and 20 (30.3%) non-IPD isolates were resistant to one or more antibiotic tested. 20.8% of the isolates were penicillin nonsusceptible (PNS), and 45.5% belonged in serotype 19F. Penicillin intermediate resistance pattern was the most frequent phenotypes accounted for 63.63% (14/22) of resistant strains and for 13.2% (14/106) of the total isolates. Multi drug resistant isolates (resistance to three or more antibiotic) accounted for 5.66% (n = 6) of the 106 *S. pneumoniae* isolates. All the isolates remained fully susceptible to amoxicillin, cefotaxime, linezolid, vancomycin and levofloxacin. 9.4% were resistant to erythromycin, 1.9% to co-trimoxazole and clindamycin.
Conclusions: The data presented here highlight not only the epidemiology of pneumococcal serotypes and the emergence of drug resistant strains, but also the impact of prevention programmes such as vaccination.

R1989

Prevalence of carriers of methicillin-resistant *Staphylococcus aureus* in 100 staff of a hospital in Kashan, Iran

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Background: *Staphylococcus aureus* is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital infections all over the world. The source of infection is nasal carrier hospital personnel. Determination of antibiotic resistance pattern of isolated strains is essential for treatment of carrier.
Objective: The aim of this study was access the incidence of methicillin resistant *S. aureus* (MRSA) carriage in the Shahid Beheshti University Hospital, Kashan (Iran).
Material and methods: To find prevalence of MRSA carrier, A prospective survey was conducted over 100 personnel in Shahid Beheshti Hospital of Kashan, from March 2001 to Sep 2002, total specimens were taken from the anterior nares of staff, were cultured on the selective media, isolates were identified based on coagulase and conventional biochemical reactions according in Standard Method. All staphylococcus isolates were screened for methicillin resistance by in oculation of Muller-Hinton agar supplemented with salt and oxacillin 6 μg/ml, according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines, other tested antibiotics included amoxicillin (20 μg), ciprofloxacin (5 μg), penicillin (10 units), gentamycin (10 μg), rifampin (30 μg) vancomycin (30 μg), then the results were presented by descriptive analysis.
Results: The results showed (12%) of Staphylococcal isolates were coagulase positive, of the total *Staphylococcus aureus*, 7 (58.3%) were resistant to methicillin.
Conclusion: Resistance pattern of *Staphylococcus aureus* to various antibiotics, especially methicillin is towards increasing trend, it seems the prevalence of MRSA clonisation in the staff of University Hospitale of Kashan(Iran) is increasing.

R1990

Prevalence of methiciilin-resistant *Staphylococcus aureus* among health care staff working in a general hospital, Yazd, Iran

A. Dehghani, M. Kalili *(Yazd, IR)*

Introduction: *S. aureus* is a common cause of pyogenic infection. Between 20% and 30% of people carry *S. aureus* in their nose and

may also carry the organism on their skin. Methicillin -resistant strains cause the same infections as sensitive, but are particularly associated with hospitalization, exposure to invasive procedures and treatment in intensive care. The aim of this study was to determine the prevalence of MRSA among Health care staff working in different wards of the Shahid Sadughi General Hospital from June to oct.2005 in the city of Yazd/IRAN.

Method: Samples from nose of 340 Health staff (155 male and 85 female) working in 17 different wards were swabbed and inoculated on the MSA medium. Following incubation at 37°C. for 24 hrs, the colonies were further examined for *S. aureus* and then Anti-microbial sensitivity testing was performed using 0.5 Mcfarland suspension of the islated organism. The entire surface of a Muller - Hinton medium was swabbed with organism and a standard methicilin disk was embedded on the medium. After 18 hrs incubation, the zone of inhibition was measured and noted.

Results: A total of 340 health staff working in 17 different wards at the Shahid Sadughi General Hospital were participated in this survey. 38 samples (11.2%) were positive for *S. aureus*, in which 22 (57. 9%) were found to be MRSA. Five wards; Emergency, ophthalmology, orthopedic and nerve department were all negative for MRSA. In comparison, person of Operation room and Gynecology were the most contaminated.

Conclusion: The results show that 22 (6.5%) of hospital workers were colonized for MRSA. Although this result is satisfactory, but it is necessary to emphasize that transmission to patients may threaten their life. Therefore, following cleaning up the carriers, intensive care and control program must be implicated to stop further transmission.

R1991

Detection of methicillin resistance in *Staphylococcus aureus*

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the major pathogens. The most common methods currently used for identifying methicillin (oxacillin) resistance in many clinical laboratories are susceptibility tests. The performance of these tests has been erratic because the expression of resistance is variable and commonly heterogeneous within strains.

Methods: A retrospective laboratory-based study carried out with clinical isolates of *S. aureus* from active infections in patients of intensive care units in a tertiary University hospital during 2001–2002. Sixty one strains of *S. aureus* recovered from various specimens, as blood cultures, tracheal aspirates, wound swabs and venous catheters. Methicillin (oxacillin) susceptibility tested by five different methods:(1) agar screening test [MH-oxacillin (6 mg/ml) agar supplemented with 4% NaCl], (2) susceptibility determination by microdilution method (Vitek 2, BioMerieux), (3) MIC determination by E-test (AB Biodisk), (4) Penicillin-binding protein (PBP) 2a detection by latex agglutination test (BioMerieux), (5) mecA gene detection by real-time PCR (Light Cycler, Roche), using specific primers and probes. The strains evaluated by using the presence of mecA gene detected by PCR, as definitive criteria for MRSA and non-MRSA. The susceptibility tests carried out as recommended by the NCCLS.

Results: Among all the isolates, 31 (51%) identified as mecA positive and the remaining 30 (49%) as mecA-negative. The percentages of sensitivity were: oxacillin agar screen 98%, latex agglutination test 95%, E-test 93%, and Vitek2 93%. Three isolates, negative for the mecA gene by real-time PCR, recognized by at least one phenotyping method as oxacillin resistant. Two strains, mecA positive, incorrectly identified as oxacillin sensitive by the oxacillin agar screening test.

Conclusions: As shown in this and other studies, no phenotypic method is completely reliable for detection of oxacillin resistance in *S. aureus*. The sensitivity was higher with the agar-screening test than with the other conventional methods. In particular, the oxacillin agar screening test and PBP2a latex agglutination test were the most accurate methods and they approached the accuracy of PCR, so they should be applied in association with the other susceptibility methods, to improve the ability to accurately detect oxacillin susceptibility in *S. aureus*.

R1992

Prevalence of methicíllin-resistant *Staphylococcus aureus* among health care staff working in a general hospital, Yazd, Iran

M.B. Khalili *(Yazd, IR)*

Introduction: *S. aureus* is a gram positive bacterium which may cause different pyogenic infection in the body. Today, it is well known that between 20% and 30% of people carry *S. aureus* in their nose and may also carry the organism on their skin. Methicillin-resistant strains cause the same infections as sensitive, but are particularly associated with hospitalization, exposure to invasive procedures and treatment in intensive care. The aim of this study was to determine the prevalence of MRSA among Health care staff working in different wards of the Rahnemoon General Hospital from June to October 2005 in the city of Yazd/IRAN.

Method: Samples from nose of 171 Health staff working in 8 different wards were swabbed and inoculated on the MSA medium. Following incubation at 37°C for 24 hrs, the colonies were further examined for *S. aureus* and then Anti-microbial sensitivity testing was performed using 0.5 Mcfarland suspension of the islated organism. The entire surface of a Muller - Hinton medium was swabbed with organism and a standard methicilin disk was embeded on the medium. After 18 hrs, incubation, the zone of inhibition was measured and noted.

Results: Out of 171 nose samples, 62 were male and 109 female.

Strain	A	B	C	D	E	F	G	H	Total (%)
S.aureus	7	2	7	4	6	-	2	5	33 (19.3%)
MRSA	5	1	4	4	6	-	2	3	25 (14.6%)
Total	12	3	11	8	12	-	4	8	

33(19.3%) were positive for *S. aureus*, in which 25 samples (14.6%) were found to be MRSA (table I).

Conclusion: The results show that 25 (19/3%) of hospital workers were colonized for MRSA. Although this results is satisfactory, but it is necessary to emphasise that transmission to patients may threaten their life Therefore, following cleaning up the carriers, intensive care and control program must be implicated to stop further transmission.

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R1993

Clinical review of *Staphylococcus aureus* bacteraemia in a Belgium tertiary centre

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Staphylococcus aureus bacteraemia (SAB) continues to be a major problem related to both community and nosocomially acquired infection. Nevertheless few clinical data are presently available about SAB in Belgium. Knowledge of our hospital population's characteristics is essential for better management.

Objectives: We aimed to identify and to compare institution specific risk factors, clinical characteristics and mortality rates associated with methicillin resistant *Staphylococcus aureus* (MRSA) bacteraemia and methicillin sensitive *Staphylococcus aureus* (MSSA) bacteraemia.

Methods: We retrospectively reviewed the charts of 147 patients, admitted between January 1, 2002 and December 31, 2004, who had at least one blood culture positive for MRSA or MSSA. The following data were recorded: demographic data, clinical course of infection, comorbidities, microbiological data, adequate treatment (at least vancomycin or teicoplanine for MRSA bacteremia and at least Beta-lactamines for MSSA bacteremia), complications and outcome.

Results: We found 59 patients with MRSA bacteraemia (40.14%) and 88 with MSSA bacteraemia (59.86%). Most cases of MRSA blood stream infection (BSI) were nosocomial (72.88%), whereas most cases of MSSA BSI (67.05%) were community acquired ($P < 0.001$). In multivariate analysis, risk factors associated with MRSA BSI were previous exposure to antibiotic therapy (OR 5.21, 95% CI 1.51–10.34; P: 0.001), and MRSA colonisation (OR 26.31, 95% CI 9.92–69.84; $P < 0.001$). Only intravenous drug use was identified as significant risk factor for MSSA BSI. Overall hospital mortality was 38.1%; there was no significant difference in terms of mortality attributable to infection between MRSA and MSSA BSI. Presence of respiratory infection (OR 3.43, 95% CI 1.37–8.53; P: 0.008), septic shock at presentation (OR 6.04, 95% CI 2.35–15.52; $P < 0.001$), endocarditis (OR 6.07, 95% CI 1.53–24.07; P: 0.01) and inadequate treatment (12/147) (OR 7.36, 95% CI 1.74–31.00; P: 0.007) were independent factors predictor of mortality.

Conclusion: Our study emphasizes the importance of early detection of MRSA nasal carriage and underlines the importance of an appropriate choice of antibiotic drugs. Vigorous management is recommended for patients presenting SAB at admission with pneumonia, septic shock or endocarditis.

R1994

Study of nasal carriage rate of *Staphylococcus aureus* in hospital personnel in Ilam, Iran

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Objectives: The aim of this study was to detect the nasal carriage rate of *Staphylococcus aureus* in Hospital personnel and determination of methicillin resistance.

Methods: In a cross sectional study two hundred nasal swabs were collected, and cultured on blood agar and manitol salt agar for isolation of *S. aureus*. Carbohydrate fermentation tests, sensitivity to lysostaphin and novobiocin disks and also coagulase test were used to confirm the organism.

Results: *S. aureus* was isolated from 76 (38%) of 200 samples. According to the results, there was no significant relationship between carriage rate and age and also carriage rate and sex ($P > 0.05$). Of 76 isolates of *S. aureus*, 28 isolates (36.8%) were resistant to methicillin.

Conclusion: Our results showed that the high rate of nasal carriage of *S. aureus* among Hospital personnel and most of *S. aureus* isolates were resistant to methicillin.

R1995

Prognosis of enterococcal bacteraemia in critically ill patients

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Objectives: The aims of this study were to know the prevalence, clinical characteristics, and prognosis of critically ill patients with enterococcal bacteremia, in an intensive care unit.

Material and methods: From 1996 to 2005, 322 patients with clinically significant bacteraemia admitted in an intensive care unit were prospectively evaluated. Clinical characteristics and related mortality of cases produced by Enterococci are presented. A multivariate analysis was performed to determine the influence of Enterococci on related mortality.

Results: Thirty (9.3%) of 320 blood stream infections were due to Enterococci. All cases except one was hospital acquired. In twenty-eight cases (93.3%) the specie isolated was *E. faecalis*, and in 2 cases *E. faecium*. Fifty percent of cases were polymicrobian: *Acinetobacter baumnannii* (n = 5), *Pseudomonas aeruginosa* (n = 4), *Staphylococcus aureus* (n = 3), *Candida* spp (n = 3), *Escherichia coli* (n = 2). The mean age of patients was 66.8 ± 1.6 years, and the relation between men/women was 1.7. The identified focus of bacteraemia were catheter (20%), urinary (17%), and abscesses (8%). The mean APACHE II score at the onset of bacteraemia was 19.5 ± 8.1. Diabetes mellitus was present in 20% of cases. Eighty percent of patients had received previous antibiotic treatment. Severe sepsis or septic shock was present in 66.6%. There was no case of vancomycin-resistant enterococci. The incidence of inadequate empirical antibiotic treatment was 23.3 %, and the related mortality rate for enterococcal bacteremia was 20% (not significantly different from the mean of mortality by other significant microorganisms, 27.4%). Septic shock and APACHE II score at the onset of bacteremia were related to mortality, but not enterococcal bacteraemia, by multivariate analysis.

Conclusions: Enterococci are a significant cause of blood stream infections in intensive care, and the related mortality to enterococcal bacteraemia (20%) is not significantly lower than the cause by other microorganisms in critically ill patients.

R1996

Distinguished features in systemic staphylococcal infections with *S. aureus* and coagulase-negative staphylococci

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Objectives: The aim of this study was to evaluate the actual epidemiological, clinical, bacteriological and evolutive differences in systemic infections with *S. aureus* (SA) and coagulase-negative staphylococci (CNS) in the era of major susceptibility variations of staphylococcal strains.

Methods: We analyzed 103 patients with systemic staphylococcal infection, between October 2002-September 2005. Staphylococcal strains were identified by conventional methods and using ID 32 Staph strips (bioMerieux®, France). Antibiotic susceptibility testing was performed by disk diffusion method

according to NCCLS standards and using ATB Staph strips (bioMerieux®, France).
Results: We isolated 73 strains (71%) of *S. aureus* (40% methicillin resistant, SAMR) and 30 strains (29%) of CNS (67% methicillin resistant, MR-CNS). Clinical isolates were recovered from blood cultures: 62%-SA, 66%-CNS, pus: 15%-SA, 3%-CNS, blood and catheter cultures: 27%-CNS, 12%-SA and other specimens normally sterile. Predisposing conditions were identified as: recent skin and soft tissue infections: 34%-SA, 7%-CNS, catheters: 27%-CNS, 12%-SA, surgery: 23%-CNS, 14%-SA, sepsis recurrences: 20%-CNS, 4%-SA, hospitalization: 15%-SA, 13%-CNS prosthetic devices: 13%-CNS, 8%-SA. The dominant clinical presentation was fever of unknown origin: 30%- CNS, 15%-SA, pyodermitis: 19%-SA, pulmonary infection: 18%-SA, 13%-CNS, arthritis: 19%-SA, 16%-CNS, septic shock: 20%-SA, 16%-CNS. Multiple secondary foci of infections were found in 33% for SA and 17% for CNS. The complications rate was 55% for SA and 33% for CNS and a severe evolution (severe sepsis, septic shock and MSOF) marked 40% of SA infections and 26% with CNS. Antibiotic switch was made in 68% of SA infections and 50% with CNS. Effective therapy was represented by associations including - betalactames: 41%-SA, 17%-CNS, glycopeptides: 37%-CNS, 29%-SA, oxazolidinones: 20%-CNS, 10%-SA, fluoroquinolones: 14%-SA, 7%-CNS. The overall rate of mortality was 16, 50%, with 21% for SA and 12% for CNS.
Conclusion: The overall rate of methicillin resistance was 48%, with a higher rate for CNS. 24% of SAMR and 10% of MR-CNS were community acquired. SSI with SA had more severe evolution, higher rate of multiple determinations and complications and higher mortality rate, while SSI with CNS had a more insidious evolution, lower rate of complications and mortality.

R1997

Monitoring of methicillin-resistant *Staphylococcus aureus* in a Greek tertiary care hospital: comparison between internal and surgical units

E. Protonotariou, M. Tsivitanidou, V.P. Pliatsika, D. Sofianou *(Thessaloniki, GR)*

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been considered as the typical bacterial populations presented in hospitals and therefore as a major cause of hospital-acquired infections. This parameter needs periodical evaluation due to its increasing frequency during the last decade. The purpose of the present study was to monitor MRSA in our hospital and compare its distribution between internal and surgical units.
Methods: A retrospective analysis was performed of microbiology records on bacterial isolates from patients admitted in Hippokration Hospital, and were infected with *S. aureus* strains during the period 2002–2004. Identification and susceptibility testing were achieved using the Vitek 2 automated system (bioMerieux, France). Statistical analysis was accomplished with Fisher's exact test.
Results: A total of 275 isolates of *S. aureus* were reviewed from hospitalized patients admitted in Internal (IU) and Surgical Units (SU). Samples were collected from wounds, bronchial secretions, blood, urine, catheter tips and other sites. The distribution of MRSA varied significantly between SU and IU (65% vs 26 %, $p < 0.0001$). In particular, 111 out of 171 strains isolated from SU patients were found to be MRSA (65%). On the other hand, only 27 strains out of 104 from IU patients were MRSA (26%). Resistance of MRSA to other antimicrobial agents was also high. Among MRSA strains from SU, 72% were resistant to clindamycin, 78.3% to erythromycin and 87.3% to tetracycline. The resistance of MRSA strains isolated from IU patients to clindamycin, erythromycin, and tetracycline was 55.5%, 59.2% and 92.5%, respectively. No strain from both units was found resistant to vancomycin or teicoplanin.
Conclusions: a) Resistance of *S. aureus* to methicillin (MRSA) was high particularly in SU (65%) and should be of concern b) Awareness resistance to other common antimicrobial agents was also important c) A continuous programme of surveillance of MRSA strains as a way of active antibiotic policy is required.

R1998

Nasal carriage of *S. aureus* among hospital personnel of a secondary general hospital

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S. aureus nasal carriage in hospital personnel has been identified as an important risk factor for nosocomial infections.
Objectives: The aim of our study was to determine the incidence of *S. aureus* nasal carriage in the personnel of a Secondary General Hospital (120 beds), without significant prevalence of staphylococcal infections in it, and the investigation of the genotypical and phenotypical characters of the strains recovered.
Methods: A total of 40 doctors and nurses (mostly of the surgical section of the Hospital) were examined during autumn 2005. Samples taken from the anterior nares were cultured using appropriate media. The strains' identification was performed by catalase production, 24-hour coagulase test, and the API Staph system (Biomerieux, Marcy L' Étoile, France). All the strains were tested for the production of PBP2a (Slidex MRSA, Biomerieux, Marcy L' Étoile, France), their susceptibility to antibiotics was performed by the disk diffusion technique and the results were interpreted according to the criteria of the N.C.C.L.S. Moreover, molecular analysis for the mecA gene and the Panton-Valentine leucocidin (PVL) - encoding genes, lukF and lukS, was performed by PCR.
Results: 1)The incidence of *S. aureus* nasal carriage among hospital personnel was 20%, and presented to be higher among doctors(31% – 5 out of 16) than among nurses (12.5% – 3 out of 24). 2) The specimens' cultures yielded 5 MSSA and 3 MRSA with total prevalence 12.5% and 7.5% respectively. 3) Three strains carried the mecA gene and produced PBP2a, whereas another 2 strains produced PBP2a but lacked the mecA gene. 4) Only one strain produced PVL leucocidin, isolated from the nasal specimen of a surgeon. The PVL-positive *S. aureus* belongs to MLST-80 clone. This clone circulated in Greece since 2002 and recently demonstrates an arising spread capacity. 5) All the strains were sensitive to quinolones, glycopeptides, cotrimoxazole, clindamycin, erythromycin, linezolid, rifampicin, mupirocin and the PVL-positive strain was resistant not only to beta-lactames, but to tetracycline and fucidic acid as well.
Conclusions: The percentage of *S. aureus* nasal carriage of doctors of our Hospital is higher compared with other hospitals of equal potency in Greece, but with low prevalence of staphylococcal infections. The isolated strains were multisusceptible. The isolation of a PVL-positive strain should arise alert in order to prevent the transmission of these strains, which may cause severe disease.

R1999

Genotyping methicillin-resistant *Staphylococcus aureus* by PFGE in a clinical hospital in Szczecin, Poland: 9-year study

K. Kopron, J. Jursa-Kulesza, S. Giedrys-Kalemba *(Szczecin, PL)*

Objective: The purpose of the study was genetic relatedness between MRSA strains isolated from different patients hospitalized in various wards of Clinical Hospital.
Material and method: A total of 270 MRSA isolates were cultured between 1994–2004 from various materials (blood, bronchoalveolar washings, sputum, secretion from wounds, catheters, drains, liquids from pleura and peritoneum) from patients hospitalized in various wards of Clinical Hospital in Szczecin: intensive care unit, surgery, internal medicine, urology, dermatology, cardiology. PFGE – SmaI was applied to analyze genetic relatedness among the isolates. Seperation of DNA fragments was achived using a CHEF DR II apparatus (Bio-Rad). The obtained patterns were compared by software Molecular Analyst Fingerprinting Software, using the Dice coefficient.
Results: Among the 264 isolates macrorestriction with SmaI and PFGE yielded 13 clonal epidemic strains, designated as A-M, and 6 strains were identified by unique PFGE patterns. During the period 1994–1997 two main PFGE types were detected: type A (48 strains) - isolated in all hospitals wards and type B (6 strains) – isolated in cardiology and urology ward only. The appearance of new genetic types were observed in the wards of Clinical Hospital from the beginning 1998 to 2004. The most frequently types were: type D included 86 strains and type E – 25 strains. Both clonal strains (D, E) were isolated for 3–4 years in surgery ward and intensive care unit. Types G, H, I, K were found in all hospital wards and the occurrence of types C, F, J, L was typical for one ward.
Conclusion: Molecular diversity of MRSA strains isolated from various wards for 9 years shows on their exchange and probably different sources of MRSA strains in hospital ecosystem. On the other hand, isolation of the same genetic types from different wards and patients proves horizontal spreading of the MRSA clones in Clinical Hospital. Maintenance of clonal strains MRSA for months or years in hospital environmental needs more effective of MRSA hospital control.

R2000

Clonal spread of community-acquired methicillin-resistant *Staphylococcus aureus* in Latvia

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Objectives: The first community acquired (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) in Latvia was isolated in 2003. Though, no study on CA-MRSA occurrence among outpatients was carried out in Latvia strains with CA-MRSA characteristics (very low oxacillin resistance, type IV SCCmec, PVL) have been isolated from patients during their admission (or within 48 h afterwards) to hospital with increasing frequency. This report provides summary of CA-MRSA found at three hospitals in Riga and Valmiera.
Methods: 513 MRSA isolates were collected at Pauls Stradins Clinical University Hospital, Gailezers Clinical Hospital and Valmiera Hospital from January 2004 to October 2005. Antimicrobial susceptibility testing on these strains was performed according to CLSI standards by disc-diffusion method and the presence of the mecA gene was verified by PCR. PVL positive strains were identified by PCR and genotyped by pulse field gel electrophoresis (PFGE) after SmaI digest, staphylococcal cassette chromosome mec typing, spa sequence typing and MLST.
Results: Screening of 513 MRSA strains revealed eight isolates (six were recovered from skin lesions and two blood isolates) harbouring genes required for the synthesis of PVL. All PVL positive strains shared common antibiogram pattern: very low resistance levels to oxacillin (MIC from 0.25 to 1.00 mg/l) and susceptibility to all antibiotics tested (ERY, GEN, CIP, CHL, CLI, RIF, TET, STX and VAN), and carried SCCmec of type IV. Among these clones, ST30-MRSA-IV was dominant – six of eight isolates belonged to this type. The ST30-MRSA-IV group can be split into three factions with distinct PFGE patterns of 4, 1 and 1 clones, respectively. This division is in agreement with spa typing which also separates the clones into three factions with strongly related spa types: t019, t012 and t021, respectively. The biggest faction of clones is indistinguishable by phenotypical and molecular characteristics from the first Latvian CA-MRSA isolated in 2003.
Conclusion: The clonal spread of CA-MRSA in Latvia is revealed. ST30-MRSA-IV with identical PFGE pattern and spa type was found in two hospitals in Riga and in northern city Valmiera hospital. Studies on CA-MRSA among outpatients are urgently required, as well as family doctors should pay special attention to this issue.

R2001

Surveillance of vancomycin-resistant enterococci in high-risk patients in a tertiary hospital

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Objective: The first vancomycin resistant enterococcus (VRE) isolate in Turkey was from a tertiary hospital in 1998 and reports from other hospitals followed afterwards. The prevalance of VRE ranges between 0 and 10% in different studies reported in our country. After the isolation of vancomycin resistant enterococcus from the blood culture of a hepatic transplant patient in our hospital, this surveillance study was planned.
Methods: The study was carried out in 8 intensive care units and oncology and nephrology departments where high-risk patients were hospitalized. In a time period of 15 months (from May 2003 to July 2004), rectal swab cultures were collected four times (five months apart) from a total of 467 patients. A questionnary form was filled for each patient in order to establish risk factors for the colonization of VRE. Enterococcosel agar containing 6 mcg/ml vancomycin and 70 mcg/ml ceftazidime was used for the screening of VRE. Enterococci were defined by standard microbiological tests and API 20 Strep (BioMerieux®) was used for species identification with some additional biochemical tests. Vancomycin and teicoplanin MIC values were determined by agar dilution. Vancomycin resistance genes (Van A, Van B) were determined by PCR analysis.
Results: Nine enterococcal isolates were defined as vancomycin resistant. The colonization rate with VRE was 1.9%. These isolates were identified as *Enterococcus faecium*. All nine isolates were highly resistant to vancomycin (MIC > 256 mcg/ml) and teicoplanin (≥64 mcg/ml). The resistance genotypes of all strains were Van A. All patients colonized with VRE were hospitalized in the intesive care unit (ICU) for a mean of 30 days. Either glycopeptide or third generation cephalosporin use was detected for these patients.
Conclusion: VRE may cause serious nosocomial infections with high mortality rates. Since fecal carriage of VRE is an important

source of hospital epidemics, surveillance cultures are recommended. It is thought that VRE might become an important nosocomial pathogen in our hospitals in a near future. The restricted use of third generation cephalosporins and glycopeptides is warranted in addition to hospital infection control precautions.

R2002

Surveillance of MRSA carriers among patients and health care workers in haemodialysis units

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Objectives: Carriage with methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent and serious problem, and appears to play a key role in the epidemiology and pathogenesis of infection. Patients on hemodialysis represent a particular high-risk group for infection caused by these organisms due to a combination of their compromised immune system and the need for repeated access to their blood system several times a week. Carriers among hospital staff in this units can also serve as source of colonisation and infection of patients. The aim of this study was to determine the carriage rates of nasal and throat MRSA in patients and hospital staff in haemodialysis units, and efficacy of mupirocin in the eradication of nasal carriage.
Methods: The study included 235 patients and 60 hospital staff from Center for hemodialysis, Clinical Center University of Sarajevo. A total of 474 nose and throat cultures of patients, and 120 cultures of hospital staff were performed. Methicillin resistance of *S. aureus* were determined with disk-diffusion methods according to the National Committee for Clinical Laboratory Standards (NCCLS), and rapid latex agglutination test (Slidex® MRSA Detection-bio Merieux) by detecting PBP2′ (penicillin-binding protein 2′) product of mecA gene. *S. aureus* ATCC 25923 strain was used for control.
Results: Nasal carriage rate of MRSA was 15.3% (36/235) among patients, while the same rate was 11.6% (7/60) among hospital staff. There were no isolates of MRSA from throat of patients, and only one isolates from throat of hospital staff. Results from the disk-diffusion test correlate almost 100% with the results of test that detect PBP2′. Nasal carriage was cleared in 34 (94.4%) of patients with 2% nasal mupirocin ointment (Bactroban) three times a day for 5 days.
Conclusion: Our results indicate a significant rate of MRSA colonisation among haemodialysis dependent patients and hospital staff, and that topical mupirocin is a highly effective treatment for the eradication of nasal carriage. Slide latex agglutination test for PBP2′ represent a rapid and reliable method for the detection of MRSA carriage. Efforts to limit the spread of MRSA in hemodialysis units must include seasonal surveillance of MRSA carriers and appropriate infection control measures to reduce opportunitities for patient-to-patient transmission.

R2003

Nasal and axillar carriage of *Staphylococcus aureus* patients among elderly population in a nursing home

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Objectives: Infections caused by *Staphylococcus aureus* are significant causes of morbidity and mortality in elderly persons in the community, hospital and nursing home. Therefore we attempted to investigate *S. aureus* colonization and risk factors for colonization in nursing home residents. To the best of our knowledge, this is the first study that investigated *S. aureus* colonization in nursing home population in our country.
Methods: In this prospective study whole residents of geriatric care unit (n = 163) were investigated between September 01 and October 31, 2005 in nursing home. Data were collected regarding gender, age, length of stay in the unit, modified daily living activity, underlying conditions, presence of skin lesions, local nasal therapy, antibiotic treatments during the previous 3 months and hospital admissions within the last 6 months. Two swabs were taken; one from the anterior nares and other from axillary area.
Results: Of these elderly persons, 123 (72.4 %) were female and 40 (23.6 %) male. Mean age was 80 (SD; 6, 6 min.60-max. 95). We determined *S. aureus* in 15 (9.3%) participants but none of them was resistant to oxacilline. Of these colonized *S. aureus*, 14 (93.3 %) isolated from nares and 1 (6.7 %) from axillary area. In 14 (93.3 %) of residents colonized with *S. aureus*, out of these risk factors investigated, length of stay in the geriatric unit was more than one year, and modified daily living activity scores were higher than 20, in spite of statistically insignificant. Other risk factors rates are shown in Table.

Risk factors	Colonized (n)		Noncolonized (n)		Total (n)
	Yes	No	Yes	No	
Antibiotic usage	6	9	46	102	163
Skin lesions	2	13	23	125	163
Hospitalization	3	12	22	126	163
Underlying disease	15	-	141	7	163
Local nasal therapy	1	14	16	132	163

Conclusion: In the study group we did not found carriage of methicillin resistant *S. aureus* in crowded living conditions of geriatric persons. However we thought that screening of carriage for the detection of methicillin resistant *S. aureus* should be performed in regular period and at the admission time to nursing home.

R2004

Antibiotic resistance of *Staphylococcus aureus*: a multicentre study 2002–2005 in a paediatric general hospital, Greece

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Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infection with a worldwide prevalence. Furthermore, community strains are beginning to arise and can exhibit increased virulence. In this study we determined the incidence of MRSA among clinical isolates during a 4-year period.
Materials and methods: A total number of 311 *S. aureus* strains from children's clinical specimens were studied during the period 2002–2005. The cultures were performed by conventional methods and identification was confirmed by API Staph system (Biomerieux). Antibiotic susceptibility testing was evaluated by disk diffusion method, according to NCCLS standards. Methicillin resistance was studied by using minimum inhibitory concentration of oxacillin (E-test strips) on Muller-Hinton agar supplemented with 2%NaCl. We also used the agglutination test with monoclonal antibodies against PBP2a, to detect the presence of the mec A gene.

Results: There was a significant statistical difference of MRSA strains during four years period. There was not isolated any MRSA strain resistant to Methicillin. The rates of resistance were as follow:

Resistance	2002	2003	2004	2005
Oxacillin	18,60%	26,50%	9,80%	42,16%
Fucidic acid	46,51%	40,9%	34,31%	42,16%
Clindamycin	4,65%	3,60%	1,96%	2,40%
Netilmicin	2,30%	1,20%	1,96%	4,81%
Vancomycin	0%	0%	0%	0%

Conclusions: There was no significant change about the rate of fucidic acid resistance. An increasing incidence of MRSA was observed. This fact can lead to serious treatment difficulties, since the MRSA strains are resistant to all beta-lactam antibiotics and often also to other groups of antibiotics. The detection of mecA gene by agglutination test against PBP2a, can be used as a reliable and rapid method in the determination of resistance to Methicillin.

R2005

Staphylococcal meningitis – an evaluation over 6 years

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Objective: To evaluate the epidemiological, microbiological and evolutive characteristics of staphylococcal meningitis cases that were diagnosticated in our hospital.
Material and methods: We conducted a retrospective study that include 49 cases of staphylococcal meningitis hospitalized in our department, between January 2000 and August 2005. Culture, isolation, identification and susceptibility were performed following standard recommendations.
Results: The patients, 15 female and 34 male with average age 48.6 years (range between one month to 76 years) were immunocompromised in 13 cases (26.5%). The meningitis occurred after neurosurgical procedures in 17 cases (34.69%) and as secondary septic determination of sepsis in 29 cases (59.18%), having otogenic source in 3 cases (6.12%). The strains, isolated from CSF in 26 cases (53.06%) and from bloodstream in 27 cases (55%) were *Staphylococcus aureus* in 39 cases (79.6%) and *Staphylococcus epidermidis* in 10 cases (20.4%), methicilino-resistant in 12 cases and 6 cases respectively. These organisms were multiresistants to 2–13 antibiotics, with resistance rates of 22.45% for Rifampicin, 30.61% for ciprofloxacine, 28.57% for cefotaxime, 8.16% for amikacine 6.12% for Imipenem and teicoplanin, 16.32% for chloramfenicol. The antibiotics, represented by fluoroquinolones (25 cases – 51%), vancomycin (20 cases – 40.81 %) especially, Rifampicin (13 cases – 26.5%) cloramfenicol (8 cases – 16.32%), Meropenem (5 cases – 10.2%), were administrated in majority of cases in bitherapy (38–77.55%), for average time of 26.6 days, with modification of therapy in 33 cases. The evolution was marked by complications in 16 cases – 32.65% (cerebral abscess – 3 cases, subdural empiema, CSF fistula and cranial nerves palsy in 2 cases each, hydrocephalus – 5 cases, septic shock in 2 cases) with double rate for patients of methicilino-resistant strains (50%) versus those with methicilino-sensible strains (22.58%). The mortality rate was 6.12%, with diference for the two types of patients (11.1% versus 3.22%).
Conclusions: Staphylococcal meningitis has a low incidence and mortality, but nosocomial context and implication of multiresistant strains lead to therapy difficulties, a higher rate of complications and deaths.

R2006

Rabbits as a human *Staphylococcus aureus* reservoir? Preliminary results of a field research

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Objectives: *Staphylococcus (S.) aureus* is responsible for human infections of difficult therapy. In intensive rabbit farms, the germ determines diseases with a heterogeneous epidemiological, clinical and pathological-anatomical picture. In this work, strains of *S. aureus*, coming from dead rabbits for *S. aureus* infections in intensive farms, have been typed. Phenotypic and genetic methodologies have been used in order to detect the species origin.
Methods: Research analyses have been conducted on 29 strains of *S. aureus* isolated from rabbits coming from 9 different intensive farms in the Region of Puglia, in South Italy. The Devriese (1984) protocol for biotyping was applied. The genetic typing has been made with Random Amplified Polymorph DNA (Hermans et al. 2000).
Results: The biological typing showed a prominence (62%) of NHS strains. In some cases, specie-specificity of isolates was found; particularly, one strain belonged to the human type, two were bovine strains, two ovine strains and two of poultry. Three isolates were not possible to be determined. Most part of the strains belonged to the CV:A-type (22 strains); the remaining (7 isolates) to CV:C-type. The genetic typing allowed us to distinguish 12 different profiles. The most frequent of them were n.1 and n 6. The human biotype showed a peculiar RAPD profile. The RAPD methodology highlighted in one farm a change in the genetic type of *S. aureus* that were infecting the rabbits in different times.
Conclusions: In this research heterogeneity in rabbit strains was found. The RAPD showed a prevalent diffusion of the RAPD-1 type infecting farms in different years Dominant genetic types are probably characterized by a higher pathogenic power. Higher virulence is associated to the CV:C type. On the contrary, the CV:A type is seen to be less pathogenic. CV:C/â-strains were often found in human being (Devriese, 1984). The dominance of strains belonging to more than one ecovar might imply that no very strict specie-specificity relations exist. Thus, one animal species could represent a reservoir for others. Consequently, the temporal replacement of the strains in intensive rabbit farms can be favoured by many vectors, including human being, a possible reservoir of the rabbit germ. The find on rabbits of a human strain may suggest the chance of a reverse transfer of the germ from rabbits to man. In this case the rabbit could have the role of *S. aureus* reservoir for human being.

R2007

Description of a methicillin-resistant *Staphylococcus aureus* hospital outbreak

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Introduction: Methicilin-resistant *S. aureus* (MRSA) is a weighty topic in hospital infections. In 2004 our Lab listed 47 MRSA positive cultures from a total of 242 *S. aureus* cultures (19.42%).

In March to April-2005 a warned number of MRSA were registered.
Objective: To describe the epidemiological characteristics of MARSA infected patients in the 2005 outbreak and to set the alert procedures for early detection.
Methods: Descriptive analysis of all the patients suffering from MRSA infection in the outbreak, the outcome and the rules and recommendations by the Infectious Commission (IC).
Results: From 01.03 to 02.05.2005 a total of 21 patients suffered from MRSA infection. Our mean bed-occupation is 270. 12 patients were male and 9 females. The mean age was 70. From 40 to 59 years were 4 cases; 60–79 were 11; 6 were up than 80 years. Previous admissions in the last year were present in 12 patients. Following the IC criteria, 4 cases were hospital acquired, 8 no certain hospital acquired (early MRSA discovery with previous near hospital admission) and 9 possible community-acquired (no previous admissions). By departments: 10 from Internal Medicine, 5 Neumology, 4 Surgery and 1 Traumatology and Hematology. Chronics obstruction to air flow was present in 7 patients, cancer in 6, heart failure and diabetes in 5 and dementia in 3. Three or more associated diseases were present in 12 patients. MRSA was isolated from wound in 10 patients, 6 sputums and 5 blood. Two cases were simultaneously isolated from wound and blood. Treatment was vancomycin in 19 cases and teicoplanine in 2. About the outcome, 12 patients became dead. IC recommendations were to order the contact isolation of the affected patients, grouping them and enforcing the precautions by the health workers; to detect carriers between patients and workers; to indicate decontamination treatments; to report to hospital and health authorities and to search retrospective cases.
Conclusions: The affected patients were mainly elder people, suffering from other health problems, with previous and lengthy hospital stays. Mortality is high, and MRSA is often a supporter cause, although usually is not directly the demise reason. There is an important rate of community source, so MRSA could be considered as an emergent community pathogen. The onset of a MRSA alert and control system must include to elder patients with near previous admissions, former antibiotic therapy and/or skin infections during the admissions.

R2008

Effectiveness of hygiene measures for preventing health care workers from acquiring undesirable pathogens in an infectious diseases ward

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Background: An alert was recently raised by the French National Centre of Streptococci (CNR) about nosocomial diffusion of group A-streptococci. More recently, the large spread among health care workers of the MRSA-clone producing Panton-Valentine leukocidin (PVL-MRSA) has been described by H. Linde et al (2005). In 2004, a woman, was hospitalised for 34 days in our Infectious Diseases Unit (IDU) for a streptococcal toxic shock syndrome (serogroup A). In 2005, another woman, was transferred to the IDU from an Algerian hospital for an antibiotic-sensitive Gram-negative rod pneumonia. At entrance she had a perineal chronic wound, but this ward does not assess the carriage of multi-drug resistant bacteria (MDR), nor perform a MDR-specific isolation unless MDR are found in pathological samples. After 3 weeks, she was transferred in the Intensive Care Unit (ICU) where MDR-specific assessment and isolation are active. Her wound samples yielded a PVL-MRSA with the typical antibiotype (methicillin-R, kanamycin-R and tobramycin-S).
Objective: We tested the hypothesis of healthy carriage of these two pathogens in health care workers after discharges of the respective patients.
Methods: After informed consent, throat swabs were obtained during the 3rd week of hospitalisation of patient-1, from 26 involved health care workers. Streptococci were isolated by routine techniques including CNA-agar incubated in anaerobic conditions. Within 2 weeks after recognition of the PVL-MRSA carrier status of patient-2, nares samples were obtained from 28 informed and agreeing health care workers of the IDU. Samples were streaked on routine media including MRSA-agar (Biorad). Patients of the ICU were not included.
Results: Following patient-1: 3 strains of beta-haemolytic streptococci were isolated belonging to serogroups A, C and F. The A strain was studied by the CNR comparatively to the patient's strain, and found different only for biochemical characters: phenotype 1403.2141.110 vs 1403.2161.111 in the API32 Strep. Following patient-2: we found 3 drug-sensitive *S. aureus*, 1 MRSA, which had not the typical antibiotype, and no PVL-MRSA.
Conclusion: Hygiene procedures currently active in the IDU, even if they are strictly based upon pathological samples and do not include specific prospective MDR detection, do not allow large spreads of *Streptococcus pyogenes* or PVL-MRSA. Genotyping the two available A-*Streptococcus* strains should be performed to strengthen this conclusion.

Epidemiology of MDR-Gram-negatives

R2009

Endemic ESBL producing *Enterobacteriaceae* among paediatric patients over 3 years

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Objective: The aim of this study was to analyse occurrence of ESBL producing *Enterobacteriaceae* in patients who were hospitalized in the Institute of Pediatrics, Medical University of Gdansk [IP].
Methods and materials: We analysed microbiologial records from 2002 to 2004. Strains were identified by classical method and VITEK cards (BioMerieux). Production of ESBL was detected by double disk method.
Results: In the studied period 10 972 patients were hospitalized in IP. ESBL + isolates were recovered from 393 patients. 60%

infecting or colonising ESBL strains were detected in patients with urinary tract infections, 14.5 % with diarrhoea, 12.5 % with haematological malignancies, 8.4% with sepsis and 3.5% suspected pneumonia. *Klebsiella pneumoniae* was isolated from 71 patients then *E. coli* (66 patients) and *K. oxytoca* (24 patients), *E. aerogenes* (22 patients), *Enterobacter cloacae* (9 patients), *Citrobacter freundii* and *Proteus mirabilis* (respectively 5 and 5 patients). Isolates were recovered from stools and rectal swabs (43.5%), urine (37.7 %), respiratory tract (10%), blood culture (1.2%) and intravenous catheter tips (0.8%). All strains were susceptible to carbapenems, about 90% to fluoroquinolones and about 60% urine isolates to nitrofurantoin, nearly 90% isolate were resistant to aminoglyozydes and co-trimoxazole.

Conclusions: High percentage of ESBL carriage among children complicates infection control and treatment of common urinary tract infection. Patients on admission and before discharge should be screened for ESBL carriage. ESBL producing bacteria are the greatest challenge in our pediatric units.

R2010

Carbapenem-nonsusceptible *Acinetobacter baumannii* in a university hospital in Poland

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Objectives: To analyse occurence and phenotype of carbapenems activity of carbapenem nonsusceptible *Acinetobacter baumannii* (CNSAB) in a university hospital in Poland.

Methods: The analysis of microbiological records with *A. baumannii* nonsusceptible to imipenem or meropenem from September 2003 to September 2005 was done. We used Vitek GNI+ cards (bioMerieux) for identification and disc-diffusion technique (CLSI) to determine susceptibility to imipenem (IMP) and meropenem (MEM).

Results: There were 655 one-per-patient *A. baumannii* isolates during the study period. The susceptibility to IMP was 87% and to MEM 80%. CNSAB was isolated from 129 patients. They were hospitalised in ICU (53), surgery departments (47), general medicine (17), haematology (4) and other units (8). From 46% patients CNSAB was isolated only once and from 8% patients there were more than 10 isolates. Two patients had isolates non-susceptible to IMP only and 43 patients to MEM only. The remaining 84 patients had isolates non-susceptible to both carbapenems. In isolates from 27 patients resistance to two carbapenems was found. CNSAB from 9 persons expressed all possible phenotypes IMP-R, IMP-I, MEM-R, MEM-I.

Conclusion: CNSAB were isolated from about 20% patients with *A. baumannii* infection/colonisation. Patients from ICU and surgery are at the greatest risk of acquiring CNSAB during their hospitalisation. MEM non-susceptibility is more common than IMP non-susceptibility. CNSAB from patients with many isolates tends to express different carbapenem resistance phenotypes.

R2011

Epidemiology of resistant *Enterobacteriaceae* in a tertiary hospital

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Introduction: An increase of *Enterobacteriaceae* (most commonly *E. coli* and *E. cloacae*) resistant to cephalosporines and fluoquinolones has been observed within the last years, whereas resistance to aminoglycosides has been stationary. Compared to their use of antibiotic and time of occurance.

Material and methods: The incidence of *E. coli* and *E. cloacae* resistant to cephalosporins (C), fluquinoloes (F) and aminoglycoside (A) were estimated for each antibiotic and combinations of the three antibiotics in different clinical in the years 2002–2004. The incidences of resistant *E. coli* and *E. cloacae* were compared to antibiotic sent from the pharmacy to the departments. To estimate the time of occurance of resistant *E. coli* and *E. cloacae* only patients with resistant bacteria in 2003 and 2004 who have not been in this hospital in 2002 were included. To investigate clonal spread of multiresistant bacteria all *E. coli* and *E. cloacae* resistant to one or more of the three antibiotics were collected and examined by automatic ribotyping.

Results: The incidence of cephlosporin resistance in *E. coli* and *E. cloacae* was found in 134 of 4990 (2.7%) patients; in 491 of 4990 (9.8%) patients strains were found resistant to fluquinolones and aminoglycoside resistant strains were found in 76 of 4990 (1.5%) of patients. Combined resistance were found in CA: 0.6%, CF: 1.1%, FA: 0.5% and CAF: 0.3%. No correlation to the use of antibiotic in the departments were found. In about 75% of patients with *E. coli* or *E. cloacae* resistant to one or more of the three antibiotics these bacteria were found within the first week after admission at a time where resistance could not yet have been developed at the hospital. The remaining 25% of the patients include those who had a susceptible *E. coli* or *E. cloacae* and those in whom these bacteria were not found at the time of admission. Typing of resistant strains revealed that three clones of resistant bacteria occurred in more than one patient. Further typing of strains is needed.

Conclusion: The incidence of *E. coli* and *E. cloacae* resistant to cephlosporines, fluquinolones and aminoglycosides are still low. The majority of the strains seem to be brought to the hospital when patients are admitted but endemic clones may also occur.

R2012

Differences in bacteraemia due to multiresistant pathogens in medical patients: a pilot study

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Objective: Our aim was to assess differences in medical patients with bacteraemia (B) due to multi-resistant (MR) pathogens regarding epidemiology, severity and outcome compared to ones with B pathogens of usually anticipated susceptibilities.

Patient-methods: All medical patients admitted in a 700 bed tertiary hospital's department with signs of severe infection were entered in a PC database and only patients with documented B were proceeded to SPSS analysis. MR pathogens defined as those resistant in at least 3 antimicrobial classes. Time: May 2004 to Oct 2005.

Results: A total of 75 patients (M:33, F:42, m.age:68.4 ± 14 yrs) were found with B. Of those MR B occurred in 17 (22.6% of Bs) due to *Klebsiella* sp: 5, MRSA: 4, *Acinetobacter* 3, *Pseudomonas* 2, *Serratia*, *Salmonella*, *S. faecalis* one each. Comparing MR B to the other bacteraemias, there were no statistical significance in previous antimicrobial use [47% v 36%] or hospitalization, comoridity, origin, SIRS criteria at admission (3 v 2.5). Appropriateness of initial regimen was, expectedly, less in MR (59% v 95%, $p < 0.001$) but did not correlate with higher mortality (6 v 18%, NS). The only significant difference noted was a considerably longer length of stay (27.6 v 9.8 days, $p < 0.0001$) in patients with.

Conclusions: Apart from a considerable longer stay patient characteristics and outcomes did not differ significantly among patients with MR B, though, obviously, a greater number may achieve this, as documented in MR literature. The main point is that seeking bacteriology documentation allows for regimen modification to allow favourable outcome in extremely difficult to treat infections.

R2013

Antimicrobial resistance of Gram-negative pathogens isolated from intensive care unit and non-intensive care unit patients with bacteraemia

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Objectives: To investigate the epidemiology of antimicrobial resistance of Gram-negative bacteria isolated from ICU and non ICU (NICU) patients with bacteraemia.
Methods: From January to October 2005, a total of 508 patients presented one or more episodes of bacteraemia. The identification and MIC determination of the isolated pathogens were performed by the VITEK II system (BioMerieux- France) and the E-test method (Solna, Sweden).
Results: Bacteraemia was detected in 67 ICU and 441 NICU patients. Polymicrobial bacteraemia with two to four pathogens was observed in 32/67 (48%) ICU patients and in 40/441 (9%) NICU patients. Of the 303 pathogens from ICU patients, Gram-negative bacteria were the predominant isolates (n = 161, 53%), followed by Gram-positive (n = 133, 44%). On the other hand, of the 745 isolates from the NICU patients 443 (59%) were Gram-positive and 267 (36%) were Gram-negative. Bacteria more frequently isolated from the ICU patients were: 57 (19%) *Klebsiella pneumoniae*, 45 (15%) *Acinetobacter baumanii* and 36 (11%) *Pseudomonas aeruginosa*. From the NICU patients 76 (10%) were *Escherichia coli*, 69 (9.3%) *Klebsiella pneumoniae*, 39 (5.2%) *Pseudomonas aeruginosa* and 28 (3.8%) *Acinetobacter baumanii*. The resistance rates (%) of *Klebsiella pneumoniae* strains isolated from ICU patients compared to those of the strains isolated from NICU patients were: imipenem 94/75, cefoxitin 95/47, amikacine 50/16, gentamicine 6/17 and colistin 2/0.0. The resistance rates (%) of *Acinetobacter baumanii* strains isolated from ICU patients compared to those of the strains isolated from NICU patients were: imipenem 98/64, cefepime 75/64, amikacine 98/82, gentamicine 48/61, ampicillin/sulbactam 54/41. The resistance rates (%) of *Pseudomonas aeruginosa* strains isolated from ICU patients compared to those of the strains isolated from NICU patients were: ticarcillin 48/14, ceftazidime 45/36, cefepime 43/25, ciprofloxacin 52/36, amikacine 49/36, aztreonam 55/64, imipenem 67/35.
Conclusion: A very high incidence of multidrug resistant Gram-negative strains, with rare and new phenotypes, causing bacteraemia were isolated especially from ICU patients. The emergence of colistin resistance in *Klebsiella pneumoniae* strains is worth noticing. Continuing regular monitoring to recognize new patterns of antimicrobial resistance is mandatory in our hospital.

R2014

Prevalence and antimicrobial resistance of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in a northern Greek rural area

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Objectives: Detection of extended-spectrum B-lactamase (ESBL)-producing *E. coli* and *Klebsiella pneumoniae* isolates is crucial for the determination of treatment since all B-lactams except B-lactams inhibitors, cephamycins and carbapenems are inactive against them. The aim of this study is to assess the prevalence of the ESBL-producing *E. coli* and *K. pneumoniae* clinical isolates in a regional hospital in northern Greece.
Methods: A total of 2342 *E. coli* and 215 *K. pneumoniae* were isolated during a period of two-years from various clinical specimens obtained from outpatients and inpatients. Identification and antimicrobial susceptibility testing was performed with the Microscan system (Dade Behring). The double-disc synergy-test (DDST) was used for ESBL production and results were interpreted according to NCCLS guidelines.
Results: During the study, 91 (3.9%) of the *E. coli* and 19 (8.8%) of the *K. pneumoniae* isolates were found ESBL-positive by the DDST. As many as 67% of the ESBL-producers were recovered from outpatient samples while 7.2% from paediatric samples. The majority of the ESBL-positive isolates (84%) were recovered from urine specimens. Antimicrobial resistance rates of the DDST-positive *E. coli* and *K. pneumoniae* isolates were: 5% and 4% to amikacin, 26% and 32% to ciprofloxacin, 17% and 15% to piperacillin/tazobactam and 67% and 25% to trimethoprim/sulfamethoxazole (SXT), respectively. Antimicrobial resistance rates of the DDST-negative *E. coli* and *K. pneumoniae* isolates were: 2% and 2% to amikacin, 7% and 12% to ciprofloxacin, 3% and 10% to piperacillin/tazobactam and 18% and 18% to SXT, respectively. There was no significant variation in the prevalence of ESBL-producing isolates during the 2 years studied ($P > 0.05$).
Conclusions: ESBL-producing isolates showed considerably higher rates of resistance to quinolones and SXT in comparison with non-ESBL-producing isolates in our area. The high incidence of ESBL-producers among outpatients' samples is worrying and is attributed to the unjustified use of antibiotics for the treatment of urine tract infections. Surveillance of those isolates is essential to prevent their dissemination and to eradicate them by following the proper antibiotic treatment.

R2015

Frequency and antimicrobial susceptibility of extended spectrum beta-lactamase producing urinary isolates

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Objectives: This study was performed to find out the frequency and antimicrobial susceptibility of ESBL producing *Enterobacteriaceae* strains isolated from urinary tract infections.
Methods: During the study period (October 2004–October 2005) 604 strains of *Escherichia coli* and 97 strains of *Klebsiella pneumoniae* were isolated from midstream urine samples in our microbiology laboratory. The identification to the species level was performed with the VITEK TWO system (Bio-Merieux) and susceptibility to antimicrobial agents was tested by the disk diffusion method according to NCCLS recommendations. Detection of ESBL expression was performed by the double-disk synergy (DDS) test and with the VITEK TWO card MIC/AST for ESBL.
Results: ESBL production was determined in 35 out of 701 strains of *Enterobacteriaceae* (4.99%). The ESBL phenotype was detected in 4.8% (29 out of 604) strains of *E. coli* and 6.4% (6 out of 97 strains) of *K. pneumoniae* urine isolates. For *E. coli*, ESBL production was detected in 25 out of 346 strains isolated from hospitalized patients (7.2%) and 4 out of 258 patients of the community (1.5%). For *K. pneumoniae* no ESBL producing strains were isolated from community patients. The antibiotic

susceptibility rates for the ESBL producing strains were for *E. coli* and *K. pneumoniae* respectively: Imipenem 100–100%, gentamicin 82.7–83.3%, amikacin 79.3–50%, netilmicin 86.2–33.3%, tobramycin 62.1–0%, chloramphenicol 62.1–83.3%, ciprofloxacin 34.5–33.3%, norfloxacin 34.5–33.3%, nitrofurantoin 44.8–33.3%, tetracycline 17.2–0%, piperacillin/ tazobactam 89.6–66.6%, trimethoprim/sulfamethoxazole 58.6–33.3%. ESBL production and fluoroquinolone resistance were detected in 19 out of 29 (76%) strains of *E. coli* and 4 out of 6 (66.7%) strains of *K. pneumoniae*.

Conclusions: Our findings demonstrate an increasing incidence of urinary tract infections with ESBL producing bacteria not only in hospitalized but also in community patients. Most of ESBL producing isolates were multidrug resistant. High resistance rates to quinolones were observed among these strains, but carbapenems had a good activity. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with urinary tract infections.

R2016

Antibiotic resistance and plasmid profiles of *Acinetobacter* spp. isolated from three hospitals in Tehran, Iran

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Objectives: This study was designed to determine antibiotic resistance pattern and plasmid profiling of Iranian isolates of Acinetobacter.

Methods: During an eight- month period, 47 Acinetobacter isolates (including 21 *A. baumannii* and 26 non-baumannii strains) were obtained from three University Hospitals in Tehran. The source of these isolates included blood, urine, wound, and respiratory tract. Their susceptibility to the 17 antibiotics was tested. The plasmid profiling method was used for typing of isolates.

Results: The results of this study showed that all *Acinetobacter baumannii* isolates were resistant to multiple antibiotics including cefoprazone, ceftazidime, ticarcillin/ clavulanic acid, aztreonam, meropenem, ceftizoxime, carbenicillin, cefixime, ceftriaxone, ticarcillin, cephotaxime, and were sensitive to colistin. All non-baumannii strains were resistant to cefexime and with one exception to meropenem and were susceptible to polmyxinB and colictin. Plasmid were found in 44 (93.61 %) of 47 isolates, and no plasmid were found in 3 isolates (6.39 %). Seven different plasmid profiles were observed among 44 Acinetobacter isolates. Similar plasmid profile was seen in two different specimens from two different hospitals. Plasmid profiles 1–7 were observed in all isolates recovered from the blood culture. All profiles except profile 2 were seen in all strains isolated from urine cultures. Plasmid profiles were identical in two isolates obtained from two different patients in the same hospital. With one exception, all *A. baumannii* strains could be differentiated by this method; among non-baumannii strains, 24 of 26 were typed using plasmid profiling method.

Conclusion: The results of this study revealed that most of Acinetobacter isoltes showed multi-drug resistant pattern. Plasmid profile analysis seems to be a rapid and simple technique for the routine screening and typing of clinical Acinetobcter isolates with epidemic potential.

R2017

Molecular typing of *Stenotrophomonas maltophilia* causing prosthetic valve endocarditis

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Objectives: At the Department of Cardiovascular surgery of the University Medical Centre Ljubljana, *Stenotrophomonas maltophilia* caused five cases of the early postoperative prosthetic valve endocarditis and one case of bacteraemia in a patient after heart transplantation in the three-year period. Because bacteria could be introduced from a common source during the operation, molecular typing was performed.

Methods: There were three male and three female patients. All patients with endocarditis fulfilled Duke criteria for definite endocarditis. All had aortic valve replacement. Three patients with endocarditis and a patient with heart transplantation were operated in 2005 and one patient with endocarditis in 2002 and one in 2004. Apart from the first patient who presented with the symptoms 13 months postoperatively, the symptoms appeared one to six months after the operation (mean 3 months). In two patients the presenting symptom was a CNS embolism and in all others temperature and signs of cardiac failure were present. Bacteria were identified and antimicrobial susceptibility was determined to trimethoprim/sulfamethoxazole, gentamicin, amikacin, piperacillin/tazobactam, ceftazidime, ciprofloxacin and imipenem. All patients were treated with ceftazidime in combination with gentamicin, amikacin or ciprofloxacin. Three of them died. Molecular typing of bacterial isolates was performed by macrorestriction analysis of chromosomal DNA by PFGE using the cutting enzyme SpeI.

Results: We detected two different PFGE patterns. The isolates of the patients with aortic valve replacement belonged to the same genotype, but the isolate from the patient after heart transplantation was different. All isolates were susceptible to ceftazidime, but only two to trimethoprim/sulfamethoxazole.

Conclusion: The results show that in five patients at the Department of Cardiovascular surgery who developed *Stenotrophomonas maltophilia endocarditis*, the infection could be connected with the contamination during aortic valve replacement. Samples from the medical equipment and the environment should be taken to detect the source of bacteria.

R2018

Antimicrobial susceptibility among *Pseudomonas aeruginosa* isolated in an infectious disease hospital in Bucharest

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The antimicrobial resistance of *Pseudomonas aeruginosa* (PA) is increasing worldwide.

Objectives: To describe the demographics and risk factors among adults hospitalized who had infection with PA, to assess the antimicrobial susceptibility of *PA* isolates.

Methods: Retrospective study of patients who were culture-positive for PA between Jan 1–Oct 31 2005. The isolates were identified using conventional methods. The sensitivity of strains to selected antibiotics amikacin (AMK), ciprofloxacin (CIP), piperacilline (PIP), piperacilline/tazobactam (P/T), ceftazidime (CAZ), cefepime (CEP), imipenem (IMI) was tested with disk diffusion method and interpretation was according to NCCLS. We classified the isolates in three categories: I-susceptible to three or more of the antibiotics tested, II-susceptible to imipe-

nem only, III–multidrug resistance MDR (resistance at least three of IMI, CAZ, CIP, AMK).
Results: We identified 75 positive cultures of PA. Clinical data were available for review for 42 patients. Mean age was 54 (±21.3) years, 24 patients were female. The organisms had been acquired in hospital in 29 cases (most of them were admitted from another hospital, from ICU in 7 cases) and from immunocompromised patients (17 cases). Mean duration of inpatient stay prior to the isolation was 31.3 (±26.3) days. The strains were isolated from wounds/soft tissues in 15 cases, urine 11 cases, respiratory tract 10 cases, blood culture 3 cases, venous catheter 2 cases and CSF 1 case. Overall, 33 of our patients had received antibiotic therapy within 30 days prior to admission in our hospital. The most frequently used antibiotics were beta-lactams, beta-lactams/betalactamase inhibitor or ciprofloxacin. The most active antibiotics were IMI (24 susceptible strains), AMK, CAZ, P/T (23), PIP (21) and the less active were CEP and CIP (18, respectively 17). Susceptibility rates varied significantly between nosocomial and community acquired infections. We noted 25, 2 and 15 isolates in category I, II and III, respectively. Most of patients with MDR PA (13) had nosocomial infections and all strains IMI resistant were isolated from nosocomial infections.
Conclusion: PA is an important cause of nosocomial infections and in immunocompromised patients. Considering the results and the high prevalence of the multidrug resistant PA strains, further studies for antimicrobial resistance surveillance are needed, as well as control measures to prevent the spreading of these strains.

R2019

Multi-resistance in *K. pneumoniae* in German university hospitals, 2002–2005

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Objectives: *K. pneumoniae* is frequently involved in nosocomial infections, the emergence of multi-resistant clones causes major problems. We want to examine the development of multi-resistance in *K. pneumoniae* within recent years using the dataset of the GENARS-project (German Network for Antimicrobial Resistance Surveillance), a prospective multi-centre surveillance study designed to provide epidemiological data for German university hospitals.
Methods: Analysis was based on non-duplicate isolates of *K. pneumoniae* from five laboratories with continuous data collection from January 2002 to June 2005. Antimicrobial susceptibility was determined as minimal inhibitory concentrations by broth microdilution method performed by different automated test systems for ceftazidime, cefotaxime, ciprofloxacin, gentamicin, meropenem, piperacillin and piperacillin/tazobactam. Resistance patterns were evaluated by using breakpoints according to DIN guidelines; multi-drug resistance was defined as resistance to at least four of the above mentioned agents.
Results: A total of 4,108 isolates was analysed. In 2002 83.4 percent of the isolates showed no resistance to any of the selected antibiotics, this rate decreased to 75.9 percent in 2005 due to significant increases in resistance rates for piperacillin (PIP: 14.7% to 23.1%; $p < 0.001$) and piperacillin/tazobactam (PIT: 3.4% to 7.5%; $p < 0.001$). Accordingly, the most common resistance pattern was a mono-drug resistance to PIP followed by co-resistance to PIT. The proportion of multi-resistant strains

Multi-resistance in *K. pneumoniae* based on seven selected antibiotic agents: number of resistances in percent by year

Resistance to ...	complete sample				intensive care units				inpatients			
	2002	2003	2004	2005	2002	2003	2004	2005	2002	2003	2004	2005
no agent	83.4	80.0	77.3	75.9	74.2	72.3	70.4	69.2	85.2	82.0	79.0	80.8
one agent	8.6	10.3	11.4	11.1	9.0	13.2	12.2	10.5	8.9	10.0	10.8	9.3
two agents	3.2	4.7	4.9	6.7	4.5	6.0	4.8	8.4	3.3	4.3	5.0	6.1
three agents	1.9	1.9	1.9	2.2	5.4	2.7	2.9	4.2	1.0	1.6	1.7	0.6
four or more agents	2.8	3.2	4.5	4.2	6.8	5.8	9.6	7.7	1.6	2.1	3.5	3.2
no. of isolates	*893*	*1264*	*1350*	*601*	*221*	*364*	*311*	*143*	*485*	*679*	*637*	*313*

accounted for 2.8% in 2002 and rose to 4.2% in 2005, this increase was not significant. Overall multi-resistance rates were significantly higher for isolates from intensive care units (ICU) than for those from non-ICU inpatients (7.4 versus 2.6%; $p < 0.001$). Increases of multi-resistance within the subsamples were not significant. The peak of multi-resistance of 9.6% in 2004 in ICUs was due to an outbreak of two phenotypes in only one centre that could be contained.
Conclusions: With an overall proportion of 3.7 percent the relevance of multi-resistance in *K. pneumoniae* as a clinical problem is proven in German university hospitals for the period 2002 to 2005. Though there were no significant increases in multi-resistance neither for the complete sample nor for subsamples of isolates from ICUs or non-ICU inpatients within this period monitoring should continue for early detection of multi-resistance.

R2020

Transfer of antibiotic resistance in multiresistant nosocomial isolates of *Acinetobacter baumannii* in three university clinics

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Objectives: The aim of this study was to evaluate transferability of antibiotic resistance in 17 multiresistant nosocomial *Acinetobacter baumannii* isolates during surveillance of antibiotic resistance in 3 University Clinics in Slovak Republic.
Methods: MIC(s) of antibiotics were determined by the agar dilution method with two-fold dilution of antibiotics. Transfer of genes for antibiotic resistance was performed in mixed liquid cultures of the donor and recipient strain. As recipient strains were used *E. coli* K-12 3110 rif+, *Proteus mirabilis* P-38 rif+, *Pseudomonas aeruginosa* 1008 rif+ and *Pseudomonas aeruginosa* 1670 rif+.
Results: 17 *A. baumannii* isolates caused severe infections in 13 patients hospitalised in anaesthesiology, traumatology and haematology ICUs of 3 different University Clinics (Kosice, Bratislava, Nitra) during years 2004 and 2005. Isolates were resistant to various beta-lactams (ticarcilin, cefalotine, cefotax-

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ime, ceftazidime, cefoperazone, cefepime, aztreonam, meropenem and mipenem), aminoglycosides (kanamycin) and quinolones (ofloxacin). We observed direct transfer of cefalotine (CFL) and cefoperazone (CFP) resistance determinants in 6 isolates from University Clinic Kosice to *E. coli* and *P. mirabilis* recipient strains. Similarly all isolates from University Clinic Bratislava and University Clinic Nitra directly transferred CFL resistance, 4 isolates from Bratislava directly transferred CFP resistance and 2 isolates from Bratislava and Nitra transferred also ticarcillin (TIC) resistance. Transfer of resistance determinants were also observed in 2 additional Ps. aeruginosa recipient strains.
Conclusions: Although transfer of meropenem resistance in *A. baumannii* isolates was not observed, strains with similar resistance patterns and transfer of resistance determinants give evidence of probable clonal spread of multiresistant meropenem resistant strains in ICUs of all examined University Clinics. CFL, CFP and TIC resistance determinants already located in plasmids showed the possibility of mobilisation of other resistance determinants. Moreover, transfer of these determinants to 4 different recipient strains demonstrates broad host range of this nosocomial pathogen. *A. baumannii* resistant to meropenem and other previously effective antibiotics is becoming more common pathogen in our country, especially in ICUs and contribute to spread of resistance in hospital environment if adequate infection control measures are not implemented.

R2021

Susceptibility profile of *Acinetobacter baumanii* isolates and evaluation of three different susceptibility test methods

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Objective: To compare and evaluate the antibiotic susceptibility of *A. baumanii* according to 3 different methods in common use in laboratories.
Methods: Thirty-four *A.baumanii* strains were isolated from patients in 2 general hospitals in Athens and from various clinical specimens (blood, urine, intravenous catheters, bronchial secretions). Identification to the species level was performed by automated VITEK II method. Susceptibility testing was done: 1) by Kirby-Bauer according NCCLS 2) by VITEK II system (AST N022 card) 3) by testing imipenem and production of metallo-b-lactamases using the E-test MBL.
Results: We classified 8 different phenotypes according susceptibility results:1) 16/34 (48%) showed sensitivity only to colistin (C). 2) 4/34 (12.5%) showed sensitivity to colistin and ampicillin-sulbactam (CS). 3) 1/34 (3%) showed sensitivity to colistin and tobramycin (CT). 4) 1/34 (3%) showed sensitivity to colistin and imipenem (CI). 5) 8/34 (24.5%) showed sensitivity to colistin, imipenem, ampicillin-sulbactam (CSI). 6) 1/34 (3%) showed sensitivity to colistin, imipenem, tobramycin, netilmycin (CITN). 7) 1/34 (3%) showed sensitivity to colistin, imipenem, tobramycin, netilmycin, cefepime (CITNF) and 8) 1/34 (3%) showed sensitivity to colistin, imipenem, tobramycin, netylmycin, cefepime, ciprofloxacin and gentamycin (CITNFPG). Among the total number of isolates, 20 (59%) were imipenem-resistant and 2 (6%) showed intermediate susceptibility by the disk-diffusion method. 8/20 (40%) R strains and 1/2 (50%) IR strains produced metallo-b-lactamases by E-test MBL. For the evaluation of susceptibilities of imipenem we used E-test as the reference method. We observed that KB correlated well with E-test results for *A. baumanii* strains. Minor discrepancies occurred comparing E-test with VITEK II system (resistance by E-test and intermediate susceptibility by VITEK II system) in 75% of the strains.
Conclusions: According results most *A. baumanii* strains of our study showed imipenem-resistance and were also multi-drug-resistant. By E-test as the reference method, Kirby-Bauer proved to be efficient in detecting those strains and also VITEK II system showed minor discrepancies.

R2022

Nosocomial pseudomonal infections in a university hospital, Adana, Turkey

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Objectives: Our aim is to determine antimicrobial resistance and risk factors for nosocomial *Pseudomonas* infections isolated from various sites in a university hospital.
Methods: This prospective study was conducted in a university hospital with 1100-bed, between January 2004 and October 2005. Nosocomial *Pseudomonas* infections were investigated in hospitalised patients in ICUs and others. Hospital-acquired infections were defined by the criteria of CDC. The identification and susceptibility to antimicrobials has been performed by Vitek 2 as described by NCCLS.
Results: One hundred and seventy nosocomial *Pseudomonal* infections were diagnosed in 170 patients. One hundred and sixteen (68%) of the patients were male. Mean age was 43.93 years. One hundred and twelve (66%) of infections were diagnosed in ICUs. Hospitalization period was more than 20 days at 78% of the patients. Nosocomial infection types were detected as follows: Urinary tract infections 35%, pneumonia 28%, bacteraemia 19%, skin and soft tissue infection 10%, surgical wound infection 8%. Risk factors were detected as, previously hospitalization 21.7%, previous antibiotic use 45%, mechanical ventilation 30.6%, burn 7.6%, diabetes mellitus 7%, malignancy 16.5%, COPD 6%, chronic liver disease 3.5%, collagen vascular disease 6%, chronic renal failure 2%. The results of susceptibility testing for Pseudomonas isolates are shown in the table below. The most active antimicrobial agents againts *Pseudomonas* spp. were carbapenems. Five percent of *Pseudomonas* isolates were found to be resistant to all antibiotics.

N:170	Ceftazidime	Cefepime	Piperacillin/taz	Amikacin	Tobramycin	Ciprofloxacin	Imipenem	Meropenem
%S	38	41	35	46.5	40	50	57	57.6
%I	42	31	19	47	59	47	20	34.7
%R	20	28	46	3	1	3	23	7.6

Conclusion: The high resistance rates in *Pseudomonal* isolates that are responsible for nosocomial infections in our hospital suggest that susceptibility pattern should be monitorised continuous and preventive procedures should be implemented. Besides, multidrug resistant *Pseudomonas* isolates are to take into consideration.

R2023

Activity of different antimicrobials against nosocomial cefotaxime-resistant *Klebsiella pneumoniae*

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Objectives: To determine the activity of different antimicrobials against cefotaxime-resistant *K. pneumoniae* in Russian ICUs.
Methods: During 2002–2004 *K. pneumoniae* strains were isolated from ICU patients with nosocomial infections. MICs of amika-

cin, amoxicillin/clavulanate, ampicillin, cefepime, cefoperazone, cefoperazone/sulbactam, cefotaxime, cefotaxime/clavulanate, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanate were determined by agar dilution method according with NCCLS. Intermediate strains were referred to resistant category.
Results: A total of 420 nosocomial *K. pneumoniae* strains were evaluated. Among them 318 (75.7%) were resistant to cefotaxime. More or equal to 3-fold decrease in MIC for cefotaxime/clavulanate vs MIC of cefotaxime was observed in 299 (94%) cefotaxime-resistant *K. pneumoniae*, thus suggesting ESBL production as the main cefotaxime-resistance driver. Resistance of cefotaxime-resistant strains to different antimicrobials was: ampicillin – 100%, piperacillin – 100%, ticarcillin/clavulanate – 99.7%, ceftriaxone – 99.1%, cefoperazone – 98.1%, amoxicilli/clavulanate – 91.8%, gentamicin – 89.6%, cefepime – 81.1%, ceftazidime – 70.4%, piperacillin/tazobactam – 52.5%, ciprofloxacin – 46.2%, amikacin – 40.3%, cefoperazone/sulbactam – 37.4%, moxifloxacin – 33.3%, levofloxacin – 31.4%, ertapenem – 3.5%, imipenem – 0%, meropenem – 0%. Among antimicrobials tested only imipenem and meropenem were active against all cefotaxime-resistant *K. pneumoniae*. Among inhibitor-protected b-lactams only cefoperazone/sulbactam was outstanding though with resistance level of 37.4%. Aminoglycosides showed poor activity – 89.6% studied strains were resistant to gentamicin and 40.3% - to amikacin. Levofloxacin was more active as compared to moxifloxacin and ciprofloxacin (31.4% vs 33.3% and 46.2% resistant, respectively).
Conclusion: The tremendous increase in resistance to III generation cephalosporins, especially to cefotaxime, in nosocomial *K. pneumoniae* demands search for antimicrobials active against these strains. Our results outline imipenem and meropenem as the most active antimicrobials against cefotaxime-resistant *K. pneumoniae*.

R2024

Activity of different antimicrobials against nosocomial ciprofloxacin-resistant *Acinetobacter baumannii*

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Objectives: Due to its unique gram-negative activity and pharmacological properties ciprofloxacin historically was one of the first-line drugs for the treatment of nosocomial infections. According to recent surveillance data *Acinetobacter baumannii* became one of the most important nosocomial pathogens manifesting various resistance patterns; in some Russian centres it is the leading nosocomial gram-negative pathogen. Thus the objective of our study was to determine the level of ciprofloxacin resistance among nosocomial *A. baumannii* in Russia and to reveal antimicrobials with potential to be active against ciprofloxacin-resistant *A. baumannii*.
Methods: Antimicrobial activity against *A. baumannii* strains isolated in 2002–2004 from Russian intensive care unit (ICU) patients with nosocomial infections was performed in accordance with NCCLS by agar dilution method. MICs of amikacin, cefepime, cefoperazone, cefoperazone/sulbactam, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam were determined. Intermediate strains were referred to resistant category.
Results: A total of 459 nosocomial *A. baumannii* strains were evaluated. Among them 339 (73.9%) were resistant to ciprofloxacin. Resistance of ciprofloxacin-resistant strains to different antimicrobials was: cefoperazone – 100%, piperacillin – 98.8%, gentamicin – 97.1%, levofloxacin – 84.4%, ceftazidime – 82.3%, piperacillin/tazobactam – 81.7%, amikacin – 76.4%, cefepime – 69%, meropenem – 4.4%, imipenem – 2.9%, cefoperazone/sulbactam – 2.4%. Obtained data demonstrate cefoperazone/sulbactam as the most active against ciprofloxacin-resistant *A. baumannii* strains followed by imipenem and meropenem. The rest antimicrobials expressed significantly poor in vitro activity against studied *A. baumannii* population. As concerning to cross-resistance between ciprofloxacin and levofloxacin it should be noticed that levofloxacin was active against 15.6% of ciprofloxacin-resistance strains, while all strains resistant to levofloxacin were resistant to ciprofloxacin as well.
Conclusion: Cefoperazone/sulbactam, imipenem and meropenem might be considered as the key antimicrobials to treat nosocomial infections due to ciprofloxacin-resistant *A. baumannii* in Russian ICUs.

Antibiotic usage

R2025

Efficacy of linezolid in treatment of non-approved indications

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Objectives: Linezolid (LZD) has been approved in the treatment of Gram-positive lower respiratory tract infections and skin and soft-tissue infections. However, few data exist about the LZD activity in other Gram-positive infections. The aim of this study was to evaluate our practical experience of LZD in the treatment of non-approved Gram-positve infections.
Methods: Patients treated with LZD in non-approved indications from June 2002 to October 2005 were assessed for the followings variables: age, gender, underlying diagnosis, isolated microorganisms, prior treatments and outcome. Only patients with more than 7 days of treatment and cultures with less than 3 isolated microorganisms were included. Finally, 14 patients (7 males, median age 59.8 years-old) were reviewed. Clinical infections included: intra-abdominal abscess (4), abdominal mesh infection (4), prosthetic joint (PJ) infection (3) and central nervous system (CNS) infection (3).
Results: A total of 18 isolates were observed in the 14 patients. Isolated microorganisms included: methicillin-resistant *Staphylococcus epidermidis* (MRSE) (8), methicillin-resistant *Staphylococcus aureus* (MRSA) (6), *Enterococcus faecalis* (2), *Enterococcus faecium* (1) and *Corynebacterium striatum* (1). LZD was the initial therapy in 3 cases and 11 patients received LZD after several

courses of antibiotics. In these cases, previous treatments included a glycopeptide in 9 patients (82%). LZD was started because of failure of previous treatment in 8 patients (72.7%) and owing to continue a treatment per os in 3 (27.3%). Included patients received LZD for a median of 44 days (range 14 to 127) and half of them received it in monotherapy. Initial therapy with LZD was intravenously in 6 cases and the orally sequence went for hospitable discharge in 5. No serious side effects were observed and only one patient, in combined therapy with cotrimoxazol, leucopenia was detected. Successful outcome was observed in 85.7% (12 of 14) of patients. No failures were observed in patients with PJ and CNS infection. Failures were observed in one patient with an intra-abdominal abscess (MRSA) and another with an abdominal mesh infection (MRSE and E. faecium).
Conclusions: LZD could be a therapeutic alternative in non-approved indications caused by Gram-positive pathogens resistant to conventional antimicrobials or in those in which standard therapy fails. In addition LZD allows continuing ambulatory treatment after clinical discharge, shortening the stay.

R2026

Bacterial and antimicrobial aspect of odontogenic infections

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Objectives: We aimed to determine bacterial agents and evaluated antimicrobial susceptibility of pus samples with odontogenic infections.
Methods: Seventy-one patients with odontogenic infections included in this study. The patients had received no antibiotic therapy in the preceding 3 months, and with chronic diseases such as diabetes mellitus, leukaemia was excluded. Pus samples were collected from abscess by aspiration after disinfection of mucosa or skin. Some part of each sample was transferred into brain heart infusion (BHI) broth for aerobic and the other was transferred into chopped meat broth (CMB) for anaerobic cultures. Samples for aerobic examination were cultured for 3 hours, and then sub-cultured on BHI Agar with 5% defibrinated sheep blood and chocolate agar for 18 hours. Samples collected into CMB for anaerobic examination were incubated for 7 days, and then sub-cultured on supplemented BHI Agar with blood. Sub-cultured plates were incubated for 48 hours. All aerobic and anaerobic bacteria were identified according to the bacterial fatty acid profiles by Microbial Identification System. Antibiotics for aerobics were erythromycin, penicillin, amoxicillin-clavulanic acid, clindamycin, tetracycline, cefazolin, and for anaerobics were clindamycin, imipenem, metronidazol, cefoxitin, penicillin-G, piperacillin-tazobactam. All practices were applied according to NCCLS standarts. A chromogenic cephalosporin impregnated on a stick was used for the detection of bacterial beta-lactamases.
Results: 19 and 13 different bacterial strains were identified as aerobic and anaerobic respectively. The most part of samples were of mix (aerobic and anaerobic) bacterial characteristic. The most prevalent bacteria were *Staphylococcus* spp., viridans group Streptococci, anaerobic Streptococci, *Fusobacterium nucleatum* and *Prevotella melaninogenica*. The incidence of beta-lactamase producing bacteria were *Staphylococcus aureus* (20 %), *Bacteroides oralis* (20%), *Fusobacterium nucleatum* (22.2%) *Bacteroides ovatus* (33.3%) *Actinomyces israelli* (33.3%) and *Prevotella melaninogenica* (33.3%). For aerobic bacterial isolates; amoxicillin-clavulanic acid and cefazolin were the most effective antibiotics, and for anaerobic bacterial isolates, imipenem and piperasilin-tazobactam were the most effective ones.
Conclusion: A combination of amoxicillin-clavulanic acid and metronidazol or a combination of clindamycin and metronidazol seems to have well effect for mixed infections.

R2027

Clinical and microbiology activity of colistin alone and in combination with rifampin or other antibiotics against MDR Gram-negative rods severe infections: four-year, single-centre experience

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Objectives: To study the in vitro and clinical activity of colistin [C] alone or in combination with rifampin [R] and/or other antimictrobial in severe MDR gram negative rods infections.
Methods: All the patients treated with C alone or in combination were reviewed. Synergism of the combination C plus R against MDR *P. aeruginosa* and *A. baumannii* were tested with the checkerboard method. Synergism was calculated using FIC index (FICI), fully synergism was considered FICI<0.5, partially synergism between 0.5 and <1, additivity = 1, indifference between 1 and 4, antagonism >4.
Results: 28 patients (mean age 56 years, 19 male) were treated with C: 9 diabetic foot infections (DFI), 7 sepsis, 6 pneumonia (1VAP), 4 post-traumatic infections, 1 arthrytis, 1 meningitis. 25 MDR *P. aeruginosa* and 3 MDR *A. baumannii* were isolated from these patients. 24 patients received C endovenously at a mean daily dose of 3 MU/day. 3 patients received C alone, 9 patients received C + R, 9 patients C + R plus another antibiotic, 7 patients received C plus another antibiotic. C therapy mean duration time was 46 days (range 3–240 days). No adverse effects were observed: neither in the three patients with renal insufficiency nor in the two patients with diabetic neuropathy. Positive clinic outcome was obtained in 24/28 patients (87%), among the 4 patients with bad outcome, three died of infection. Synergism was tested against 10 MDR P. aeruginosa: C + R was fully synergistic aginst 2/10 strains, partially synergistic against 7/10 strains and indifferent against only one strain. C + R was partially synergistic against the only one strain of MDR *A. baumannii* tested.
Conclusion: Colistin alone and in combination with rifampin and /or other antibiotics is safe and effective in the treatment of severe MDR Gram-negative rods infections.

R2028

A five-day ceftriaxone and levofloxacin regimen plus early sequentation to oral levofloxacin in heart transplantation recipients with community-acquired pneumonia

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Objective: To determine the efficacy of a 5-day regimen of ceftriaxone and levofloxacin followed by a 7-day regimen of the oral quinolone in heart transplantation recipients with community-acquired pneumonia (CAP) in comparison to established regimens of 14-day betalactams.
Methods: Heart transplantation recipients attended in emergencies that presented clinical signs and symptons of CAP

combined with a radiologic evidence of an infiltrate were included. Before antimicrobial therapy was started, samples for respiratory cultures were obtained when possible. Clinical outcome of patients was assessed at discharge.
Results: Study included 14 patients (median age, 62.5 years) with a gender distribution of 13 males and one female. Radiological tests interested right lung in 78.5% of patients (57% lower lobe). Microbiologic testing was positive in 57.1% (sputum collected in 9 patients). The most common isolated pathogen was *Streptococcus pneumoniae*. In 10 patients regimen was followed as pre-established. In two patients oral administration was not possible due to acute allograft rejection and a concomitant Herpes simplex systemic infection. In another patient cefepime was used instead of ceftriaxone due to the presence in blood cultures of *Propionibacterium acnes*. Finally, in one patient treatment was interrupted after 5 days due to the isolation of Parainfluenzae virus in bronchoscopic cultures. Mean duration of antimicrobial treatment was 13.9 days: 6.6 intravenous and 7.2 oral administration. Mean duration of the in-patient period was 5.8 days being the 78.6% of patients admitted in conventional hospitalisation. Clinical successful outcome was observed in 85.7% of patients per intention to treat and in a 100% per protocol.
Conclusions: 5-day regimen with intravenous ceftriaxone and levofloxacin followed by a 7-day regimen of oral levofloxacin reveals as a successful outcome as established regimens in heart transplantation recipients with CAP; otherwise, reduces the in-patient period in 12 days.

R2029

Treatment of chronic bone and joint infections with peroral linezolid

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Objectives: To evaluate the efficacy and side effects of long-term treatment of chronic osteomyelitis and joint infections caused by multiresistant Gram-positive bacteria with the oxazolidinone Linezolid.
Methods: Included were patients (ptt) with chronic osteomyelitis and joint infections with multiresistant Gram-positive bacteria, sensitive to Linezolid, who were treated primarily with vancomycin i.v. followed by Linezolid 600 mg × 2 p.o. for 2 to 4 weeks. Ptt were monitored weekly for haematologic and hepatic parameters. All ptt had biopsies taken for culture in relation to operation. Biopsies were cultured aerobic and anaerobic for 5 days and bacteria identified according to clinical microbiological standard procedures.
Results: Eleven ptt, eight women and three men, median age 75.6 (range 57–92) had chronic osteomyelitis or joint infections with multiresistant coagulase negative staphylococci and/or enterococci and were treated with Linezolid 600mg × 2 p.o. Eight ptt had hip prosthesis and two had knee arthroplasty, only one had primary soft tissue infection of the knee (popliteal cyst). All received vancomycin therapy as primary antibiotic treatment (1–4 weeks) followed by Linezolid for either two (three patients) or four weeks (eight patients). Five ptt were cured of the chronic infection, they all got four weeks Linezolid treatment. Three of them had no side effects, but two had nausea. Four ptt died due to cardial insufficiency soon after the Linezolid treatment stopped (age 71, 77, 90, 92 years). They had no clinical signs of infection when they died. One woman had nausea and diarrhoea during the treatment, but died from hepatocellular carcinoma. One pt was not cured despite six weeks treatment with vancomycin and four weeks treatment with Linezolid. Nine ptt had no haematologic side effects. The patient with hepatocellular carcinoma both had hepatic and haematological side effects. One patient had leucocytosis after four weeks of Linezolid.
Conclusion: Half of the patients were cured of their very complicated infections. The patients with chronic multiresistant Gram-positive infections were able to get Linezolid per oral treatment and were discharged either to own home or to nursinghome.

R2030

An intervention to reduce irrational surgical antibiotic prophylaxis by evidence-based guidelines, education and feedback

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Objectives: Previous research showed overconsumption of antibiotics in teaching hospitals in Surabaya (SBY) and Semarang (SMG) on Java, Indonesia. We studied the effect of an intervention to reduce irrational prophylaxis.
Methods: Prospective intervention study conducted concurrently in 2 hospitals. The most frequently performed procedures in the departments of obstetrics and gynecology (Ob/Gyn) and surgery (Surg) were selected. Consecutive cases were included from July 2003 till August 2004. Data on procedures and antibiotic use were collected. Four periods were defined: a baseline period (I) during which an evidence based update of prophylaxis guidelines was developed by consensus of surgical staff, period II following dissemination of the guidelines on paper, period III following an interactive training for residents and period IV, after feedback of the data on practice in previous periods. Antibiotic use was calculated in Defined Daily Doses (DDD)/procedure. Quality criteria to evaluate prophylactic prescribing were derived from the guidelines. We report here on 2 criteria 1) DDD/procedure for clean procedures without indication for prophylaxis 2) Number of postoperative days (>24 h) of prophylaxis; according to the guideline both should be zero.
Results: Between July 2003 and September 2004, 3173 procedures were studied in the 4 departments.

		DDD/ procedure for clean procedures					Postoperative days of prophylaxis (mean) for all procedures				
		N	Period				N	Period			
			I	II	III	IV		I	II	III	IV
SBY	Ob/gyn	300	11.6	7.8	6.0	5.3	1281	4.0	3.0	1.4	0.9
	Surg	629	4.1	2.6	1.3	1.8	884	4.1	3.4	1.6	1.4
SMG	Ob/gyn	115	11.3	10.6	2.2	2.7	600	2.8	5.8	0.6	0.3
	Surg*	339	5.2	3.3	4.9	3.7	408	4.1	3.4	3.9	3.3

ANOVA: The mean differences between periods were significant at the 0.05 level except *

Conclusion: An identical intervention consisting of updating consensus guidelines, educational training and feedback in 2 hospitals resulted in significant improvements of prescribing in 3 out of 4 departments. Sustainability of the improvements needs to be explored.

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R2031

Seasonal trends in antimicrobial consumption in Russia in 2004

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Objectives: The long-term program on the antimicrobial (AM) consumption evaluation in Russia was implemented by the IAC and TRGC in 2003. The purpose of this study was to compare seasonal changes in AM consumption in Russia in 2004.
Methods: Data containing products names, ATC classification codes (WHO, version 2005), drug forms, dosages and number of packages were collected during pharmacy and hospital audits in 53 and 27 regions of Russia, respectively. Variations of the data of both audits did not exceed 10%. The use of AM was expressed as the number of defined daily doses (DDD) per 1000 inhabitants per day (DID). We compared seasonal variations in AM consumption as winter (quarter 1 + quarter 4) vs summer (quarter 2 + quarter 3). To find out trends the formula: ([quarter1 + quarter4/quarter2 + quarter3] -1) x 100% was used.
Results: AM consumption increased in 18.1% in winter quarters compared with summer ones. Major changes were found for sulfonamides and trimethoprim (J01E) (+ 41.6%), beta-lactams, penicillins (J01C) (+30.7%) and macrolides, lincosamides, streptogramins (J01F) (+25.2%). Amphenicols (J01B) consumption had the opposite trend (-18.6%). The seasonal changes in J01C group mostly resulted from the increase in ampicillin/sulbactam (+130.8%), benzathine phenoxymethylpenicillin (+66.7%) and phenoxymethylpenicillin (+50.0%) usage, in J01E group by sulfalen (+72.0%), sulfametrol/trimethoprim (+52.5%) and sulfadimethoxine (+52.0%) and in J01F group – by midecamycin (+41.3%), erythromycin (+28.9%) and roxithromycin (+28.6%) consumption.
Conclusion: Based upon foregoing findings seasonal changes in AM consumption with increase in winter quarters might be related to the increase in incidence of respiratory tract infections, and in summer quarters – gastrointestinal disturbances which in the majority of cases are of viral origin and self-limiting. More detailed survey is ongoing to justify this correlation.

R2032

Intravenous, intrathecal and intraventricular administration of colistin for the treatment of multiresistant *Acinetobacter baumannii* meningitis

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Background-Aim: Infections due to Gram-negative multiresistant bacteria pose a common therapeutic challenge for clinicians. Colistin has been increasingly used to treat infections caused by these bacteria. We present two cases of nosocomial meningitis caused by multiresistant *Acinetobacter baumannii* that were succesfully being treated by colistin.
Results: Case 1: A 20-year-old male patient was admitted to our unit due to severe cranial trauma. The patient developed a hydrocephalus and a ventriculoperitoneal shunt was placed. A few days later he developed high fever, meningismus and lethargy. The cerebrospinal fluid (CSF) examination revealed: glucose 2 mg/dl, protein 295 mg/dl, LDH 642 iu/dl, cells 48.000/mm, while CSF cultures yielded *Acinetobacter baumannii* susceptible only to colistin. The shunt was removed and the patient was treated with colistin intravenously. After 3 days the patient was afebrile and CSF was sterile after 13 days of therapy, respectively. Case 2: A 24-year-old female patient was admitted to our unit because of subarachnoid haemorrhage with concomitant hydrocephalus due to a ruptured cerebral aneurysm. After 10 days the patient became febrile (40°C) and the CSF examination showed: glucose <2 mg/dl, protein 130 mg/dl, cells 32.000, LDH 1274, while CSF cultures yielded *Acinetobacter baumannii* susceptible only to colistin. Colistin was initiated intravenously and the drainage was removed. A lumbar drainage was placed and colistin was administered intrathecally, but after 3 days the catheter was removed due to thrombosis. As the patient was still febrile and sterilization of CSF has not been achieved a ventriculostomy tube was inserted in the left ventricle with synchronous placement of an Omaya catheter in the right ventricle for the intraventricular administration of colistin. The patient was cured 26 days after the beginning of antibiotic treatment.
Conclusion: Intravenous and/or intraventricular administration of colistin may be life saving in cases of meningitis caused by multiresistant Gram – negative bacteria.

R2033

The treatment of acute bacterial meningitis with meropenem

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Objectives: The study of the clinical-biological efficiency of meropenem therapy at patients with acute bacterial meningitis.
Methods: The authors studied a group of 23 patients who were admitted in the Infectious Diseases Clinic from Timisoara with acute bacterial meningitis. The diagnosis of the bacterial meningitis was based on clinical findings (malaise, fever, headache, stiff neck, vomiting, confusion) and on examination of cerebrospinal fluid (CSF) sample (aspect, pressure, Pandy reaction, PMN leucocytes counts, proteins, clor, glucose and culture on special mediums). All patients were treated with meropenem (Meronem® - Astra-Zeneca, 1g), 3 × 2 g/24 hours, iv, during a period between 10 and 14 days and supportive treatment with Glucosis 10%, Manitol 20%, AINS, corticoterapy, vitamins, antipyretics, etc.
Results: The biological tests like ESR, fibrinogen, CRP, the parameters and the bacteriologic data of the CSF, were registered in the individual files, following the agreed protocol. The CSF culture and the direct bacteriological examination have confirmed: 7 patients with *Streptococcus pneumoniae* meningitis, 9 patients with Neisseria meningitis, 5 patients with *Staphyloccocus aureus* meningitis and 2 patients with *Klebsiella penumoniae* meningitis. The results of the effectuated antibiograms have confirmed the sensitivity of the isolated germs at meropenem. The registered side effects were minor and transitory, nausea, abdominal discomfort and soft stools, after the administration of the first doses of meropenem.
Conclusions: Through its large antibacterial spectrum, high clinical-biological efficiency and reduced side effects, the meropenem (Meronem® – Astra-Zeneca) can be used with success in the treatment of acute bacterial meningitis.

R2034

Hospital consumption and resistance of fluoroquinolones between 1996 and 2004

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Objectives: The excessive and inadequate consumption of fluoroquinolones (FQ) has serious consequences for public

health, such as the increase of bacterial resistance. Therefore our hospital is evaluating trends in antimicrobial use since 1996.
Methods: A 652-bed general hospital in Belgium (184 230 bed-days in 2004, including 63% acute beds). Antibiotic committee was created in 1998 and has many missions like to develop formalized treatment guidelines for commonly encountered infectious diseases and to control the consumption of extended-spectrum (E-S) antibiotics (ATBs). The annual consumption of ATBs (Class J01 in the ATC classification from WHO 2004) in Defined Daily Doses (DDDs) and total patient days (PDs) for 1996 through 2004 were determined from electronic capture of billing records. The DDDs/100 PDs ratio was calculated for each antimicrobial. The microbiology laboratory provided the committee with data about the frequency of various resistant pathogens.
Results: Total antimicrobial use (J01) increased between 1996 and 2004 and consumption was respectively 30.27, 31.47, 31.88, 30.90, 30.26, 30.23, 35.37, 36.60 and 43.09 DDDs/100PDs. The FQ class (J01MA) is one of the most dispensed antimicrobials with a yearly average of 4.32 DDDs/100PDs. The resistance of various pathogens to FQ raised between 1996 and 2002 from 37% to 48% for *Pseudomonas aeruginosa*, 35% to 45% for *Staphylococcus aureus*, with an increased consumption from 2.56 to 7.35 DDDs/100PDs. Intravenous (IV)/per os (p.o.) ratio (%) were 23/77 in 2002, 25/75 in 2003 and reached 32/68 in 2004.
Conclusion: Total consumption of ATBs did not change significantly between 1996 and 2000, except in 2001 where an increase of almost all classes of ATBs and FQ especially was observed. In 2003, a 72-hour stop order system of prescribing was implemented to control the use of E-S ATBs. In 2005, clinical guidelines, educational campaigns and a prescription of IV FQ restricted to 72-hour were implemented. These measures, which are presently under evaluation, are hoped: 1) to discourage the excessive use of IV FQ, 2) to promote p.o. usage because equivalent biodisponibility and lower cost, 3) to contain resistance development, and 4) to safeguard the value of this ATBs class.

R2035

Benchmarking antimicrobial drug use in university hospitals in five European countries

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Objectives: Benchmarking antimicrobial drug use in similar hospitals can be used to identify potential problem areas in prescribing practice and to aid in establishing appropriate and attainable goals. We compared the pattern and the extent of antimicrobial drug use in five European university hospitals aiming at establishing best practices.
Methods: A modified point prevalence study design was used. All in-patients at the University Hospital Rijeka, Croatia, Tartu University Hospital, Estonia, Stradins University Hospital, Riga, Latvia, Vilnius University Hospital, Lithuania and Karolinska University Hospital-Huddinge, Sweden were surveyed for antimicrobial drug use during one day in May 2003. The patient charts of all hospitalized patinets were reviewed and annonymous data regarding antimicrobial drug use were entered into a uniformed data sheet. We determined the prevalence and the main purpose of antimicrobial drug use in each hospital, as well as the prevalence of antimicrobial drug use according to departments. The pattern of antimicrobial drug use was assessed using the DU90% methodology. The main route of antibiotic administration was also evaluated. The prevalence of nosocomial infections with risk factors for such infections and the type of infections treated were determined.
Results: The prevalence of antimicrobial drug use was 24% in Rijeka, 30% in Tartu, 26% in Riga, 14% in Vilnius and 32% in Huddinge. Surgical patients were treated with antimicrobials more often than medical in Riga (53% vs. 31%), Tartu (39% vs. 26%) and Vilnius (54% vs. 25%). In Rijeka, Tartu, Riga and Vilnius 2/3 of the patients received antimicrobials intravenously, and in Huddinge less than one half. Broad-spectrum antimicrobials were most commonly used in Rijeka. The prevalence of nosocomial infections treated with antibiotics was 9% at Huddinge with the highest number of risk factors, and 3–5% in the other centres.
Conclusion: Benchmarking antimicrobial drug use in five different university hospitals identified differences and problem areas. The high rates of intravenous administration, poor compliance with the guidelines and prolonged surgical prophylaxis were general problems, which deserve specific attention in all centres. Changing antimicrobial prescribing practices may reduce unnecessary antimicrobial drug use and decrease resistance.

R2036

Attitudes of population to antibiotic use in country with high self-medication: analysis of 103 face-to-face interviews

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Self-medication is one of the main reasons of microbial resistance. Survey in 19 European countries revealed Lithuania as the country with the highest rate of self-medication (24% of all respondents). It prevailed in almost the half of questioned residents, who used antibiotics during the last 12 months.
Objective: The aim was to get insight about the attitudes of population towards antibiotic use.
Methods: The study was carried out as a part of multinational survey on self-medication with antibiotics in Europe. The respondents, who agreed to be questioned from the randomly selected (3000) and responded (746) in urban and rural area during the first phase of the study, were target population. Sample was made of two equal comparable groups: exposed group (self-medication) and reference group (non-users). Respondents were allocated to any of them according to the given answers in the first questionnaire. 103 face-to-face double-blind interviews were performed in order to ascertain attitudes towards antibiotics and their use, self-care attitudes, illness behaviour, etc.
Results: After the performance of interviews shifts between original groups occurred – 80% of 103 respondents pointed out about taking antibiotics any time in life.Analysis of basic knowledge about this medicine showed that a lot of the respondents (46.6%) considered it as bacteria killer (55.3% prescribed users, 41.7% self-medicators, 31.3% non-users). However people often thought, that antibiotics could cure infections (21.4% cases), kill viruses (9.7%) or have analgesic (2.7%), antipyretic (9.7%) and anti-inflammatory (9.7%) effect. Respondents tended to take antibiotics for different indications, even for easy symptoms such as of respiratory tract, diarrhoea, etc. The major source for self-medication was indicated as over-the-counter acquisition in community pharmacies. 50% of self-medicators (30% prescribed users) indicated them as the possible very easy way to get antibiotics. Leftovers as easy source prevailed in self-medicators' group (about 70%), while only a bit above 30% among the rest.

Conclusion: Rather often Lithuanian people consider antibiotics as 'universal' drug. It became evident that it's not enough to ensure the sales of antibiotics only by prescriptions to control self-medication. The attitudes of people have to be changed by proper educational campaigns.

R2037

Changes in antimicrobial use in Lithuania outpatient clinics

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There is no monitoring system of antimicrobial use in Lithuania neither in hospitals neither in community. So the data from studies are the single source of information on this topic.
Objective: The aim of the study was to analyze the changes in use of antimicrobials and diagnostic tests in Lithuanian paediatric and adult outpatient clinics.
Materials and methods: The 1 year prevalence study was carried out. Study population was children and adults who were treated in outpatient clinics. Individual patient charts from paediatric and adult outpatient clinics were randomly selected and analysed: 4200 records (20 clinics) – in 1997 and 3056 records (11 clinics) – in 2004.
Results: It turned out that antimicrobial prescription rate in outpatient clinics decreased for children (66.4% in 1997 and 51.9% in 2004) and haven't changed for adults (34.9% in 1997 and 33.1% in 2004). Penicilines with beta lactamase inhibitors (40.3%) and macrolides (29.5%) were the most frequently prescribed antibiotics for children in 2004 while aminopenicillins (31.6%) and macrolides (14.7%) in 1997. For adults aminopenicillines (44.5%) and tetracyclines (17.9%) were the most commonly prescribed in 2004 while tetracyclines (28.4%) and aminopenicillins (26.2%) in 1997). About 80% of all antibiotics prescriptions were issued for upper respiratory tract infections, common cold, acute bronchitis, that usually are caused by viruses. It was found out that 53.2% of children and 50.6% of adults receive antibiotics without any clinical tests (blood, CRE, RO, microbiological and urinary tests). Data for 1997 are not available. Microbiological tests are rarely used for out patients while prescribing antibiotics: they were performed only for 1.2% children in 2004 (2.8% in 1997) and for 2% of adults in 2004 (0.4% in 1997).
Conclusions: No big changes in antimicrobial prescribing rates were observed in 7-year period – antibiotics were prescribed for more than half children and every third adult. Structure of prescribed antibiotics is changing with highly increasing prevalence of broad-spectrum antibiotics for children. The analysis of antibiotic prescription indications confirmed the hypothesis that antibiotics are used irrational in Lithuanian out patient sector. The need of multisectoral interventions, including education, issue of guidelines and regulations etc. to influence the antibiotic usage and combat the resistance problem is essential.

Molecular bacteriology

R2038

Actinobaculum urinale in a 94-year-old male with sepsis

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Objectives: To show an example of the interaction between a central reference laboratory – with sequencing facilities – and a peripheral unit when it comes to speciation of a seldomly diagnosed bacterium from blood culture.
Methods: Blood cultures were drawn according to standard procedures. There was growth on blood and chocolate agars aerobically and on the same media under anaerobic conditions plus lactose agar under aerobic conditions. The bacterium was subject to resistance testing with our standard antibiotic panel for such organisms. 16S rRNA sequencing was done, on a central level, according to their standard procedures.A MEDLINE search as of 12/10/05 showed 13 ''hits" for ''Actinobaculum" and two ''hits" for ''*Actinobaculum urinale*" alone.
Results: A male aged 94 years presented with pneumonia, sepsis and fever of 40°C. From a blood culture isolate the following was observed microscopically: a small Gram-variable, corynebacterium-like bacterium. The colonies on blood agar were non-haemolytic, and were catalase and oxidase negative. The bacterium was resistant for metronidazole but sensitive for vancomycin. API 20 A showed *Actinomyces meyeri/odontolyticus* (ID% 55.2) and *Lactobacillus fermentum* (ID% 43.3.). Sequencing data showed that using a 499 bp fragment of the 16S rRNA gene demonstrated a 100% homology with the 16S rRNA gene of *Actinobaculum urinale*. Our laboratory had previously had a blood culture positive islolate – also from an elderly male – aged 91 – where the sequencing data demonstrated *Actinobaculum schalii*.
Conclusion: The findings demonstrated above show that there may be significant synergy when it comes to interaction between laboratories on a peripheral and a central level, given that it is not always cost-effective to do sequencing decentralized.
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R2039

Using PCR with an unusual purpose of blood culture positive *Brucella* spp. infection: a case report

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Brucellosis is an endemic disease in Turkey. Brucellosis cause a systemic infection which can involve any organ or system of the body. A 46-year-old man history of nausea, weakness, icterus, anorexia, and fever of up to 39°C was admitted to the Infectious Diseases policlinic. with 10 day's Physical examination revealed scleral, mucous membrane and cutaneous icterus, hepatomegali, pain and tenderness in the upper right quadrant. Laboratory test results was found as follows: white blood cell count: 6810/mm^3;

erythrocyte sedimentation rate: 12 mm/h; C-reactive protein: positive; total bilirubin: 8.17 mg/dl; direct bilirubin: 5.94 g/dl; AST: 311 U/L; ALT: 291 IU/L; alkaline phosphatase: 535 IU/L; GGT: 626 IU/L; protrombin time: 19.3 s; activation: 52%. Hepatic markers were negative. After cultures were taken, antibiotic therapy (ampicillin/sulbactam 4×2 g IV + gentamicin 1×160 mg IV) was began with the diagnosis acute cholangitis. Ultrasonographic examination of the abdomen has showed hepatosplenomegali. Upper gastrointestinal endoscopic examination was found normal. During the clinical follow-up the patient was subfebril. We found Rose Bengal test positive. After that, we performed Wright standard tube agglutination test. The titres of brucella antibodies in serum were 1/320. *Brucella melitensis* was isolated from blood on the 8th day. We found with PCR IS 711 gene region of *Brucella* spp. (*Brucella melitensis* strain 133 IS 711 845 bp : SEQ-2 5′-ATC TTC CGG GGC GAG TTG GTA-3′; SEQ-1 5′-GGA TCT GAG CCG TTG CCT TGA-3′) positive in the isolated bacteria. Antibiotic treatment switced to oral doxycycline 2×100 mg and streptomycin 1×1 g IM. We use PCR here to show gene region, which is specific for *Brucella* spp. in the isolated Gram-negative coccobacille. Gastrointestinal, cardiovascular, genitourinary, haematopoetic, nervous, skeletal systems may be involved in brucellosis as a complication. PCR can be used in isolated bacteria from different specimens of cases, which came with different presentations.

R2040

Phenotypic typic and genotypic detection of virulence factors in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains from a Tunisian hospital

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Enterococci are recognised as important human pathogens that are responsible for serious nosocomial infections. Several virulence factors have been described in enterococci, for instance, aggregation substance (Agg), gelatinase (GelE), cytolysin (Cyl), enterococccal surface protein (Esp), collagen-binding adhesin (Ace) and Sex pheromone (Cpd). Phenotypic and genotypic determination of virulence factors were carried out in all the 46 high-level-gentamicin resistant (HLGR) clinical *Enterococcus faecalis* (n = 34) and *Enterococcus faecium* (n = 12) strains recovered from unrelated patients in La Rabta Hospital of Tunis during 2000–2003 (all these strains harboured the aac (6′)-aph (2") gene). The genes encoding virulence factors (agg, gelE, ace, cyl, esp, cpd and fsrB) were analysed by PCR and sequencing. The production of gelatinase and hemolysin as well as the adherence to caco-2 and hep-2 cells or the capacity for biofilm formation were also investigated in all 46 HLGR enterococci. The percentages of *E. faecalis* strains harbouring virulence genes were as follows: gelE and cpd (100%), fsrB (62%), agg (56 %), cylLLS (41.2%), esp (26.5%), and ace gene (6%). The only virulence gene detected among *E. faecium* strains was esp (58%). Gelatinase activity was detected in 22 *E. faecalis* strains (65%, most of them with genotype gelE + -fsrB+), being the remaining 12 strains gelatinase-negative (with genotype gelE+-fsrB-, and a deletion of 23.9 kb of fsr locus). Fifty-seven per cent of the cylLLS-containing *E. faecalis* strains showed B-haemolysis, showing a negative B-haemolysis reaction the remaining cylLLS-positive strains (most of these strains lacked cylABM or cylB genes). Our HLGR *E. faecalis* strains, in contrast to *E. faecium*, showed strong biofilm formation or adherence to caco-2 and hep-2 cells, although it was not observed a clear association between those activities and the presence of esp or agg genes, respectively.

R2041

Bacteraemia caused by *Corynebacterium diphtheriae*

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The common administration of prophylactic vaccination against diphtheria in Poland has led to elimination of infections caused by toxigenic *Corynebacterium diphtheriae* (*C. d*) strains. Single cases of this disease reported in Poland between 1988 and 1996 were associated with common migration of people from countries in which the vaccine-induced acquired immunity rates against diphtheria were low (Russia, Ukraine, Belarus) and in which infections caused by *C. d* reached epidemic proportions. Given only a single case of the disease noted in Poland in 2000, it has been claimed that the country is free from diphtheria since 1997. The *C. d* strains have not been isolated in Poland for the last 5 years. The case of bacteraemia caused by *C. d* and reported in the present study has been the second encountered within the last year in Poland. The analysed patient was implanted an aortic valve 2 years before the isolation of *C. d* in his blood sample. The patient had a sudden onset of high fever not accompanied by any laryngological symptoms. The strain cultured from the blood was identified as *C. d* on the basis of its biochemical features (API Coryne-bioMerieux). The preliminary identification of the species was further confirmed by the PCR assay (the analysis of the 16sRNA gene sequence). As a result of the diagnostic procedure and the analysis of the clinical picture of the patient diphtheria was excluded. The ability of the cultured strain to produce the diphtheria toxin was evaluated using the Elek precipitation test. The strain was negative for the tox gene by the PCR method, which unequivocally confirmed that the microorganism was not toxigenic. The results of the employed assays were compared to the reference *C. d* strain [PW-8 (tox+)]. The antimicrobial sensitivity testing (MIC) was conducted using the MHS medium (bioMerieux) and E-tests (AB BIODISK). In order to broaden the diagnostic procedure and to improve the knowledge of the patient's clinical picture the serum antibody levels directed against *C. d* were analysed using the ELISA assay. The above presented case indicates that the nontoxigenic *C. d* strains are encountered in Poland as etiologic agents of infections. These strains give rise to the diagnostic and therapeutic problems and constitute a challenge for physicians and microbiologists. Their reporting and a detailed characterisation are of outstanding importance from the epidemiological point of view.

R2042

Prevalence and clinical presentation of *Staphylococcus aureus* positive for Panton-Valentine Leukocidin in a university hospital in the southwestern region of Germany

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Objective: The presence of Panton-Valentine Leukocidin (PVL) in *S. aureus* has been associated with lethal pneumonia in young people and persistent severe skin infection. It has also been found in most strains of the recently described community acquired MRSA (cMRSA). This study was performed to obtain information about prevalence, clinical features and resistance patterns of PVL-positive *S. aureus* strains in our hospital and region. Data from January to October, 2005, were available for first results.

Material and methods: Only the first *S. aureus* isolate from clinical samples of each patient was investigated for PVL (lukS-lukF) using published primers including an internal control amplifying sequences of the nuc-gene of *S. aureus*. For antimicrobial resistance we used the BD Phoenix automated system. All strains resistant for oxacillin, ciprofloxacin or gentamicin, or positive for PVL were additionally checked for the presence of the mecA-gene.
Results: MSSA: A total number of 760 isolates could be included, yielding 21 PVL+ strains (2.8%). PVL+ strains were predominantly found in younger outpatients with superficial infections of the head, whereas they were not involved in blood stream infections, or infections of the urinary tract, bones, or foreign bodies. Resistance patterns of PVL+ vs. PVL- groups showed no significant differences.
MRSA: Of 136 MRSA strains, only 1 PVL+ strain was identified and confirmed as cMRSA. The majority of our MRSA isolates corresponds to the south German epidemic strain and are of nosocomial origin.
Conclusion: This study underlines that the prevalence of PVL is still low in *S. aureus* isolates from patients in the southwestern region of Germany.

R2043

Phenotypic and genotypic characterisation of *Staphylococcus aureus* isolates from patients with cystic fibrosis

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Objectives: *Staphylococcus aureus* is one of the most common pathogens isolated from patients with cystic fibrosis (CF). Despite intensive antibiotic therapy the microorganism is rarely eradicated from the respiratory tract of CF patients. The ability of *S. aureus* to form biofilm, is considered an important virulence factor responsible for chronic airway infections in these patients. The aim of this study was to determine the frequency of slime production and the prevalence of icaA and icaD genes known to play an important role in biofilm formation in *S. aureus*.
Methods: During 1997–2004, 221 *S. aureus* strains were isolated from sputum of CF patients (age range 1 month–20 years) treated in the Children's Memorial Health Institute, Warsaw. The presence of the icaA and icaD genes was determined by PCR with primers derived from the icaADBC locus of *S. aureus* (GeneBank accession no. AF086783). Slime formation was detected with Congo red agar and a standard biofilm assay in tissue culture plates (TCP) with Tripticase soy broth (TSB).
Results: All *S. aureus* strains carried the icaA and icaD genes. Majority of tested strains (211/221; 95%) showed a black colony-forming phenotype on Congo red agar, whereas less than 50% of isolates were biofilm-positive in TCP-TSB assay.
Conclusions: Although all *S. aureus* strains isolated from airways of CF patients possess icaADBC locus, and nearly all show a black colony phenotype on Congo red agar, only part of them produce biofilm.

R2044

Detection by PCR method of fastidious micro-organisms in urine from patients with UTI symptoms

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Objectives: We aimed to demonstrate to clinicians the advantage of detection by PCR of the fastidious microorganisms in urine from the patients with UTI symptoms, when standard cultures were negative and antimicrobial drugs were not applied to patients.
Methods: During the period of 08–12, 2004, 1326 urine samples were sent to the laboratory of microbiology from the nephrological department of the hospital. In standard cultures onto the CLED agar and blood agar the samples from 662 patients (49%) showed no growth. From among these patients, 113 were selected for further investigation with PCR method (within age range of 17–47 years, not cured with antibiotics). Urine samples of these patients were investigated with Roche Cobas Amplicor for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and with in-house PCR for *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis*. The patients were divided into 3 age groups. Out of 113 patients 71% were women, what witnesses once again that UTI is prevalent among women due to anatomical peculiarities of their urogenital tract.
Results: From 113 samples investigated, 42 (37%) gave positive results with PCR methods, with microorganisms detected as follows: *C. trachomatis* – in 4% of samples, *U. urealyticum* – 29%, *M. hominis* – 12%. *N. gonorrhoeae* and *T. vaginalis* were not found. The age group of under 25 years, containing 36 samples, gave the following results: *C. trachomatis* – in 11% of samples of the respective group, *U. urealyticum* – 28%, *M. hominis* – 11%. In the age group of 26-35 years (44 samples), *C. trachomatis* was not found, *U. urealyticum* – 27%, *M. hominis* – 14%. In the age group of over 35 years (33 samples), *C. trachomatis* was not found either, *U. urealyticum* – 33%, *M. hominis* – 12%. In samples from women (80 samples), microorganisms were detected as follows: *C. trachomatis* – 11%, *U. urealyticum* – 31%, *M. hominis* – 11%. Among men (33 samples), percent of microorganisms found was: *C. trachomatis* – 9%, *U. urealyticum* – 24%, *M. hominis* – 15%.
Conclusion: In the case of negative results of standard urine cultures, investigation with PCR methods for detection of fastidious micro-organisms is of great help to clinicians. But it should be considered that 60% of healthy women carry *U. urealyticum* and 20% carry *M. hominis* in their urogenital tract. Unlikely, in male urogenital tract these microorganisms should not appear. If present in male urethra, they should always be treated as pathogens.

R2045

Influence of a mutation in the gyrA gene on the relative abundance of many *Escherichia coli* proteins

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Objective: The purpose of this study was to analyse the role of the acquisition of a mutation in the gyrA gene in the expression of proteins in *E. coli*.
Materials and methods: Three *E. coli* strains were used in this study: 1) the 14366 wild-type *E. coli* clinical isolate, 2) this strain was submitted to repeated in-vitro subinhibitory concentrations of ciprofloxacin to generate a ciprofloxacin-resistant mutant (*E. coli* 14366 M) with a mutation in the gyrA gene (Ser83-Leu). 3) This mutant was further transformed with a plasmid carrying the wild-type gyrA gene, generating a complementation of the gyrA gene (*E. coli* 14366 MC). Purification of whole proteins was performed using a sonicator-based method. Protein extracts from these three strains were displayed by two-dimensional gel electrophoresis and then stained using a silver staining protocol to compare their patterns. The spots in the 14366 wild-type *E. coli*, which experienced a variation in their level of abundance compared to the mutant strain (*E. coli* 14366 M) and which were

restored in the transformed *E. coli* were sliced and characterized by MALDI TOF/TOF.
Results: To date 14 out of 24 proteins have been characterized. Among them, 10 proteins have shown an increase in their abundance with respect to the mutant strain and the other 4 proteins have shown a decrease in their abundance. The characterized proteins can be distributed into different groups: i) proteins implicated in cellular permeability (OmpA), ii) proteins implicated in metabolic functions (aspartate ammonia-lyase, glycerol kinase, aminomethyltransferase) and ii) proteins implicated in DNA replication (RpoA).
Conclusions: We can infer from this work that a mutation at the gyrA gene might play an important role in regulating the expression of several proteins in *E. coli*, which can be linked to the supercoiling status of the promotor of the genes.

R2046

Frequency of enterotoxin, toxic shock syndrome toxin 1 and panton-valentine leukocidin genes in MRSA vs. MSSA isolated from blood

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Materials and methods: The presence of enterotoxin (sea–see, seg–ser), exfoliativtoxin (eta, etb), TSST-1 (tst) and leukocidin (lukS-F, lukD-E, lukM) genes was detected by the PCR technique in 164 *Staphylococcus aureus* (*SA*) strains. *SA* isolates were collected at the Medical University Hospital Vienna from blood cultures (=BL) from patients with sepsis. Primers for the detection of 3 recently published ET genes (sel, sep, seq) were newly designed for this study. Antibiotic susceptibility was determined by disc diffusion (Kirby-Bauer Method) and PCR technique (mecA).
Results: In the 164 BL-strains toxin-gene distribution was as follows: sea: 31%, seb: 6%, sec1: 0.5%, sec2: 12%, sec3: 1%, sed: 6%, see: 0%, seg: 62%, seh: 2%, sei: 62%, sej: 6%, sek: 1%, sel: 14%, sem: 57%, sen: 62%, seo: 62%, sep: 8%, seq: 1%, ser: 6%, tst: 9%, eta: 4%, etb: 1%, lukS-F: 1%, lukD-E: 54%, lukM: 0%. mecA positiv strains (n = 45). MRSA (%)/MSSA (%): sea: 93/8, seb: 4/7, sec1: 0/0.5, sec2: 0/16, sec3: 0/1, sed: 2/7, see: 0/0, seg: 57/63, seh: 0/3, sei: 57/64, sej: 2/8, sek: 0/2, sel: 0/19, sem: 60/56, sen: 60/63, seo: 62/63, sep: 6/9, seq: 0/2, ser: 4/6, tst: 2/12, eta: 2/5, etb: 0/1, lukS-F: 0/2, lukD-E: 86/42, lukM: 0/0.
Conclusion: The most commonly found ET genes were seg, sei, sem, sen, seo, which were mostly found in association with one another. Genes second most frequent found in combination were sel with sec. The SA toxin genes sea and luk D-E were dominant in MRSA strains. MRSA did not harbour sec, seh, sek, sel and seq. The recently described genes sep, seq and ser were rarely found in BA strains. Three MSSA strains were lukS-F (Panton-Valentine leukocidin) positive.

R2047

Anaerobic meningitis due to *Peptostreptococcus anaerobius* diagnosed by broad-range PCR. Case report

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Introduction: Anaerobic bacteria are recovered from <1% of patients with acute bacterial meningitis. There have been few cases of acute bacterial meningitis associated with *Peptostreptococcus* species reported in the literature up to now. The most common predisposing factors are meningorectal fistulae and head-and-neck surgery. Because of their fastidious nature, these organisms are difficult to isolate from biological materials and can be often overlooked and underdiagnosed. Molecular detection methods based on amplification of microbial 16S rRNA genes seem to be a promising diagnostic tool in these situations.
Case report: Here we report a case of 55-year-old man developing sinusitis, which rapidly progressed to acute bacterial meningitis with unconsciousness. He underwent surgery after head injury 20 years ago. *Peptostreptococcus anaerobius* was detected from cerebrospinal fluid (CSF) using broad-range PCR followed by direct sequencing and sequence similarity analysis. On the basis of this result, metronidazole was added to conventional antimicrobial therapy with ampicillin and cefotaxime. Several days later *Peptostreptococcus* sp. was cultivated from maxillary sinus material while cultures from CSF remained negative. Unfortunately, the patient had to undergo several neurosurgical intervences and the course of disease was accompanied by severe complications such as repeated sepsis caused by G- microorganisms, serious gastrointestinal bleeding episodes and leg amputation due to ischaemia. Finally, he recovered with neurological sequelae.
Discussion: A method of broad-range PCR was demonstrated to be useful in diagnosis of anaerobic meningitis.

Molecular virology

R2048

Linkage of CTL epitopes of HPV16 E7 gene to HSP70 gene using three-steps PCR

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Objectives: DNA vaccines represent an attractive approach to generating antigen-specific immunity because of their stability and simplicity of delivery. For immunotherapy of HPV16 associated disease, the E7 gene is considered a prime candidate, as it is expressed in all HPV16-positive tumours. Unfortunately, the E7 gene is a poor inducer of a cytotoxic T-cell response, when being used as antigen in DNA vaccination. Linkage of antigens to HSP represents a potential approach for increasing the potency of DNA vaccines.
Methods: Truncated HPV16 E7 gene is selected from a region proximal to the COOH-terminal end of the HPV16 E7 gene (codons 246–279) that encodes several overlapping CTL epitopes. These have been defined by peptide binding libraries, immunological screening assays and epitope elution/sequencing methods. Then the E7 segment is fused to HSP70 gene and for read through translation of it, stop codon of HSP70 gene is eliminated and a polyglycine linker is considered between two parts. In order to have protein expression, one stop codon is applied at the end of E7 segment. All of the procedures are done with three-steps PCR using one forward and three reverse primers.
Results and conclusion: The HSP70/E7 segment is cloned in pcDNA3 and resulted construct was sequenced by SEQ-LAB Company. The construct is consider to be used in future for CTL

cytotoxicity assay and we will compare it with CTL response to E7 complete gene.

R2049

Evaluation of IFI versus real-time PCR as the gold standard for diagnosis in genital HSV

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Objectives: Herpes simplex virus genital infections have increased in the last avoiding years. A sensitive and specific method for detecting herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) is important for diagnosing genital infections and HSV-1 and HSV-2 transmission. The aim of this study is evaluating the serological diagnostic (IFI) in comparison with Real Time PCR for the detection of HSV-1 and HSV-2.
Methods: A total of 93 specimens (39 genital ulceration, 30 vesicle, 21 endocervical, 3 urethral) were collected from 93 patients (54 women and 39 men) with an average age of 30.9 years. All the patients were followed at STD clinic, Seville, Spain. The samples were collected following the manufactures rules (STD, Swab Specimen Collection and transport Kit, Roche). Magnapure (Roche Diagnostic System) was used to extract nucleic acids. The diagnostic was performed using IFI (Path-finder Herpes Simplex Virus types 1 and 2, Bio-Rad) and Light Cycler, real time PCR (Roche Diagnostic System LC HSV ½ detection). To evaluate IFI we used real time PCR as the gold standard. Positive and negative controls were used in all cases.
Results: Of the total of 93 samples, 45.16% (38/93) were positive (66% vesicle, 38.09% endocervical and 35.8% genital ulceration). The percentage of positive to VHS-1 and VHS-2 were 67.4% and 32.5% respectively. The 40.86% (38/93) were positive by PCR and 21.5% (20/93) by IFI, PCR detected 22 samples that were negative by IFI and 4 samples were positive by IFI and negative by PCR. The sensitivity and specificity obtained were 42.10% and 92.7% respectively and the positive and the negative predictive value were 80% and 69.8%.
Conclusions: 1. IFI presents a low sensitive and a high specific test for the detection of HSV infections. 2. PCR is an easy, effective, non-time consuming and objective method for the diagnosis of herpes simplex genital infection. 3. The incidence of VHS-1 has increased compared to VHS-2 in genital infection in this study.

R2050

Molecular characterisation of non-polio enteroviruses recovered from patients with aseptic meningitis in a tertiary care hospital in Korea

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Objectives: Non-polio enteroviruses are the most common cause of aseptic meningitis worldwide. In Korea, a few big outbreaks of aseptic meningitis have been reported thus far and, from July to September 2005, there might be another outbreak. In the present study, we performed molecular characterization of non-polio enteroviruses recovered from patients with aseptic meningitis during this period in a tertiary care hospital in Korea.
Methods: Twenty-four out of 36 enteroviral isolates recovered from CSF or stool of patients with aseptic meningitis were included in the study. Serotyping was performed by seroneutralization. Reverse transcription-polymerase chain reaction (RT-PCR), and direct sequencing of 5′ untranslated regions (5′-UTR) followed by a phylogenetic analysis using the PHYLIP software were done for molecular characterization.
Results: Coxsackie B virus (CVB) serotype 5 was the most frequently isolated serotype (15/24), followed by CVB1 (4/24), echovirus (ECV) serotype 9 (4/24), and CVB3 (1/24). The 5′-TTTCCTTTT-3′ region in the 5′-UTR, which is known to be associated with neurovirulence, were conserved in most strains except 5 strains including 1 CVB5 and all 4 CVB1 strains. Phlyogenetic analysis of 5′-UTR showed three major groups and CVB5 strains clustered into a single group.
Conclusion: In the present study, we demonstrated that the predominant enteroviral strain recovered from patients with aseptic meningitis was CVB5. In addition, the CVB5 isolates might be closely related according to the molecular analysis results.

R2051

Our first experiance with RT-nPCR for detection of HIV-1 RNA among seropositive persons in Republic of Macedonia

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The aim of the study was to establish PCR method in the routine laboratory work, as well as to detect HIV RNA in seropositive patients in our country.
Methods: 33 examined persons (age 25 to 52 years) were divided in two groups: the first group consisted of 20 healthy blood donors randomly selected; and the second group of 13 seropositive persons for HIV. According the mode of the transmission 10 of them were heterosexual, 1 heterosexual and intravenous drug user, and 2 homobisexual. 10 persons were male and 3 female. ELISA test for detection of antibodies against HIV-1 and HIV-2, and ELFA test for combined detection of HIV p24 antigen and anti HIV-1, 2 IgG were performed for each examined person. The RNA from the whole blood was extracted with a commercial kit based on salt precipitation combined with high effective inhibitors of RN-aza activities. Detection of HIV RNA was performed with RT-nPCR kit. The product was separated with electrophoresis on 1.5% agarose gel. The result was positive if the band of 210bp has been seen with any intensity. Measures of precaution were taken during the all steps of the work and disposition of the HIV infected materials.
Results: In the group of blood donors ELISA, ELFA and RT-nPCR were negative. Assuming that prevalence of HIV infection is zero, the clinical specificity of RT-nPCR is 100%. The analytical specificity of the RT-nPCR test was performed with attempt to amplificated nucleic acids from HCV, HBV, HPV, CMV, HSV, RBV, TBC, Chl. trachomatis. None of these showed reactivity. From the group of 13 seropositive persons, 33 samples were analysed. HIV RNA was detected in 15 samples. ELISA and ELFA test were positive in all samples. Different aliquots of the samples were tested independently and show the same results. After different period of freezing at –70ordm;C of RNA samples, the RT-nPCR reaction was identical to the first one. Gained amplicons were frozen at –20ordm;C for a week and the electrophoresis was identical to the previous one. The reaction is fast, simple for manipulation; with low detection level of 60 IU/ml. RT-nPCR needs a small amount of RNA, as well as a small volume of sample. HIV RNA was detected in different period of time with different clinical presentations of patients, with or without antiretroviral therapy.
Conclusion: The qualitative RT-nPCR method is recommended for use in routine laboratory for documentation of HIV infection, as well as for the clinical management of HIV infected patients.

R2052

Evaluation of a molecular beacons real-time PCR method for the diagnosis of enteroviral meningitis

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Objective: To compare the efficiency of a real-time PCR (RT-PCR), Cepheid, with cell culture (CC) for the diagnosis of enterovirus (EV) meningitis.
Methods: An EV strain and a rhinovirus strain were used to determine the sensitivity and specificity of the test. RNA was extracted by QIAamp® Viral RNA Mini Kit (QIAGEN). Reverse transcription and amplification were done by QuantiTect® Reverse Transcription and QuantiTect® Multiplex PCR noROX kits (QIAGEN); and the Enterovirus Primer and Probe Set ASR (Cepheid). Forty-five cerebrospinal fluid (CSF) samples from 44 patients with suspected viral meningitis were processed by RT-PCR. Thirty-eight CSF specimens were evaluated by CC. Samples were inoculated into four cell lines (MRC-5, A-549, Vero and RD) and viral growth was confirmed by immunofluorescence (Chemicon). Eighteen of all CSF samples were analysed for DNA of HHV-1/HHV-2 by RT-PCR (Roche).
Results: RT-PCR detected 0.05 PFU of the enteroviral strain and was negative for the rhinovirus isolate. EV was detected in 51.1% of samples (23/45) by RT-PCR and in 23% (9/38) by CC. Concordance between 2 techniques was 63% (24/38). Of the 38 CSF specimens tested by both methods, none were positive only by CC, but 13 were exclusively positive by RT-PCR (13/38, 34%). Six of the 8 CSF RT-PCR positive/CC negative cases, had other positive viral cultures (1 NPA; 1 urine; 3 stool [from the same patient]; and 1 NPA and stool from one patient). One of the 2 remaining cases was negative for NPA and the other was negative for NPA and urine. RT-PCR required 5 h to perform and median time to isolate the virus was 7 days. No CSF was positive for HHV-1/HHV-2.
Conclusions: 1) RT-PCR from Cepheid showed a good analytical sensitivity and specificity with the strains tested. 2) In our experience, Cepheid RT-PCR methods were more sensitive than CC to detect EV meningitis. 3) Results from RT-PCR were obtained faster than those from CC (5 h vs 7 days).

Molecular typing

R2053

SCCmec typing of methicillin-resistant *Staphylococcus aureus*

W. Witte for ESGEM

Objective: MRSA have evolved by integration of SCCmec elements into the chromosome of particular clonal lineages of *S. aureus*, which can be discriminated by multilocus-sequence typing (MLST, reference method) and spa-sequence typing. As MRSA belonging to the same clonal lineage can have acquired different SCCmec elements (five different basic types known so far), molecular typing of SCCmec elements has to supplement typing based in chromosomal genes.
Methods: Different PCR based approaches have been reported which recognize sequences specific for different SCCmec-elements (1, 2, 3). Most reliable are PCR's for the ccr-complex and the upstream neighbouring sequence, for KDP (group II elements) as well as for ORF EO24 (group I and subtypes of IVa) and sequences upstream to the ccrA complex for subdivision of group IV elements. Furthermore, sequencing of a 400 bp stretch of ccrA determinants allows differentiation of the different basic types.
Results: Examples for necessity of SCCmec typing are MRSA exhibiting MLST ST05 and spa t002, which can contain SCCmec I, II, or IV elements, MRSA of ST45 (elements IVa, IVd), and ST254 (IVc or IVd), ST30 (IVa, IVc). 1. Oliviera, P., De Lencastre, H. AAC 46 (2002) 2155–61. 2. Okuma et al. JCM 40 (2002) 4289–94. 3. Cuny et al. Eurosurveillance (2005) in press.

R2054

Serotyping of *Toxoplasma gondii* in pregnant women. Predominance of type II in the Old World and type I and III in the New World

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Background: *Toxoplasma gondii*, a worldwide protozoan, usually induces asymptomatic infections. It can be life threatening in immunodeficient patients or in fetuses whose mothers are acutely infected during pregnancy. Moreover the clinical outcome of congenital infections cannot be predicted, ranging from fetal loss to sub-clinical infection. Infected newborns with no clinical signs are at risk of developing visual impairment in childhood or adolescence. Factor of virulence are not yet defined. Genetic analysis indicated an unusual parasite population structure consisting in three clonal lineage of different virulence as defined in mouse model. Type I is highly virulent for mice and rarely found in Europe, whereas type II is avirulent and predominant among Europeans strains. Type III is relatively virulent and uncommon in Europe. Yet, it is not clear whether genotypic pattern is associated with clinical profile of the disease in humans or with geographical distribution. The main reason is the difficulty to obtain parasitic DNA from patients. Hence, data are limited and originate from acute or congenital infections or animals. For addressing the question of type strain, geographical distribution and severity of clinical toxoplasmosis, a non-invasive assay is needed.
Method: We developed an ELISA test for serotyping *Toxoplasma gondii* strains using polymorphic peptides specific of the 3 clonal lineages derived from GRA5 and GRA6, 2 dense granule organelles. Two hundred and twelve sera from chronically infected pregnant women from 3 different European countries were investigated and compared with 40 sera from Colombia where clinical expression of the disease is more severe and strain genotypes are mainly from type I and III.
Results: The analysis of genotype-specicifc antibody response showed a homogenous type II structure in the European samples and a type I and III in the Colombian population.
Conclusions: We demonstrated that, despite some limitation due to antigen/antibody specificity, serotyping gives results consistent with genotyping and is a promising assay for investigating relationship between type of strain and toxoplasmosis severity.

R2055

Poultry as a potential source of *Campylobacter jejuni* and *Campylobacter coli* infections in children in Poland

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Objectives: Contamination of poultry meat with *Campylobacter* is considered an important risk factor for human infections. The aim of this study was to determine the relatedness of *Campylobacter* strains isolated from children with diarrhoea and chicken carcasses.
Material and methods: *Campylobacter* isolates were cultured from anal swabs from children with diarrhoea and swabs from chicken carcasses obtained in supermarkets and slaughterhouses. Species identification was performed using standard methods, and confirmed by PCR with specific primers. The isolates were typed using randomly amplified polymorphic DNA (RAPD).
Results: A total of 115 *Campylobacter* isolates were cultured from chickens (71 *C. jejuni* and 44 *C. coli*), and 80 were obtained from children (63 *C. jejuni* and 17 *C. coli*). Cluster analysis of the RAPD patterns revealed substantial diversity between human and chicken isolates, both *C. jejuni* and *C. coli*; however RAPD profiles of 6 human *C. coli* isolates were identical to chicken isolates.
Conclusions: Our results suggest that *Campylobacter* infections in children in Poland may come from additional sources, other than poultry meat.

Molecular biology, including diagnosistics - others

R2057

Diagnosis of *Toxoplasma gondii* by polymerase chain reaction method in pregnant women from Turkey

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Objective: To evaluate the prevalance of toxoplasmosis in various samples sent from Obstetrics and Gynaecology clinic to Gazi University molecular diagnosis laboratory by PCR.
Methods: One hundred and eighteen samples (90 blood, 27 amniotic fluid, one cord blood) obtained from 109 pregnant women during the 20th week of pregnancy for genetic diagnosis were enrolled in the study. *Toxoplasma gondii* DNA was extracted by Heliosis^{TM} DNA Extraction kit (Metis Biotechnology, Turkey). Amplification was performed in two steps by using specific primers for the *Toxoplasma gondii* B1 gene. The second PCR products were analysed on a 1.5% agarose gel stained with ethidium bromide, and the expected 190 base pair length was confirmed.

Results

	Toxoplasma DNA PCR (+)	Toxoplasma DNA PCR (-)
Blood (n:90)	4 (%4.4)	86 (%95.6)
Amniotic fluid (n:27)	2 (%7.4)	25 (%92.6)
Cord blood (n:1)	1 (%100)	0 (%0)
Total (n:118)	7 (%5.9)	111 (%94.1)

Conclusion: PCR method can be used in prenatal diagnosis of congenital toxoplasmosis and acute infections in immunosuppressed patients by using various clinical samples such as blood, cerebrospinal fluid, amniotic fluid, bronchoalveoler lavage and urine especially in patients with atypical clinical and serological findings in the diagnosis of toxoplasmosis. In conclusion, we recommend to use PCR, as a direct and rapid assay to support the serological assays in pregnant women.

R2058

Classification of bacterial isolates of the Jordanian Oil refinery petroleum sludge

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Objective: The aim of this study is to characterize the bacterial isolates of the Jordanian oil refinery sludge for the purpose of utilizing microorganisms in treating industrial wastewater effluents that contains hydrocarbons.
Methods: Morphological, physiological, biochemical, antimicrobial susceptibility tests and 16S-23S rRNA spacer region polymorphism were used to characterize the isolated thermotolerant *Bacillus*, with specific reference *Bacillus* strains. Data were coded and analysed by numerical techniques using the Gower coefficients and by average linkage (UPGMA) analysis.
Results: Study was resulted in allocation of strains into two areas at 50.0% similarity levels and ten major phenons at 78.0 % similarity level. Amplification of 16S-32S rRNA genes divided all strains in two areas at 48.0% similarity level however; at 78.0% similarity level five taxonomically distinct phenons were evident.
Conclusion: The aim of this study was to evaluate the taxonomy of *Bacillus* strain isolated from petroleum sludge in Jordan, which might be valuable for solving future industrial pollution problems.

R2059

PCR-based identification and verification of *Burkholderia cepacia*

H.D. Binnet *(Ankara, TR)*

Objectives: *B. cepacia* is a problematic pathogen in patients. Identification of *B. cepacia* complex is too much difficult for routine clinical microbiology methods. In this research, we demonstrated the efficacy of the PCR in the epidemiological analysis of *B. cepacia*.
Methods: *B. cepacia* strains were isolated with using API20 NE. Bacterial DNA was isolated and analysed by PCR amplified 16S rRNA gene fragments with primers PC480-PC1250. All isolates were digested with enzymes (Tsp 509I, RsaI and TaqI). Target sequences were amplified by using forward and reverse primers labelled with various fluorescent dyes. The labelled PCR products were determined with automated DNA sequencer

(CEQ 2000XL, Beckman Coulter, USA). Data were compared with Gen-Bank database of *B. cepacia* strains.
Results: 28 clinical isolates were identified as *B. cepacia* with API20 NE, PCR with using PC480–PC1250. Bacterial genomic DNA was successfully prepared from all *B. cepacia* cultures. The 16S rRNA gene product was successfully amplified from the genomic DNAs. In order to verify the analysis, amplified product samples were digested with the Tsp509I, RsaI and TaqI enzymes. With TaqI enzyme 473, 300 bp fragments were detected in 17 isolates. In 11 *B. cepacia* any fragment was not observed. With RsaI enzyme 365, 150, 100 bp fragments were detected in all *B. cepacia*. With Tsp509I enzyme 341, 204 bp fragments were detected in 23 isolates. These sites were not detected in remaining 5 isolates. It was also considered that disappearance of fragments in this sites as a result of mutation. In our study, t has been aimed to get nucleotide sequences (16S rRNA) of *B. cepacia* isolates, which verified with restrictions endonuclease enzymes. As a result the nucleotide sequences of the 16S rRNA were found to be completely identical.
Conclusion: We occurred the oligonucleotide primer pairs PC480–PC1250 target the 16S rRNA region. This gene has region of heterogeneity at the genus species and strain levels. We suggest that the PCR procedure be used in clinical microbiology laboratories. During this study, when we compared the sequences both nucleotide sequences produced by the 16S rRNA gene sequence and *B. cepacia* sequence existing at GeneBank data base. We observed that they were entirely similar. Moreover, at our study we also confirmed all PCR products belong to *B. cepacia*.

R2060

Detection of stx genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction

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Objectives: The purpose of this study was to determine the presence of selected virulence genes in avian pathogenic *Escherichia coli* (APEC).
Methods: We examined 12 APEC isolates, which belonged to the most common serotypes in Iran. All 12 isolates were tested for the presence of stx1, stx2, eae, espB and hly genes by multiplex polymerase chain reaction in two protocols. In the first protocol the isolates were tested with EC and with hly primers and in the second protocol the isolates were examined with eae, stx1, stx2 and espB primers.
Results: Seventy five percent (9) of the isolates carried only stx2 gene sequence and just one isolates had both stx1 and stx2 genes. Furthermore, 2 isolates (16.66 %) possessed eae sequence and 3 isolates carried espB (25%). The hly gene was not detected in any of the isolates.
Conclusion: The findings of this study indicated that the Stx2 may be widespread among APEC in Iran.

R2061

Diagnostic use of two serologic tests for invasive candidiasis

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Objective: To check the diagnostic utility of two tests (detection of mannan antigen-Ag- and antimannan antibodies–Ab-) for invasive candidasis in neutropenic patients.
Methods: A total of 583 sera from 89 neutropenic patients presenting risk factors for invasive fungal infection were studied. Patients were attended at the Hospitals V. del Rocio, and V. Macarena from Seville and Hospital de Jerez, from September of 2004 to June 2005. Two sera specimens weekly were extracted from each patients from the inclusion to the patient discharge from the study. Other samples as blood culture, LAB, bronquial aspirates or others were taken following clinical criteria. Culture of these samples were carried out following the usual techniques of a mycological laboratory. Antigen detection was performed using AG Platelia *Candida* and the corresponding antibody detection using Ac Platelia *Candida*, both kits from Bio-Rad.
Results: Of the total 583 studied sera, 124 showed at least one Ab positive (≥20 U/ml of antimannan antibodies) specimen, corresponding to 22 patients. Of them, only 5 patients showed positive results both to Ag and to Ab in two or more specimens. These positive results corresponded to patients with radiologic signs or positive cultures for *Candida albicans* who responded to treatment.
Conclusion: Calculating significant sensitivity for both detection methods was not feasible due to a low number of probable/possible cases. Nevertheless, Ag detection showed a higher correlation with suspicion of *Candida* infection, but it should be used in unison to other techniques for detection of invasive *Candida* infections.

R2062

The father of modern medicine: the first research of the physical factor of tetanus

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Hippocrates in Ko-island (460–370 BC) was the one who changed the ancient medicine in the scientific direction. After extended research, the father of modern medicine proposed the replacement of dark and religious theories about diseases with the direct clinical observations and logical relation between reason and result. Our purpose is to describe the knowledge of Hippocrates about tetanus.
Material: Hippocrates – in the work ''About air, water and places'' – gave the advice of sufficient washing of hands in order to avoid contaminations around birth. He realized the big danger of open wounds because of the ''contaminating'' air and he first described a disease with territory results, the tetanus. In his work ''Aphorism'' he writes ''…if there are spasms in case of trauma the prognosis is fatal …all patients with tetanus die in four days''. He describes the clinical signs with the following words ''in case of tetanus, the patient is not able to open his mouth, the eyes are full of tears, and the lower limbs are not possible to come in contact''.
Conclusion: What Hippocrates said about tetanus remain valid for 23 centuries, up to 1884 when Arthur Nicolaier (1862–1945) described the clostridium responsible for tetanus after injecting experimental animals.

R2063

Aetiological role of *Chlamydia pneumoniae* in chronic adenotonsillitis

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Objectives: Palatine tonsils and nasopharyngeal tonsils (adenoids) are lymphoepithelial tissues located in oropharynx and nasopharynx attempt to defense mechanism against ingested or inhaled foreign pathogens and induced natural immunity. Infections caused by many bacteria, fungi and viruses in these tissues called adenotonsillitis attending with clinical symptoms

like sore throats, chronic pharyngitis and generalized diffuse hypertrophy. However, identification of pathogens is not easy from swab samples though clinical symptoms seems to occur bacterial infections. Consequently, ineffective antibacterial therapy caused chronic infection and surgical procedure is performed. *Chlamydia pneumoniae*; is an intracellular microorganism cause to many acute, chronic and persistant respiratory tract infections like pharyngitis, tonsillitis, bronchitis and pneumonia. Lately, is claimed, palatine tonsils and nasopharyngeal tonsils can be act as a reservoir for *Chlamydia pneumoniae* so persistant upper respiratory infections and diffüse hypertrophy are seen frequently and also the reason of chronic adenotonsillitis. In this study we aimed to investigate *Chlamydia pneumoniae* in lymphoepithelial tissues and search the aetiology of chronic adenotonsillitis in order to avoid surgical process.

Methods: This study, includes 51 patients, performed tonsillectomy or adenoidectomy because of chronic adenotonsillitis were included to this study. Swab samples were taken before operation and one of the tonsil or adenoid tissue samples were placed into 2SP Chlamydia-transport medium and sent to the microbiology laboratory for PCR detection. The other tissue sample was sent to pathology laboratory in %10 formalin solution for immunohistochemistry (IHC) method.

Results: *Chlamydia pneumoniae* DNA was positive in 2 adenoid tissue and in 2 swab samples with PCR method though patients were different. However, *C. pneumoniae* was confirmed only in 1 adenoid tissue by IHC method as literatures claim the adverse.

Conclusion: As a result, we need more studies to suggest the effective antibacterial therapy in chronic adenotonsillitis due to *C. pneumoniae* instead of surgical procedure and to investigate the correlation between the two laboratory methods.

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Diagnostic/laboratory methods (other than molecular)

R2064

Microbiological findings in patients with chronic prostatitis using Meares-Stamey method

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Microbiological diagnosis of chronic prostatitis is still an important clinical problem. We evaluate 32 patients (pts) with chonic prostatitic symptoms using segmented urinalysis culture according to Meares–Stamey protocol (4 samples; first voided urine U1, midstream urine U2, expressed prostatic secretion EPS and urines after prostatic massage U3). 12 pts (27%) have a negative culture for all urines specimens. We looked for all wellknown uropathogens and more unusual ones as *Chlamydia trachomatis*, *Mycoplasma*, *Ureaplasma* using PCR methods. 20 pts (63%) had at least a positive urethral culture. 10 pts have only a significant EPS positive culture and 3 pts showed a 10 fold greater for U3 and/or EPS cultures than for U2. 7 pts have all urines samples positive with more than 10^3 CFU/ml, which means non-causative organisms or in some cases indeterminate, based upon patient's clinical status, microorganism, quantitative culture, bacteriological criteria…. The recovered uropathogens were: *Enterococcus* (3), *Haemophilus parainfluenzae* (2), *Streptococcus agalactiae* (2), *Escherichia coli* (2), *Klebsiella pneumoniae* (1), *Prevotella* spp. (1) *Corynebacterium* spp (1), *Staphylococcus coagulase* negative (1). The others putative agents for the 7 pts with all positive specimens were; *E. coli* (3), *Citrobacter koseri* (1), *K. pneumomiae* (1) non-typable *Streptococci* (2). In 10 cases EPS was the only positive sample obtain. About the 13 cases diagnosed, 11 micro-organisms were recognized uropathogens, the *Corynebacteria* and the *Prevotella* were recovered from EPS with a count of 10^4–10^5 CFU/ml. The results showed that performed the cost effective and time consuming 4 glasses test allowed the laboratory to bring microbiological evidence of chronic bacterial prostatitis for 13 pts, 65 % of the positive samples.

R2065

Proficiency testing in Iranian microbiology laboratories: survey results of 2121 laboratories

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Objectives: The aim of this study was to evaluate proficiency testing among 2121 microbiology laboratories in Iran.

Methods: In January 2005 16th run of proficiency testing carried out among 2121 microbiology laboratories located in all provinces in Iran. Microbiology laboratories divided in two groups: Hospital microbiology laboratories (631 labs) and non-hospital laboratories (1490 labs). These laboratories include all governmental and private sector microbiology laboratories. Three unknown microorganism including *Serratia marcescens* (WHO approved strain) *Pseudomonas aeruginosa* (ATCC27853) and *Enteroccus faecalis* (ATCC29212) were chosen. *Serratia marcescens* and *P. aeruginosa* were sent to hospitals microbiology laboratories. *S. marcescens* and *Enterococcus faecalis* to non-hospital microbiology laboratories. We asked all laboratories for identification unknown microorganism and susceptible testing of *S. marcescens*. We also asked laboratories to return their answers within 2 weeks receipt of samples. Scoring of answers determined according to WHO external proficiency testing criteria. The maximum score for identification of each organism was 3 score and 5 score for susceptibility testing.

Results: Of 2121 laboratories we received answer from 1482 (69.8%) laboratories, 639 (31.2%) laboratories did not participated in this survey or sent their answer after deadline. The mean score of point for identification *S. marcescens* in hospital laboratories was 1.037(SD ± 0.78040) and 2.13SD (SD ± 0.75707) for *P. aeruginosa*. The main score of point for identification *S. marcescens* in non-hospitals labs was 1.045 (0.60115) and 1.70(SD ± 0.83304) for *Enterococcus faecalis*. The mean score of points for susceptibility testing of S. marcescens in hospital labs was 3.85 (SD ± 1.46494) and 3.79 (SD ± 1.53595) in non-hospital microbiology labs. The results of this survey reveals that there are many difficulties for identification of microorganisms such as *S. marcescens* and *E .faecalis*. In total the results of susceptibility testing were relatively satisfied.

Conclusion: The results of this survey showed that unfortunately the majority of microbiology laboratories unable to identify ordinary microorganism at species levels. It is need for education and providing culture media and regent for our laboratories.

R2066

The innovation of the STAPHYtest 16 identification kit produced by PLIVA-Lachema Diagnostika s.r.o

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On the basis of requirements of the National Reference Laboratory for Staphylococci (NRLS), National Institute for Public Health, the innovation of the STAPHYtest 16 commercial identification kit was performed. The innovation study was based on replacement of tests for novobiocin susceptibility (GLN) and pyrrolidonylarylamidase activity (PYR), two tests in the old STAPHYtest 16 having low discrimination power. The innovation started at Czech Collection of Microorganisms (CCM) in cooperation with PLIVA-Lachema Diagnostika s.r.o. by chosen media testing. The tests for utilization of cellobiose, galactose and lactose were concerned, and from these three tests, the tests for the detection of galactose and lactose were chosen. The novel STAPHYtest 16 to be innovated using both the clinical and reference staphylococcal and micrococcal strains were tested in the National Institute for Public Health. The result of a correct identification to the species level was 95%. The excluded tests for detection of pyrrolidonylarylamidase and susceptibility to novobiocin can still be performed using the detection strip PYRAtest and the detection disc NOVOBIOCIN which are offered by PLIVA-Lachema Diagnostika as well.

R2067

Possibilities in the use of BacT/Alert SA bottles for diagnosing vascular catheter infections

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Objectives: Infection caused by the insertion of vascular catheters is responsible for a high percentage of nosocomial infections. Catheter infections cause increased morbidity and mortality along with the raising cost of treatment. The new method is based on the transport of bacteria from catheter surface into solution and cultivation of this solution Bact/Alet system. Bacteria quantity present on the catheter is determined on the base of isolated agent and detection time (TTD). The TTD of positive vials is proportional to quantity of inoculated bacteria and their metabolic activity.
Methods: 4 ml of sterile physiological solution was added to a test-tube containing sample of vascular catheter and the tube was agitated for 2 minutes. The whole volume of the solution from the tube with vascular catheter was inoculated into the bottle BacT/Alert SA. Bacteria quantity was determined by substitution TTD to the equation of TTD dependence on the number of inoculated CFU for separate groups of species.
Results: In total 187 vascular catheters were elaborated. After seven days of cultivation 10 catheters were negative (5.34%). At the evaluating criterion 103 and more detected bacteria, 18 catheters were positive (9.62%). With respect to the correlation with clinical conditions 2 false positive results (1.06%) and 12 false negative results (6.41%) were found. On the basis of clinical conditions 28 (13.46%) cases of catheter sepsis in the set of 187 examined patients were diagnosed. Agreement of quantitative catheter examination with clinical finding occurred in 179 cases (92.7%). Coincidence of positive findings on the catheter with positive evaluation of clinical condition was seen in 16 cases (57.14%). The most frequent detected pathogens were coagulase negative staphylococci.
Conclusion: The method is applicable in clinical practice and is able to detect most frequent agents of catheter infections. Number of bacteria caught on the surface of a catheter is a simple an easy evaluation criteria, but for the correct interpretation it demands correlation with clinical conditions of the patient.

R2068

Determination of metabolic activity and detection of microbial cells by electrophysical analysis

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Objectives and methods: Measurement of electrophysical properties of microbial cells may be used for creation of new kind of biosensor systems. There were investigated the microbial cells of some strains with preliminary metabolic enzyme system of toxic compounds. These processes conduce to the redistribution of the charges in the microbial cells and may be register by electro-optical (EO) methods. The electro-optical analyzer (ELUS EO), which has been developed at the State Research Center for Applied Microbiology, Obolensk, Russia, was used as the basic instrument for electro-optical measurements EO analysis is based on the recording of changes in optical characteristics of cell suspensions under the orienting effect of an electric field. Redistribution of the charges in the microbial cells may be used for determination of substrates of enzyme reaction and finally for determination of substrate concentration.
Results: Same approach may be used for studies of microbial cells and some biological agents (antibodies and phages) binding. Since the AC electrokinetic effects depend on dielectric properties of bioparticles, their composition, morphology, phenotype, the medium, and the frequency of applied electrical field, the EO properties of cell suspensions were used for discrimination of bacteria before and after selective binding with antibodies. There were shown the determination of the presence of particular bacteria within a mixed sample may be achieved by selection and matching of antibodies specific to individual bacterium types and by comparing spectra of bacterium in the presence and in the absence of specific antibody. Same principles were used for investigations of bacteria – phage interaction. Integration of the electro-optical approach with a bioselective binding agents has the following advantages: 1) bacteria from biological samples need not be purified and 2) exogenous substrates and mediators are not required for detection.
Conclusions: So electro-optical analysis of cell suspensions provide new opportunities for creation of new biosensor methods in biotechnology, environmental control and medicine.This work was supported ISTC grant 615 and 3170PDG.

R2069

Nocardia sp. in cystic fibrosis patients in a Greek tertiary hospital

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Objectives: 1) To determine the incidence of *Nocardia* sp.in Cystic Fibrosis (CF) patients, 2) to identify them to the species level and 3) to investigate their susceptibility to common used antibiotics.
Methods: During an 8 years period, multiple sputum specimens of 68 (CF) adult patients were cultured in appropriate

media. The identification was performed on the basis of adenine, casein, xanthine, hypoxanthine, tyrosine utilization, urea hydrolysis and antimicrobial susceptibility patterns. The susceptibility testing was obtained by disk diffusion and microdilution methods according to CLSI/NCCLS guidelines and by epsilometer test (E-test) (AB BIODISK, Solna, Sweden) according to the manufacture's recommendations. Bita-Lactamase production was detected by nitrocefin disks (Mast Diagnostics, UK).
Results: In a period of 8 years 7/68 (10.29%) CF patients had *Nocardia* sp. in their sputa, without any significant change in their clinical status, radiology findings and pulmonary function. The following species were isolated: *N. asteroids* (I, II, VI) (4/7), *N. farcinica* (1/7), *N. otitidiscaviarum* (1/7), *N. transvalensis* complex (1/7). The above isolates were found resistant to: ampicillin (100%), ciprofloxacin, clarithromycin, erythromycin (71.14% each), cefamandole (42.86%), imipenem, tobramycin, gentamicin (28.57% each), amikacin, amoxicillin-clavulanic acid, cefepime, ceftriaxone, (14.28% each). All isolates were sensitive to linezolid, trimethoprim-sulfamethoxazole (TMP-SMX), cefotaxime and doxycycline.
Conclusions: 1) The incidence of *Nocardia* sp. in our Cystic Fibrosis (CF) patients is remarkable (10.29%). 2) The predominant isolated *Nocardia* sp. was: *N. asteroides* (I, II, VI). 3). All *Nocardia* sp have to be tested for their susceptibility profile, since routinely used antibiotics in CF patients such as Ciprofloxacin or Imipenem may be inactive.

R2070

New rapid flow-through immunofiltration assay system for rapid detection of *Trichomonas vaginalis* in ThinPrep specimens

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Objectives: The ThinPrep Pap Test (TP) is preferred over the traditional Papanicolaou (Pap) smear, and the liquid-based cytology is being increasingly employed for detection of the sexually transmitted HPV. Such fixative renders the number one, non-viral sexually transmitted parasite, *Trichomonas vaginalis*, undetectable. Given the association of trichomonosis with cervical cancer, we wanted to develop a rapid flow-through (Filtration) assay system to detect *T. vaginalis* in TP specimens.
Methods: Spleen lymphocytes of mice immunized with trichomonads after treatment with TP fixative were fused with NS1 myeloma cells to generate hybridomas producing mAbs. Supernatant of hybridomas were tested for reactivity with organisms exposed to TP fixative coating microtitre wells. Immunoreactive mAbs of single cell-cloned hybridomas were tested for detection of trichomonad antigen in ThinPrep specimens under optimized conditions. Finally, mAbs were examined using a Filtration assay system on disposable porous filter-membranes with bound antigen. Finally, TP specimens from patients with trichomonosis and uninfected individuals were tested for reactivity by mAbs using the Filtration assay.
Results: The mAbs were identified by standard colorimetric ELISA at 405 nm that immunoreacted with trichomonads exposed to TP liquid fixative coated onto microtitre wells. Then, mAbs detected trichomonad antigen immobilized onto membranes in a Filtration assay system, and this was done by both colorimetric ELISA and emission chemiluminescence (ECL). The Filtration ECL assay was very sensitive and detected antigen from as little as 10 organisms immobilized on membranes. Filtration ECL yielded rapid, accurate results with fewer fixed organisms within 30 sec compared to colorimetric ELISA. The mAbs reacted with organisms added to the ThinPrep fixative and with antigen in samples of patients with trichomonosis. No immuno-crossreactivity was ever detected with the mAbs with other trichomonad species, in the absence of *T. vaginalis* antigen, and in samples with materials from uninfected individuals.
Conclusion: A rapid flow-through immunofiltration assay system was developed for detecting *T. vaginalis* antigen. Individual mAbs readily detect in a sensitive and specific fashion trichomonad antigen in TP specimens. This demonstrates the ability to now detect this sexually transmitted infection in the liquid-based ThinPrep specimens.

R2071

Serological markers for cytomegalovirus infection in newborns

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Objectives: Cytomegalovirus (CMV) infection in newborns is often a cause of clinical illness. The purpose of this study was to investigate the diagnostic value of different serological markers for diagnosis of acute CMV infection in newborns.
Methods: During study period 2004–2005 years 34 mother – child pairs and 8 newborns were tester for presence of anti-CMV IgG, IgM and IgG avidity by commercially available enzyme immunoassay (''Diagnostic systems'', Russia). To distinguish between maternal and neonate immune response, IgG to individual CMV proteins (pp150, pp52, pp38, pp28, gB) were analysed. The children were monitored for at least 3 months.
Results: Acute CMV infection were confirmed in 7 out of 42 children by laboratory research and clinical signs. In newborns with proven CMV infection 28.6% was anti-CMV IgM positive, 33.3% had low-avidity IgG and 57.1% had significant IgG elevation during the observation period. Five out of 7 (71.4%) had IgG profile different from maternal. Presence of anti pp52 in newborn and absence in their mother samples was the reason for difference between the mother's and child's IgG profiles in all cases.
Conclusions: Paired blood samples from both the mother and infant are necessary for reliable serological analysis. Transferred maternal and neonate-synthesized CMV specific IgG can be differentiated by detection of antibodies to individual CMV proteins. These results indicate that raised IgG, detection of anti-CMV IgM, in particular low avidity immunoglobulin, and especially different from maternal IgG profiles are useful markers for the identification of congenital CMV infection.

R2072

Extremely elevated C-reactive protein

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Objective: Measurements of C-reactive protein (CRP) are commonly used in clinic to differentiate inflammatory from non-inflammatory conditions. We sought to delineate the clinical significance of extremely elevated CRP levels.
Methods: From the 2004 lab files of a university hospital we extracted all patients with at least one CRP value >500 mg/L. We reviewed the electronic records and assessed the clinical diagnosis of the inflammatory event, comorbid conditions and mortality. Infections were differentiated from non-infectious inflammatory conditions.
Results: CRP surpassed 500 mg/L in 212 samples from 130 patients, representing 0.065% of all samples. Median age was 62 years (range, 2–94). In 80% of patients, maximal CRP was

reached within the first week of admission. A wide variety of infections caused the CRP elevation in 114 of the 130 patients (88%), with the lower respiratory tract and the abdomen as prevailing foci. In the minority without readily identifiable infection, sterile necrosis, ischaemia or heavy tumour burden were responsible for the excessive acute-phase response. In 88 of 114 (77%) of patients with infection, a causative pathogen could be identified. Apart from 5 mycotic infections, all were bacterial. Blood cultures were positive in 44 of 109 patients (40%), demonstrating gram-negative bacteraemia in 22, gram-positive bacteraemia in 20 and candidaemia in 2. Overall, 47 of the 130 patients (36%) died, mostly within the first month. Mortality was especially high in patients with active cancer (28/46, 61%) and with neutropaenia (11/15, 73%). Of 38 patients without debilitating conditions, 5 died (13%).

Conclusion: A wide variety of mostly bacterial infections, that are generally readily identified, accounts for the majority of cases of extremely elevated CRP. Mortality is high, especially in patients with active malignancies, underscoring the importance of prompt identification and control of the source of inflammation.

R2073

The added value of using recombinant-versus purified membrane preparation-based antigens in sero-diagnostics of *Mycoplasma pneumoniae*

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Objectives: *Mycoplasma pneumoniae* (MP) is an etiological agent responsible for 10–30% of community-acquired pneumonia cases. MP may be detected in all parts of the respiratory system, and its effects are well recognized and documented. In respect to diagnosis and treatment, the most prominent structural feature of MP is the lack of a cell wall. It has been shown that surface-exposed polypeptides elicit immunogenic response, in particular those that are involved in the attachment organelle of MP. This attachment organelle is composed of a complex of polypeptides, in which P1 Cytadhesin Protein has a major role. The current SeroMP test of Savyon uses a purified fraction of MP membrane proteins that contains the P1 as the major antigenic factor. In an effort to produce a recombinant antigen-based assay we evaluated the added value of using the recombinant methodology.

Methods: Various candidates for the recombinant antigenic mixture were defined and their coding sequences were amplified, cloned in an expression vector, and the His-tagged polypeptides were affinity-purified using Ni columns. The effects of different combinations of recombinant proteins were evaluated by testing characterized sera in ELISA utilizing detection systems specific to IgG, IgM and IgA MP antibodies.

Results: Applying various combinations of recombinant antigens revealed a lower prevalence in healthy population. An apparent phenomenon was the clarification of those sera which were previously determined as borderline, and defining them as negatives. In sick population the amount of borderline samples was substantially reduced as well, by defining them as negatives or positives. This partition increased the amount of positives, and compensated for any possible decrease in sensitivity, which might have been expected due to increasing specificity. This led to a better separation between the sick and non-sick patient's results. The increase in specificity was in particular noticeable when binding to potential cross-reacting antibodies, for example anti-EBV, was completely abolished. These findings were apparent in IgG, IgM and IgA tests.

Conclusions: This work points out specific improvements by using recombinant antigens. Moreover, the output parameters mentioned-above served as a tool for choosing the optimal combination of recombinant polypeptides to be used as the antigen in the test. These observations enable the serology test to provide a better tool for the correct diagnosis of MP.

R2074

Evaluation of four chromogenic media for surveillance cultures of methicillin-resistant *Staphylococcus aureus*

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Screening of MRSA carriage in hospitalized patients is part of the measures to control MRSA. Thus, rapid and reliable laboratory procedures for detection of MRSA is of utmost importance. New chromogenic media have been recently developed for this purpose. The aim of the study was to evaluate the performance of 4 chromogenic media, MRSA ID (bioMérieux, France), CHROMagar MRSA (Chromagar, France), ORSAB (Oxoid, UK), and MRSA Select (Biorad, France). 466 samples were homogenized in NaCl 0.9% before inoculation onto the media (primo-cultures) and in an enrichment broth. Most were swabs from nose, throat, perineum and wounds. In primo-cultures, 93 samples showed the presence of MRSA on at least one medium. Results for each medium are presented in the following order: MRSA ID, CHROMagar MRSA, ORSAB and MRSA Select. After 16–18 h and 42 h of incubation, sensitivities were 56%, 65%, 52%, 71% and 90%, 82%, 73%, 88%, respectively. The colour of MRSA colonies was not characteristic for 2 samples on MRSA ID and ORSAB at 16–18 h; and for 7 samples on ORSAB at 42 h. The specificity of each medium to identify MRSA by colony color was 100%, 99%, 99%, 100% after 16–18 h of incubation and 98%, 97%, 98%, 98% after 42 h, respectively. The performance was evaluated by calculating the mean time to report a positive result, and was 1.65 d, 1.72 d, 2.31 d and 1.35 d, respectively. The use of an enrichment broth increased the detection of MRSA by 16 to 24% for all media. In conclusion, in order to detect most MRSA, all media should be incubated for at least 42 h and/or and enrichment broth should be used. These chromogenic media improve the procedure of MRSA surveillance cultures and so contribute to reduce hospitalization costs.

R2075

High blood levels of procalcitonin identify critically ill patients at high risk of mortality

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Objectives: Serum procalcitonin (PCT) has been shown to be a reliable sepsis marker and elevation of PCT blood levels occurs early in the course of a bacterial infection. We investigated blood levels of PCT to evaluate the association between high levels of Procalcitonin and mortality in 1). The intensive care unit, 2) In a 30 day follow-up period of time basis and 3) In a 90 day follow-up period of time.

Methods: We enrolled all patients admitted to a multidisciplinary Intensive Care Unit (ICU) in 2002. Daily PCT measurements, were carried out during the study period. The clinical end point was all cause mortality during the stay in the intensive care unit, at 30 days after admission to the ICU and at 90 days after admission to the ICU.

Results: PCT measurements evaluated in 472 critically ill patients. Overall ICU mortality of critically ill patients in the one-year study was 19.1%. Mortalities at 90 days ranged between 11.6% and 59.6% according to maximum PCT level obtained (PCT max) in the ICU. For details of mortality and PCT levels, see table 1.

PCT-max level (ng/ml)	Intensive Care Unit mortality	30-day mortality	90-day mortality	Patients in this PCT-max interval
< 1.0	4.7%	10.1%	11.6%	129
1.0 - 5.0	10.7%	26.4%	35.7%	140
5.0 - 20.0	27.6%	37.8%	41.8%	98
20.0 - 50.0	36.5%	46.2%	59.6%	52
50.0 -1000.0	41.5%	47.2%	58.5%	53
All PCT-max levels	19.1%	28.8%	35.6%	472

Conclusion: Strikingly high mortality risk was seen in patients with a high PCT max. A PCT max of 1.0 ng/ml seemed to divide high and low mortality risk in these ICU patients. PCT measurements may be a useful tool in fast stratification of critically ill patients in high and low risk patients.

R2076

Galactomannan and mannan antigens and anti-mannan antibodies detection for the diagnosis of invasive aspergillosis and candidiasis in liver transplant recipients

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Objectives: Fungal infections in liver transplanted patients may decrease the survival rate at 120 days after transplant from 90% to 65%. Hence it is important a rapid diagnosis to allow an early antifungal therapy. We studied the utility of galactomannan (GM), mannan (MAN) and antiMAN antibodies detection for the rapid diagnosis of invasive aspergillosis (IA) and candidiasis in liver transplant recipients.
Methods: Four hundred and sixty-five serum samples were collected from 46 liver transplanted patients, during the post-transplant hospitalization in the intensive and sub-intensive care unit. After a collection period of 13 months, samples were analysed with the *Platelia Aspergillus*, *Platelia Candida* Ag and *Platelia Candida* AB tests (BIO-RAD). Positivity cut-off for GM detection was a GM index (sample OD/control cut-off OD) ≥0.7. For MAN detection the test was positive if MAN concentration was ≥0.5 ng/ml, while for antiMAN assay the test was positive if antibodies content was ≥10 arbitrary units (as defined by the manufacturer).
Aspergillus results: No cases of IA or Aspergillus colonization were found in the 46 pts. However 3 of them tested positive for GM and they were considered as false positive cases.
Candida **results:** MAN and/or antiMAN test were positive in 20 of 46 pts. Within the first 100 days post transplant 98.5% of MAN and 100% of antiMAN test were positives, vs. 93% of MAN and 84% of antiMAN negative results.*Candida* infections: Five pts. had probable candidiasis (2 with positive cultures from SwanGanz catheter and 3 with *Candida* colonisation index ≥0.5). Three of 5 infected patients tested positive for MAN and/or antiMAN, while 17 of 41 non-infected pts. were positives for antigens and/or antibodies assays. Cumulative sensitivity and specificity of the 2 *Candida* tests were 60% and 58.5%.
***Candida* colonisation:** Twenty-six of 46 patients (56.5%) had *Candida* colonization (included the 5 pts. with probable candidiasis) and 14 of 26 colonized patients (53.8%) were positive for MAN and/or antiMAN test, vs. 6 of 20 between the non-colonized patients (30%). Moreover the time of first colonization and first positivity of the two Candida tests were almost all within 20 days after transplantation.
Conclusions: Due to the absence of IA, only the specificity (93%) was valuable for the GM detection. As for *Candida* assays, MAN and antiMAN tests performed poorly and *Candida* colonization also seems actively influence the detection of *Candida* antigens and antibodies.

R2077

Cultural findings of *Lactobacillus* in pregnant women with and without bacterial vaginosis

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Objectives: To determine the presence of lactobacillus in vaginal swabs of pregnant women with and without bacterial vaginosis (BV) and their mode of metabolism.
Method: The study involved 137 pregnant women at the age from 18–45 years out of which 54 pregnant women had the BV diagnosis and 83 were without BV. BV diagnosis was given based on Amsel's clinical criteria and Nugent's score system. Isolation and identification of lactobacillus was done on MRS (deMan, Rogosa, Sharpe) plate, produced by Biolife, with the period of incubation of 72 hours anaerobe and 48 hours aerobe on separate Petri dishes. Characteristic small colonies were analysed on catalase test and microscopic preparation. Gram-positive bacillus catalase negative were identified as lactobacillus.
Results: Obtained results showed that only 24 (44.5%) pregnant women with BV had lactobacillus in cultural finding, wears 78 (93.9%) pregnant women without BV. Out of total 54 pregnant women with BV 4 (7.4%) had lactobacillus that grows up aerobe and 6 (11.1%) had facultative anaerobe lactobacillus, while anaerobe lactobacillus were dominant and they were found in 14 (25.9%) pregnant women with BV. In pregnant women with BV facultative anaerobe lactobacillus was dominant and they were found in 36 (43.4%) cases together with aerobe lactobacillus that were present in 25 (30.1%) pregnant women, while anaerobe lactobacillus were found in 17 (20.5%) women.
Conclusion: On the basis of χ^2 tests it was proved that cultural finding of lactobacillus has been frequently isolated from vaginal swab of pregnant women without BV with statistical significance ($\chi^2 = 50.94$ $p < 0.01$) where the least presence of anaerobe lactobacillus was found.

R2078

Evaluations of differents selective mediums to isolate *Helicobacter pylori*

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Objective: Development of a new medium to culture and isolate *Helicobacter pylori* from faeces, could enable to eliminate in the future the gastric biopsy as the only way to obtain strains. The aim of the present study was to optimize mediums of culture to isolate *H. pylori* when it is present with other intestinal tract bacteria.

Methods: Twenty-nine selective culture mediums were evaluated in several and consecutive assays. They were composed of 11 nutrient and component combinations; as well as by 14 antibiotic combinations (Amphotericin B, Cefalotin, Cefoperazona, Cefsulodin, Colistin, Polymixin B, Sulfamethoxazol, Trimethoprim and/or Vancomycin). We cultured between 2 and 13 *H. pylori* strains and 5 intestinal tract bacteria species (Et) isolated from clinical samples. Pylori agar (PA) was used as control of the selective medium, and Columbia agar with 5% sheep blood (both bioMérieux, Lyon, France) and the same nutrient combinations without antibiotics, were used as control of non-selective plates. Pure *H. pylori* and mixed (*H. pylori* + Et) suspensions were prepared, cultured and incubated at 37ordm;C on microaerophilia during 4 and 7 days. *H. pylori* growth was evaluated as pure and mixed suspensions, tacking into account the number of colony-forming units (CFU) on each plate and the colony visualization.
Results: *H. pylori* was isolated on 3 selective mediums, in spite of there being 104 CFU Et/ml, where the number and visualization of *H. pylori* colonies were better with regard to the obtained on PA. The most selective antibiotic combination was Amphotericin B, Cefsulodin, Polymixin B, Trimethoprim and Vancomycin. The number and size of *H. pylori* colonies decreased in the presence of other bacteria species.
Conclusions: The new culture mediums evaluated could be useful to isolate *H. pylori* from faeces, because increase number and visualization of *H. pylori* colonies with regard to commercial medium used to isolate *H. pylori* from gastric biopsies.

R2079

A recombinant line-blot avidity assay for the detection of Epstein–Barr virus infection

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Epstein–Barr Virus (EBV) is responsible to primary and secondary infections frequently found in clinical routine. Acute mononucleosis associated to EBV can be determined with specific IgM detection but there are many situations with doubtful clinical interpretation. We present the results of a line-blot immunoassay for the detection of EBV IgG antibodies including his avidity as marker of recent infection. We have studied 50 sera from patients with primary infection and past or secondary infection. The serological status was determined by immunoassay detecting EBV IgM, VCA IgG and EBNA IgG (Liaison, Diasorin). We have evaluated a line-immunoassay based in EBV recombinant antigens composed by EBNA-1 antigen, VCA antigens (p18 and p23) and EA antigens (p138 and p54) (Mikrogen). We have detected specific IgG and the avidity was determined doubly using or not a wash buffer with 8 M urea. Twenty-four sera corresponding to mononucleosis associated to primary infection. Even if we use anti-IgG conjugate we detected reactivity in 20 sera (83.3%). The more frequent reactive bands was VCA p23 (15), EA p54 (14) and EA p138 (13). However these bands were erased with 8 M urea in 10, 11 and 11 sera respectively. There is not reactivity to EBNA-1 antigen in these samples. Twenty-six sera corresponding to past o reactivated EBV infection. In these samples was critical the presence or not of EBNA-1 antibodies. Not presence is associated to subacute infection and is demonstrated by his low avidity VCA and EA antibodies. When there are EBNA antibodies 20/22 patients presents VCA p23 and EBNA-1 with high avidity. Two patients was missed EBNA-1 and was related with subacute process. The presence of high level of EBNA-1 antibodies (>600U) is strongly correlated with high avidity reactivity against EA antigen (6/7) and reactivation process. We concluded that this line-blot immunoassay can be useful for to resolve subacute or reactivated infections studying the reactivity to EBNA and/or EA antigens and improves the EBV serological diagnostic.

R2080

Rapid diagnosis of pneumococcal meningitis using Binax NOW Urinary Antigen Kit

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Objectives: The Binax NOW *Streptococcus pneumoniae* urinary antigen assay (Binax NOW test) was developed to detect *S. pneumoniae* antigens in urine samples in patients with suspected pneumococcal pneumonia. Sensitivity and specifity are 65.9–70.4% and 89.7–100% respectively in adults with community-acquired pneumonia. Maria A. Marcos used Binax NOW test for the diagnosis of pneumococcal meningitis in CSF samples and urine. Sensitivity and specificity were 100% in specimen from eight patients. Cross-reactivity of rapid antigen tests are well known for *S. pneumoniae* and *Streptococcus* spp. of the oralis Group, i.e. *S. oralis* and *S. mitis*. Cross-reativity results from the fact that all three species share the C-polysaccharide, which is detected by Binax NOW. However, bacterial meningitis with oral streptoccoci are rare and usually iatrogenic.
Methods: We used the Binax Now test manufactured by Binax, Maine 04103 USA to test CSF-samples from 77 patients with suspected bacterial meningitis: 14 CSF-samples with Gram-positive cocci in the Gram-stain, 4 with Gram-negative rods (3) or Gram-negative cocci (1), 7 CSF-samples with negative direct stain but positive CSF culture. In addition, 52 CSF-samples with negative direct stain and negative CSF culture were tested.
Results: In 6 out of 14 CSF-samples with Gram-positive cocci in the direct stain culture grew *S. pneumoniae*, 4 grew coagulase negative *Staph*., 1 *S. haemolyticus* and 1 *S. milleri* and anaerobic flora, 1 *Enterococci*, 1 *S. oralis/mitis*. Binax Now test was positive for all CSF-samples growing *S. pneumoniae* and weakly positive in one sample with *S. milleri*. In 4 direct stain positive CSF (3 rods, 1 Gram-negative cocci) the Binax Now test was negative. In 7 Gram-stain negative CSF-samples cultures grew other species (5 coagulase negative *Staph*., 1 *Enterobacter cloacae*, 1 *Propioni bacterium*) and Binax Now test was negative. It was negative too in the 52 CSF-samples with negative direct stain and negative culture. (ppV 86 %, npV 100 %). The patient with the false positive Binax Now test had a brain abscess (S.milleri and anaerobic flora).
Conclusion: Binax NOW antigen test on CSF revealed positive results in all culture positive pneumococcal meningitis. There was no false negative result and only one false positive (weak) reaction. In patients with suspected bacterial meningitis Binax NOW test seems to be a reliable, rapid diagnostic tool to identify patients with pneumococcal meningitis.

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Methods for antibacterial susceptibility testing

R2081

A new *in vitro* method for testing antimicrobial properties of volatile phytotherapeutic compounds

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Objective: To develop a new system for antimicrobial susceptibility testing of phytotherapeutic compounds containing volatile essential oils.
Methods: A commercially available phytotherapeutic preparation of nasturtium (N) and horseradish (H) (Angocin® Anti-Infekt N; Repha, Langenhagen, Germany) was used as test drug. The active ingredients (gently dried and ground N and H, in a ratio of 2.5:1) were applied to the covers of Columbia blood agar plates and mixed with sterile H_2O. A variety of different bacterial species including *Haemophilus influenzae*, *Moraxella catarrhalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, methicillin-susceptible and resistant *Staphylococcus aureus* [MSSA., MRSA] were tested (20 isolates each). The test organisms were plated onto the blood agar plates and placed above the test substance. The plates were sealed with adhesive tape and incubated for 24–92 h at 37°C. Following incubation, colony forming units (CFUs) were counted and the MHK90 was determined for each bacterial species.
Results: The new system designed for testing volatile compounds/essential oils produced reproducible and reliable results. Relevant antimicrobial activity of the test drug was found against *H. influenzae* (MHK90 50 mg N/20 mg H), *M. catarrhalis* (100 mg/40 mg), *E. coli* (400 mg/160 mg), *P. aeruginosa* (400 mg/160 mg), MSSA (400 mg/160 mg), MRSA (400 mg/160 mg), and *S. pyogenes* (400 mg/160 mg).
Conclusions: A newly developed *in vitro* technique for antimicrobial susceptibility testing of volatile compounds produced highly reproducible results. A phytotherapeutic compound tested with this method showed broad antibacterial activities against clinically relevant pathogens.

R2082

Effect of beta-lactamic antibiotic on the electrophysical characteristics of *Escherichia coli*

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Objectives: Study of the adaptation of microbes to antibiotic action is an important problem that is of theoretical and applied significance. Ampicillin is a beta-lactamic antibiotic that is produced by aminophenylacetic acid acetylation of 6-aminopenicillanic acid. It is a broad-spectrum agent that is active specifically toward *E. coli*. The aim of this work was to study the effect of ampicillin on the electro-optical (EO) parameters of cell suspensions of *Escherichia coli* strains differing in there resistance to this antibiotic. One can assume that changes in cell morphology and cell-wall disturbances in antibiotic-resistant microorganisms should lead to changes in their electrophysical characteristics. These changes are reflected in alterations in the electro-optical characteristics of cell suspensions, which are recorded during experiments using electric-field cell orientation. On the basis of these alterations, one can draw tentative conclusions about the presence or absence of resistance to a given antibiotic in the cells under study.
Methods: The orientational spectra (OS) of the cells were measured with an ELBIC EO analyzer at a wavelength of 670 nm.
Results: We examined the effect of ampicillin on the electrophysical characteristics of ampicillin-sensitive and ampicillin-resistant *Escherichia coli* cells. Substantial changes in the OS of suspensions of cells incubated with various ampicillin concentration took place only at the first five frequencies of the orienting electric field (10×1000 kHz) The maximal change in the magnitude of the electro-optical signal occurred at 50 μg/ml of ampicillin. The suspension-OS changes did not depend on the antibiotic-action period. Under the action of ampicillin, sensitive and resistant *E. coli* strains gave different EO effects. After ampicillin incubation of the sensitive strains, K-12 and XL-1, there occurred a considerable change in the EO-signal magnitude. Suspensions of the resistant strains, K-12 (pUC-18) and XL-1 (pHEN1), showed no changes in this EO parameters after ampicillin incubation.
Conclusion: Thus, the suspension-OS changes occurring under the effect of ampicillin may be used as a test for resistance to this antibiotic in the cells studied. The possibility is suggested of using electrophysical methods to study the mechanism of antibiotic action on bacterial cells, with a view to check antibiotic action of microorganisms.This work was supported by Russian Science Support Foundation and CRDF Y1-P-06-09.

R2083

Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical specimens: first report from Iran

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Objectives: The aim of this study was to determine incidence of erythromyvin-induced resistance to clindamycin in *Staphylococcus aureus* isolated from clinical specimens in Milad hospital of Tehran, Iran.
Methods: A total of 175 strains of *S. aureus* isolated from clinical specimens identified by conventional microbiological methods. To detect inducible clindamycin resistance a 15 mcg erythromycin and 2 mcg clindamycin were placed at distance of 15–20 mm on Muler Hinton agar, Disk diffusion methods was performed for other antibiotics including linozolid, vancomycin, mupirocin and co-trimoxazol as recombined by CLSI.
Results: Of 175 isolates of *S. aureus* 53 (35%) were resistant to methicillin (MRSA). All isolates were susceptible to linozolid, and vancomycin and 1.6% were resistant to mupirocin and 28.5% of isolates were resistant to co-trimoxazol. Of 175 isolates of *S. aureus* 17 (9.7%) isolates showed inducible cindamycin, resistance. Of 17 inducible clindamycin isolates of *S. aureus*, 11 isolates were methicllin resistant (MRSA) and 6 isolates methicillin susceptible (MSSA). 27 isolates were resistant both erythromycin and clindamycin (constitutve resistant) and 9 isolates were resistant to erythromycin and susceptible to clindamycin.
Conclusion: This study reveals that 35.7% of *S. aureus* isolates in our hospital were resistant to methicillin and 17 (9.7%) isolates had inducible clindamycinn resitanc. All isolates were susceptible to linozolid and vancomycin.

Diagnostics – New automated techniques

R2084

Comparison of identification and susceptibility testing in the MicroScan WalkAway and the VITEK 2 for nonfermenting Gram-negative bacilli

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Objectives: The MicroScan WalkAway 96 SI and the VITEK 2 are automated instruments for rapid organism identification and susceptibility testing. The purpose of this ongoing study is to compare the performance of identification (ID) and antibiotic susceptibility testing (AST) of nonfermenting gram-negative bacilli in both instruments.
Methods: 177 clinical isolates of nonfermenting bacilli were tested using the MicroScan WalkAway 96 SI with Synergies plus Neg Breakpoint Combo for ID and AST and the VITEK 2 with the GN card for ID and the AST-N021 for susceptibility testing.
Results: Identification results were concordant in 141 isolates (79.7%). Essential agreement of susceptibility testing results was defined as a MIC value within one doubling dilution of each other. This was observed for piperacillin in 86%, for piperacillin/tazobactam in 92.7%, for ceftazidime in 93.7%, for cefepime in 92.1%, for imipenem in 93.6%, for meropenem in 98.2%, for gentamicin in 98.9%, for tobramycin in 97.7%, for levofloxacin in 98.9% and for ciprofloxacin in 98.9% of the strains tested.
Conclusion: Good agreement between the results of AST with the two instruments was found for most antibiotics especially for the aminoglycosides gentamicin and tobramycin, for the fluorochinolones levofloxacin and ciprofloxacin and for meropenem. However, different results were found for identification in a substantial portion of nonfermenting gram-negative bacilli. In order to solve discrepant results susceptibility testing by standard microdilution methods and identification by molecular methods will be performed in our ongoing study.

Public health and community-acquired infections

R2085

Psoas muscle abscess and pyogenic arthritis due to *Streptococcus milleri* in an intravenous drug abuser

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Objectives: *Streptococci* of the milleri group are part of the normal flora of human mucous membranes. They can cause serious pyogenic infections. Our objective is to stress the fact that an abscess should be sought when *Streptococcus milleri* is isolated from the blood.
Case report: A 33-year-old man was admitted in our department because of fever and painful swelling of his right leg. He had a history of regular intravenous heroin use for at least three years and was positive for hepatitis C; he had a stomach operation due to ulcer; he was injured in the spine because of an accident. The patient had temperature 39.6°C, blood pressure 90/60 mmHg, pulse 140/min and respirations 22/min. The physical examination revealed a red, hot, painful swelling of the right groin and leg and mild tenderness over the right and left lower abdominal quadrants. Laboratory values showed erythrocyte sedimentation rate of 110 mm/h, haemoglobin 9.3 g/dl, haematocrit 27.6%, leucocytes 20340/mm^3 (granulocytes 78%), platelets 20000/mm^3, C-reactive protein 18.9 mg/dl, creatinin 3.2 mg/dl. The triplex echo revealed a deep venous thrombosis of the right leg, from the common femoral vein and below. Chest radiograph, brain and chest CT were normal. Echocardiogram was negative for endocarditis. Bone scan revealed chronic inflammation of O2–O5 lumbar vertebrae due to injury. CT of the abdomen showed large abscess of the right and especially the left psoas muscle. A percutaneous drainage of the left psoas muscle was performed under CT assistance. Treatment was started with penicillin G and gentamicin intravenous. The fourth hospitalization day the patient developed swelling, pain and functional incapacity of his right knee. The arthrocentesis yielded a yellow, turbid fluid with 20560 cells/mm3 (95% granulocytes). Cultures of blood, abscess and arthric fluid grew streptococcus milleri. He continued his intravenous antibiotic treatment for six weeks and oral antibiotics for a further six weeks and his clinical evolution was completely satisfactory.
Conclusion: Careful history taking and meticulous physical examination remain crucial for patients with a psoas abscess. CT is the most efficient diagnostic method. Management consists of early and effective drainage and long-term antibiotic treatment. Because of the close contact with iliac veins there is a risk for thromboembolic complications.

R2086

Research of bacterial contamination in dental unit waterlines

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Introduction: The water used in dental unit waterlines (DUWLs) acts as a coolant for the high-speed drills and as an irrigant during dental procedures. There are a number of possible sources of water supplied to DUWLs, including public main water supply connections or water tanks. DUWLs provides an ideal environment for microbial biofilm and proliferation primarily due to the high surface/ volume ratio in the tubing and the character of fluid dynamics in narrow, smooth-walled waterlines. Biofilms can harbour opportunistic pathogens such as *Legionella* sp., *Pseudomonas* sp., and therefore dentists, immunocompromised patients may be at risk. The aim of our study was to determine *Legionella*, *Pseudomonas*, Total Aerobic Heterotrophic Mezophilic Bacteria (TAHMB) contamination of DUWLs in private dental offices Istanbul city of Turkey.
Methods: A total of 59 water samples were taken from high-speed drill, air-water syringe, oral rinsing cup. R2A Agar and Nutrient Agar (NA) were used for TAHMB. Pseudomonas Agar Base with CFC supplement was used for Pseudomonas. Buffered Charcoal Yeast Extract Agar containing glycine, vancomycin, polymyxin, cycloheximide was used for *Legionella*. In addition two DUs biofilms from the high-speed drill were investigated according to Method of the Sanden et all.

Results: In the United States, the American Dental Association (ADA) and the Centers of Disease Control and Prevention have suggested a standard for DUWLs water of no more than 200 cfu/ml of aerobic heterotrofik bacteria. We found that all of air-water syringe (range 317–46320 cfu/ml), all of high-speed drill (range 370–52240 cfu/ml) and 90% of oral rinsing cup (range 183–119117 cfu/ml) exceed ADA recommendation for dental unit water. We compared bacterial counts on R2A Agar versus NA; results of R2A Agar were significantly higher than NA. Of the 59 water samples examined, 14 (24%) were positive for *Pseudomonas* sp. All water samples were negative for *Legionella* sp.
Conclusion: We suggested that bacterial contamination and also biofilms in DUs should be controlled to eliminate opportunistic pathogens and to provide water for dental treatment, which meets public health standards for ADA recommendation.

R2087

The prevalence of genital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in women and men attending different health care areas in Kuwait

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Introduction and objective: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most common sexually transmitted diseases (STDs) worldwide. They may cause pelvic inflammatory disease in females, which contributes to infertility. The objective of this retrospective study is to determine the prevalence of genital chlamydia trachomatis and Neisseria gonorrhoeae infection in women and men attending different health care areas in Kuwait.
Materials and methods: A total of 931 specimens were tested for both *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) with BD Probe Tec Et system. A total of 604 clinical specimens from women consisting of 46 urine and 558 endocervical swabs and 327 clinical specimens from men consisting of 119 urine and 208 urethral swabs were tested.
Results: The overall prevalence of CT was 6.7% (63) and 14% (131) for GC. Eleven samples (1.2%) were positive for both CT and GC. The prevalence of CT in different health areas as follows: Adan 16.8% Amiri 4%, Sabah 3.8%, Jahra 2.6%, and 0.9% for Farwania. The prevalence of GC in different health areas were 31.4%, 28%, 12.8%, 7%, 0.6% for Adan, Farwania, Jahra, Sabah and Amiri respectively. The overall prevalence of CT and GC in men was 75% of the total positive specimens.
Conclusion: The prevalence of CT and GC was high but differed at various health areas in Kuwait. The findings indicate that a major problem of STDs exist among the population in Kuwait.

R2088

Diagnosis and management of congenital toxoplasmosis in Slovenia

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Objectives: Toxoplasmosis, especially its congenital form caused by the parasite *Toxoplasma gondii*, may cause in women who acquire Toxoplasma infection in pregnancy the severe lesions in the foetus or even death of the foetus. In accordance with our previous results stressing the risk of congenital toxoplasmosis in Slovenia, compulsory screening of pregnant women for Toxoplasma infection was introduced in 1995 (Official Gazette, Art. 41, 1995; 69: 5297).
Methods: The current policy of the Institute of Microbiology and immunology, Medical Faculty Ljubljana and 7 regional laboratories of Institute Public Health in Slovenia is to screen the sera of pregnant women for the primary Toxoplasma infection by ELISA IgG and ELISA IgM tests as soon as possible, at the latest by 10-20 weeks' gestation.Those who are seronegative on the first visit are re-tested at 20–24 weeks and in the 3rd trimester for the infection acquired in pregnancy. These women are given also a booklet containing information on how avoid this infection. Primary infected pregnant women are evaluated also by Toxo-IgG avidity test and by PCR of amniotic fluid. In all women ultrasound scans are performed every four weeks. Women with confirmed primary *T. gondii* infection are administered therapy and followed up at the Department of Gynaecology and Obstetrics, University Medical Centre. In the 1st trimester the women are treated with spiramycin but later pyrimethamine and sulphadiazine with folinic acid alone is given or alternating with 3 weeks courses of spiramycin until delivery. In primary infected but PCR-negative women spiramycin is continued beyond the 20th week. Serologically IgG, IgM and/or IgA positive newborns are treated and followed up at the Department of Infectious Diseases and Febrile Illnesses and at the Department of Ophthalmology, University Medical Centre Ljubljana.
Results: In the period from 1995, two congenitally affected infants were born to mother who were screened to late in pregnancy and were therefore not treated in time. To all primary Toxoplasma infected women but treated in time no affected child has been born.
Conclusions: Serological screening for Toxoplasma infection during pregnancy can considerably reduce the incidence of congenital toxoplasmosis but timely testing, performed repeatedly at least 3 times during pregnancy and clinically long-term monitoring of Toxoplasma infected children is strongly recommended if this disease is not to be missed.

R2089

Pyogenic liver abscess: experience over a 10-year period

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Objectives: The purpose of this study was to determine the demographic, clinical laboratory, imaging, microbiologic and management characteristics of pyogenic liver abscess.
Methods: We carried out a descriptive study of 70 patients with pyogenic liver abscess from January 1995 to July 2005 in Carlos Haya Hospital, Malaga, a tertiary hospital in southern Spain.
Results: The mean age was 62.3 ± 16.3 years. 57% were male. Underlying medical illnesses included diabetes mellitus (31.4%), malignancy (4.3%), alcohol abuse (7.1%), chronic renal failure (5.7%) and immunosuppressive therapy (4.3%). Biliary tract disease was the cause in 45.7%, pylephlebitis in 17.1%, direct extension in 4.3%, and cryptogenic abscesses in 35.7%. Fifty-three abscesses were solitary, 52 (74.3%) were right sided, 14 (20%) were left sided, and 2 (2.9%) were bilateral. The most frequent presenting symptoms and signs were fever (94.3%), chills (75.7%), nausea and vomiting (40%), upper right quadrant pain (52.9%), jaundice (18.6%), hepatomegaly (27.1%), abnormal chest findings (35.7%) and sepsis (14.3%). The most common laboratory abnormalities noted were leukocytosis, elevated alkaline phosphatase levels, and increases in ESR and C reactive protein levels. Ultrasonography was performed in 65 patients,

with findings in 84.6%, and computed tomography (CT) in 66 patients, with findings in all patients. Blood cultures were performed in 60 patients (85.7%) and abscess cultures in 54 (77.1%), which were positive in 27 (45%) and 40 (74%) cases, respectively. The most common organisms isolated included *E. coli* in 16 patients, *K. pneumoniae* in 13, *S. intermedius* in 14, and anaerobes in 6. All patients were treated with intravenous antibiotic drugs. Ten patients underwent ultrasound or CT-guided percutaneous aspiration of the abscess. A percutaneous drainage catheter was inserted after aspiration in 57%. Surgical drainage was performed in nine patients, due to an elective operation or failure of antibiotic treatment (6 patients), failure of aspiration (2 patients), or failure of percutaneous drainage (1 patient). The overall mortality was 10%.
Conclusion: Biliary tract disease remains the most common cause of pyogenic liver abscess. In our series, *E. coli*, streptococcus intermedius and *Klebsiella pneumoniae* were the most common pathogens. Percutaneous drainage combined with intravenous antibiotics was the most common treatment. Pyogenic liver abscess remains a disease with a significant mortality.

R2090

Spectrum and antimicrobial resistance of pathogens in community-acquired urinary tract infections in a general hospital

E. Draghijeva, P. Egbase, S. Mathai *(Kuwait, KW)*

Objective: The aim of the study was the aetiology and antibiotic resistance of clinical isolates from patients in London hospital, Kuwait – general hospital, with different forms of uncomplicated community-acquired urinary tract infections (CA-UTIs).
Methods: Between October 2003–2005, a total of 3634 urine specimens were obtained. The urine cultures were performed in the laboratory according to conventional methods. The strains isolated from the patients who had significant bacteriuria were included in the microbiological analysis. The identification of the strains was performed using the API 20E system (Bio-Merieux). The sensitivity to antibiotics (ampicillin – AMP, amoxicillin-clavulanate – AMX-CLV, cefuroxime-CFR, ceftriaxone-CRO, gentamicin-G, nitrofurantoin-NTF, norfloxacin-NOR, penicillin-P, amikacin-A) were determined by disk diffusion method. The interpretation of the results was realized according to NCCLS guidelines.
Results: 636 out of 3634urine samples (17.5%) fulfilled the criteria for significant bacteriuria and they were analysed. The isolates were: *E. coli* 324 (51%), Streptococcus agalactiae (Group B Streptococcus) 117 (18%), *Klebsiella* spp. 78 (12%), CNS 45 (7%), *Enterobacter* spp. 27 (4%), *Candida* spp. 18 (3%), *Pseudomonas* spp. 15 (3%), *Proteus* spp. 9 (1.5%), *S. aureus* 3 (0.5%). There is high resistance of *E. coli* isolates to Ampicillin, Amoxicillin-clavulanate and Cephalotin – 62%, 61% and 82%, respectively. Similar distinction in *Klebsiella* spp. was found – 100%, 48% and 50% respectively. *Streptococcus agalactiae* isolates from women in our study showed resistance to Penicillin and quinolones – 38% and 58% respectively.
Conclusions: *E. coli* continuous to be the most common clinical isolate in community-acquired urinary tract infections. About 62% of *E. coli* isolates were resistant to Ampicillin and Amoxicillin-clavulanate. There is comparative high resistance to nitrofurantoin – 31% of clinical isolates. Similar distinction in *Klebsiella* spp. isolates was found – 29%. *Streptococcus agalactiae* strains isolated from pregnant and non-pregnant women in our study showed high resistance to Penicillin and quinolones.

R2091

In vitro antibiotic resistance of *E. coli* isolates in community-acquired urinary tract infections during 2001–2005

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Objectives: The purpose of this study is to compare the in vitro antibiotic resistance of *E. coli* isolates during 2001–2005 in community – acquired urinary tract infections.
Methods: During 1/1/2001–30/10/2005, a total of 3262 *E. coli* isolates caused community – acquired urinary tract infections (1144 *E. coli* isolates during 2001–2002, 1500 during 2003–2003 and 618 during 1/1/2005–30/10/2005). Isolation was performed by standard method. The identification and the susceptibility test to other antimicrobial agents were performed by the Vitek or the mini API system (Biomerieux).
Results: The resistance of *E. coli* isolates to antibiotics for 2001–2002/2003–2004/2005 respectively was: Ampicillin 32.8%/35%/42.2%, Amoxicillin/Clavulanic acid 5.0%/2.4%/4.0%, Cefalothin 11.6%/10.2%/15%, Cefotaxime 1.0%/1.5%/4.7%, Imipenem 0%/0%/0%, Tobramycin 1.7%1.5%/1.0%, Amikacin 0.9%/0.4%/0%, Netilmicin 1.0%/0.3%/0.6%, Gentamicin 2.1%/2.7%/3.7%, Trimethoprim – Sulfamethoxazole 15.8%/16.5%/25.0%, Nitrofurantoin 2.4%/2.5%/2.7%, Nalidixic acid 8.6%/9.2%/12.6%, Ciprofloxacin 7.0%/7.7%/10.0%.
Conclusions: We noticed a rapidly increase in resistance rates in *E. coli* isolates to Ampicillin and Trimethoprim–Sulfamethoxazole. Resistance rates to cephalosporins and quinolones were significant raised. Resistance to Amoxicillin/Clavulanic acid, Imipenem, Nitrofurantoin and aminoglycosides remained almost stable.

R2092

Quality of life in brucellosis

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Objectives: Brucellosis is a systemic disease that may cause several complications of different systems and it may affect quality of life of the patients. In our study, we aim to investigate the quality of life on patients with brucellosis. To our knowledge, this is the first study that investigates the quality of life in brucellosis.
Method: In this prospective study, 57 adult patients with brucellosis who were hospitalized in our clinic at Ankara Research and Training Hospital between September 2003 and September 2004 were included. The difference between quality of life before and after therapy was measured by using the Medical Outcomes Study 36-Item Short Form Health Survey (SF-36). Patients who had treatment history for brucellosis, patients with relapsed brucellosis, and patients who have underlying diseases like collagenous diseases diabetes mellitus, chronic renal disease or autoimmune diseases were excluded. In our study, we used a survey, which had two parts. In the first part of survey, social-demographic changes of patients and variants of the disease were investigated while in the second part the SF-36 Health Survey was taken place. The survey was applied before and after therapy.
Results: The differences between physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems, mental health points before and after

therapy were found as statistically significant (p = 0.000). At the end of the study each score of 8 scales of SF-36 found as increased. In addition to these findings, we found out that the vitality score was higher in 56–75 age group than the others. The physical functioning scores of patients were higher if the patients had fever or did not have chills. The vitality scores of patients were higher if they did not have any neurological involvement and also higher in widows when compared to singles. The scores of social functioning and mental health of patients without underlying diseases were higher than those of the patients with underlying diseases.
Conclusion: We concluded that the quality of life increases with the therapy in brucellosis cases and the SF-36 Health Survey is suitable to be used in brucellosis.

R2093

Prevalence of *Chlamydia trachomatis* in female population in Athens, Greece

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Chlamydia trachomatis is a common sexually transmitted pathogen in developed countries. It causes genital tract infection in both men and women, leading to sometimes serious consequences such as infertility, ectopic pregnancy and chronic pelvic pain. Infection with *C. trachomatis* is often asymptomatic, delaying its diagnosis and treatment.
Objectives: To determine the incidence of *Chlamydia trachomatis* (CT) in women who attended the Outpatient Dept of our hospital, for their routine annual visit.
Material and methods: A total of 2.528 women were studied for the presence of *Chlamydia trashomatic* in their endocervical samples. Two age groups were studied: group A 20–40 years (n = 1138) and group B 40–60 years (n = 1390). CT detection was confirmed by direct immunofluorescence method using a monoclonal antibody against MOP antigen of the microorganism.
Results: CT was detected in 233 out of 2.528 samples (9.2%). The percentage of CT detection was 13% (149/1138) in group A and 6% (84/1390) in group B. It is worth to note that a percentage of 72% of the infected women (168/233) were asymptomatic.
Conclusion: The *Chlamydia trachomatis* infection is common and largely asymptomatic, so early detection strategies with screening programs among young women are important in prevention and control.

R2094

Characteristics of 102 tetanus cases in southeast region of Poland

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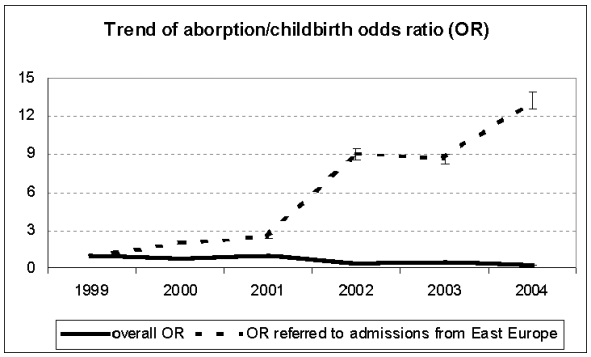
Introduction: Tetanus is acute infectious disease caused by anaerobic Gram-positive rod *Clostridium tetani*. Disease is affecting nonimmunised persons after inoculation of wound with *Clostridium tetani* spores, which are present in the soil. Regular immunisation with tetanus vaccine is the only effective way of prophylaxis against this disease. The morbidity rate in Poland is low (0.004/100 000 citizens). Mainly old people, from rural region of Southeast part of the country are affected.
Material and methods: 102 patients with tetanus, hospitalized in the Department of Infectious Diseases of the Jagiellonian University Collegium Medicum between 1995 and 2004 were investigated.
Results: Among hospitalised patients 48 (47%) were men, 54 (53%) were women, age ranged from 45 to 90 years (mean age 71 years). 26 patients were younger than 65 years old, and 75 were older than 65. 17 (16%) were town citizens, 85 persons (84%) were from rural area. Wound caused by injury was the portal of entry in 89 cases, in 12 patients infection was associated with chronic varices ulcerations, and in few cases with chronic skin fistula and animal bite. Only 8 patients visited doctor after injury, no one has received accurate post exposure prophylaxis. 5 (5%) patients developed localised tetanus, benign course of the disease was observed in 29 (29%) patients, 68 (67%) had severe, generalised tetanus. 34 (34%) of patients died, among them 31 were older than 65 years.
Conclusions: 1. Tetanus is mainly observed among old patients. 2. Lack of medical consultation after injury is the principal reason of tetanus occurrence among non-immunised individuals. 3. Active immunisation against tetanus, especially in rural area should be propagated.

R2095

Temporal trend of health care needs of extra-European Union citizens coming from developing countries admitted to a large hospital in Northern Italy, 1999–2004. Focus on infectious diseases among 6003 assessed hospitalisations

S. Sabbatani, E. Baldi, R. Manfredi *(Bologna, IT)*

Objective: All hospitalizations of foreign patients (p) coming from extra-European Union developing countries during the last 6 years was performed.
Methods: Data regarding foreign p admitted at all Divisions in the period 1999–2004 were extracted focusing on infectious diseases (ID)-related diagnoses.
Results: P aged ≥14 years had 6003 admission and 7231 overall diagnoses. During the 6-year study period, female hospitalizations had a steady increase with a peak in 2002 ($p < 0.001$). This trend is due to the rise of women from Eastern Europe ($p < 0.001$) which occurs at a younger mean age vs that of males ($p < 0.001$). The admission of clandestines carried out on emergency basis, accounted for a mean 9.4%. This phenomenon, very frequent in 1999 (43% of admissions), had a dramatic drop since 2002 ($p < 0.001$) depending on an appropriate deed of indemnity Italian law. The prevalent women diagnoses were obgyn ones: voluntary pregnancy interruption, spontaneous aborption or pregnancy complications in 30.6% of p and childbirths or controls of pregnancies with a favourable outcome in 18.2% of p. These diagnoses covered nearly 50% of hospitalizations of migrant women: other admissions were due to organic, dysmetabolic, or functional disorders while ID (4.6%) were less frequent. Among men, dysmetabolic disorders, organic-degenerative diseases, or functional illnesses (36.2%) were prominent and significantly more frequent vs women ($p < 0.001$), as well as post-traumatic diseases (16.5%) and ID (12.1%; $p < 0.001$). Also generic-undefined diagnoses were proportionally numerous (6.6% of diagnoses): cultural-language deficiencies strongly affected the physician-patient relationship. Among ID the main causative organisms were *Myc. tuberculosis* (14.9%), HIV (7.1%), HBV (3.3%), and HCV (2.6%). Upper-lower airways represented the most involved organ system (45% of discharges) followed by the gastroenteric tract (16.4%) and skin-soft tissues (7.4%) while systemic ID accounted for 14.9% of episodes. Such disorders predominated (up to 90% of cases) among non-regular migrants during years 1999–2000, while after 2002 an increase of ID was observed among p from Eastern Europe.



Conclusions: From an health care-social perspective, although a reduced incidence of ID did not occur during time, the possibility to attribute them to p of ascertained identity and housing makes possible to trace index p towards well-planned and effective therapeutic-preventive interventions.

R2096

Systematic review of fever of unknown origin in Turkey

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Objectives: In this study it was aimed to review the adult fever of unknown origin literature from Turkey systematically.
Methods: To find out the published series three national databases (Ulakbim Turkish Medical Literature database, http://www.turkishmedline.com, http://medline.plek-sus.com.tr) and two international databases [Pubmed and Science Citation Index (SCI)] were searched. Keywords for national databases were ''nedeni bilinmeyen ate'' or ''nba'' or ''fever of unknown origin'' or ''pyrexia of unknown origin'' or ''fuo'' or ''puo''. Keywords for Index Medicus and SCI were (''fever of unknown origin'' or ''fuo'' or ''pyrexia of unknown origin'' or ''puo'') and Turkey. In addition articles, which were cited by the extracted published articles were included in the study. In case of presentations from a single study with intersecting periods, the one with longer period was chosen. Articles published before 1990, paediatric series, series below 5 patients were excluded. Fever of unknown origin (FUO) was defined as fever over 38.3°C, that continues at least for 3 weeks, with no diagnosis reached after 1 week of inpatient investigation in all series (Petersdorf and Beason criteria).
Results: Data for 726 patients with the diagnosis of fever of unknown origin were obtained from 11 articles. Infections, collagen vascular diseases and neoplasms were found to be the reason of fever in 345 (%47.5), 114 (%15.7) and 110 (%15.1) of 726 patients. The most common infectious disease was tuberculosis (131/345, %37.9) followed by brucellosis (52/345, %15.0) and infective endocarditis (27/345, %7.8). The most common collagen vascular disease was adult-onset Still's Disease (46/114, %40.3), followed by vasculitic syndromes (22/114, %19.2) and SLE (18/114, %15.7). The most common neoplasm was Hodgkin's disease (29/110, %26.3), followed by non- Hodgkin Lymphoma (28/110, %24.5) and solid tumours (12/110, %10.9). Reason of fever could not be defined in 116/726 (%15.9) patients. The invasive procedures helped diagnosis in (234/726, %32.2) patients.
Conclusion: An infectious disease, especially tuberculosis and brucellosis, remains a common cause of FUO, in Turkey. Although several diseases may lead to FUO, lymphomas, Adult-Onset Still's Disease and particularly tuberculosis should be considered in the differential diagnosis of a patient admitted with FUO. Biopsy, aspiration, serology, bacteriology, radiology and observation of the clinical course were the most useful diagnostic procedures.

R2097

Relationship between *Chlamydia pneumoniae* seroprevalence and reproductive health of female health care workers

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Objectives: To evaluate the relation between infectious agents and reproductive health hazards of health care workers (HCWs), a cross-sectional study consisted of 73 HCWs and 65 bureau workers were designed.
Methods: Reproductive health problems of both groups were compared with a questionnaire, and then serologic examinations for *Chlamydia pneumoniae* IgG, IgA and IgM were performed by indirect immunofluorescence assay.
Results: The mean age of HCWs (32.9 ± 5.4 years) is lower than that of the controls (38.0 ± 5.9 years) and the difference was statistically significant ($p < 0.05$). Most of the HCWs (56/91) were positive for *Chlamydia pneumoniae* IgG whereas approximately half of the controls (25/54) were positive but the difference was not statistically significant ($p = 0.053$). According to the presence of *Chlamydia pneumoniae* IgA, no significantly difference was found between HCWs (46/91) and controls (20/54). Six HCWs were positive for *Chlamydia pneumoniae* IgM while none of the controls were positive. Smoking rates were similar between HCWs (25/91) and controls (19/54). The mean weight of the children of subjects positive for *Chlamydia pneumoniae* IgG (2896.4 ± 511.7 gr) was statistically significantly lower than that (3656.8 ± 398.5 gr) of subjects negative for *Chlamydia pneumoniae* IgG. Preterm birth was significantly ($p = 0.02$) higher in subjects positive for *Chlamydia pneumoniae* IgG (20/91) than in subjects negative for *Chlamydia pneumoniae* IgG (4/54).
Conclusion: These data showed that HCWs had a high rate of *C. pneumoniae* seropositivity and the rate of low birth weight was associated with this infection. We considered that future studies should be focused on the relation of infectious diseases and reproductive health problems of the HCWs.

R2098

Menengitis caused by *Streptococcus pyogenes* in a previously healthy woman

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Objectives: *Streptococcus pyogenes* is a wellknown cause of variety clinical expressions including local symptoms like tonsillopharengitis, cervical lymphadenitis, otitis media, cellulytis, eryspelas and more severe diseases like scarlet fever, osteomyelitis, necrotizing fasciitis, sepsis or the toxic shock syndrome. *Streptococcus pyogenes* is not well recognised as a cause of bacterial menengitis, although there are several reportsin the literature over the last years. Because of this reason we reported a case of *Streptococcus pyogenes* menengitis here.
Case: A forty-year old previously well woman presented a history of fever, headache, vomiting and sore throat of 3 days duration. On physical examination, she was unconscious, with a poor general condition. The temperature was 38.5°C, pulse rate 96/minute, respiratory rate 48/minute and blood pressure was

110/60 mmHg. Examination of the respiratory system, cardivascular system and abdomen were found to be normal. Neurological examination revealed diminished consciousness and neck rigidity. Laboratory investigations revealed a haemoglobin 8.3 g/dl and total leucocyte count of 22700/mm^3 (polymorphs 94%), C reactive protein was elevated up to 351 mg/dl, ESR was 57 mm/h. Serum electrolytes, renal and liver function tests were within normal limits. The cerebrospinal fluid (CSF) was turbid, with 10000 leucocytes/mm^3 (%95 polymorphs and %5 lymphocytes). The CSF sugar was 61 mg/100 ml and proteins were 474 mg/100 ml. Direct examination of CSF showed Gram-positive cocci and diplococci, and cultures yielded *Streptococcus pyogenes*. Also her blood cultures yielded growth of *Streptococcus pyogenes* too. She was treated initially with ceftriaxone (4 gr/day). Control lumber puncture was done at the third day of the treatment, but there was no change in CSF examination. So vancomycin was added to the treatment. But she died at the fourth day of the treatment.

Conclusion: *Streptococcus pyogenes* menengitis is not a complication of invasive GAS diseases but usually occurs in association with other illness such as otitis media, pharyngitis or head trauma. *Streptococcus pyogenes* menengitis is uncommon and the incidence seems to be persistently low, neverthless, clinicians should be aware that sporadic cases may occur and may have a fulminant course with relevent neurolological sequelae.

R2099

Osteoarticular complications of brucellosis: evaluation of the clinical, laboratory and radiological findings of 19 patients

O. Ural, D. Findik, G. Ural, U. Arslan *(Konya, TR)*

We carried out a retrospective study of 58 in or outpatients with brucellosis in our hospital. We evaluated the osteoarticular involvement, involvement sides, clinical specialities, relapse ratios and response to therapy. Of the 58 brucellosis patients who had therapy during the last five years 19 (32.7%) had osteoarticular complications. Eleven (57.9%) of the patients were female and 8 (42.1) male. Sacroiliac joint was the most frequent involved side found in 11 (57.9%) cases, followed by spondylitis in 6 patients (31.6%) and knee joint involvement in 2 patients (10.5%). These patients were prescribed a 6–12 weeks course of streptomycin, doxycycline and rifampicin. During the follow-up period after antibiotic therapy relapse was seen in two patients with osteoarticular involvement. These two patients was taken under therapy again for 12 weeks with doxycycline and rifampicin combination. After the treatment of these two patients, no relapse or damage was encountered in all of the patients.

R2100

Epidemiology of acute cystitis: the first Russian multicenter study

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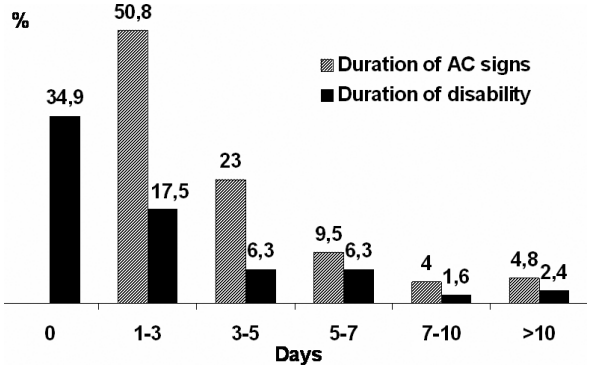
Objectives: To evaluate prevalence of AC among young women in Russia and their behaviour when AC episode appeared.

Methods: Questionnaire design study was carried out among students in three Russian and one Kazakhstan cities – Smolensk, Krasnoyarsk, Yekaterinburg, Karaganda. Questionnaire contained 19 questions concerning frequency of AC onset during the last year, data about AC relapsing, sexual activity, visits to a physician for AC management, possible self-treatment of AC, concomitant diseases and family history of UTI.

Results: 660 females (mean age 19.2 ± 4.3, median – 18.0 years) were included in the study. Symptoms of AC noted 126 responders (19%), mean age of respondents with symptoms of AC was 20.9 ± 5.5, median – 19.0 years. Among women with AC history 32.5% had 1 episode of AC during the year, 15.9% – 2 episodes, 8.7% – 3 episodes, 4–6 episodes had 4.8%, more than 6 episodes – 4.8% of respondents. Duration of AC signs in 50.8% of respondents was 1 to 3 days, in 23% – 3–5 days, in 9.5% – 5–7 days, in 4% – 7–10 days, in 8% – 10–14 days. Among women with AC 44% did not miss their studies or work when AC appeared, 17.5% could not attend studies or work during 1–3 days, 6.3% – during 3–5 days, 6.3% – during 5–7 days, 1.6% – during 7–10 days, >10 days – 2.4% of respondents (fig 1). Family history of UTI was noted in 46% women with AC and only in 17.9% of respondents, who had never suffered from AC. 35.7% of women with AC had never had sexual intercourse whereas 60.3% of respondents with AC history had sexual activity. Number of intercourse per month during last year were in women with AC history - ≤ 1 in 11.9%, 1 to 5 – 12.7%, 6 to 10 – 11.1%, 11 to 20 – 11.9%, every day – 8.7% and in women without AC history: ≤ 1 – 4.9%, 1 to 5 – 8.5%, 6 to 10 – 5.7%, 11 to 20 – 5.5%, every day in 3.0%. When AC appeared 18.6% of respondents do not seek for medical care, 21.4% were consulted by non physician (relatives, friends, etc.), and only 60% were under appropriate medical care – 17.1% were consulted by gynaecologist, 15% – by outpatient urologist, 11.4% – GP, 4.3% – pharmacist, 0.7% – nephrologists, 11.4% – other physicians.



Conclusion: AC affects 19% of young women in Russia and Kazakhstan and 34.2% of women have relapses observed during the year. Risk factor for development of AC is sexual activity and positive family history of UTI. When AC appeared 18.6% of respondents do not seek for medical care at all, and only 60% are under appropriate medical care.

Emerging infectious diseases

R2101

Review of infections due to *Pseudallescheria boydii*

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Infections due to *Pseudallescheria boydii* (Scedosporium apiospermum) are known already since 1899. An analysis of 528 published cases in humans was performed to reveal the spectrum of disease, risk factors, geographic distribution and outcome of infection. Initially the fungus was observed mainly as a cause of mycetoma in temperate climate zones after traumatic inoculation. Since the seventies of the previous century, other disease entities have become preponderant. Particularly pulmonary infections in immunocompromised patient became relatively frequent, so that the species can be viewed as a truly emerging fungus in the growing hospitalised population with severe immune disorders. However, two disease entities are noted in patient populations without apparent changes in frequency. The species regularly colonises lungs of patients with cystic fibrosis, but thus far escaped attention because its presence was concealed by co-occurring *Aspergillus fumigatus*. Another, unique syndrome is delayed cerebral infection after near-drowning. Since it is unlikely that the frequency of *P. boydii* in surface waters, lakes and ditches has increased dramatically since 1970, we have to assume that also this clinical picture has long been misdiagnosed. Given the low susceptibility of the fungus against current antifungals, development of proper diagnostics for *P. boydii* is highly significant.

R2102

Alkhurma haemorrhagic fever virus, an emerging tick-borne flavivirus with high fatality rate in Saudi Arabia

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Alkhurma haemorrhagic fever virus (AHFV) has been first isolated in 1995 (reported in 1997) from patients presenting with either hemorrhagic fever or central nervous system manifestations. We present here a review of most of the cases reported to date in patients with emphasizing the clinical, biological and epidemiological data. Fatality rate is 25% which represents the most deadly flavivirus recognized at this time.

Objectives: To investigate the transmission routes of AHFV; to better understand the microevolution of AHFV in Saudi Arabia.

Methods: Ticks were collected in different regions of Saudi Arabia, identified using morphological keys and sequencing, and tested with Flavivirus generic primers. Biological material found to be positive by PCR amplification was sequenced for final identification, and used to attempt viral isolation onto Vero cells and/or via intracerebral inoculation to suckling mice.

Results: One tick (*Ornithodoros savignyi*) collected from a camel-resting place near the city of Jeddah was found to be positive for AHFV RNA. Full coding sequence of one isolate of AHFV (the first one to be detected in ticks) was determined and sequence analysis was performed for comparison with sequences derived from human specimens aiming at a better understanding of AHFV phylogeny and evolution. Different aspects of the potential transmission routes for AHFV will also be addressed. Field evidence for the tick-borne transmission of AHFV will be presented; Additional results provided by tick collection campaigns that are currently organized will be performed. Additional investigation of the microevolution will be presented based on human sequences determined in samples collected over the last 7 years.

Conclusions: Although AHFV is the most deadly flavivirus to circulate in the world, few investigation have been performed to date. In these times of surveillance of viruses candidate for bioterrorism use, a better knowledge of AHFV natural history and epidemiology is required, as well as a development of collaborative studies intending to field investigation of the medical, veterinary and entomological aspects.

R2103

Comamonas testosteroni bacteraemia and perforated acute appendicitis in a patient

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Objectives: *Comamonas testosteroni* (*C. testosteroni*) is an uncommon isolate in the clinical laboratory as a human pathogen. However the organism is found worldwide in environmental sources such as soil and water. We report in our hospital a case of bacteraemia in patients with perforated acute appendicitis due to this organism.

Case report: A 22-year-old man; presenting with onset of abdominal pain as bilateral flank pain followed by periumbilical localization of pain, anorexia, nausea and vomitting. At the time of application, he was looking moderate, cooperated and oriented. Laboratory values are: haemoglobin 15.2 gr/dl, haematocrit 44.9%, leucocyte count 12.000/mm^3, differential white count shows 91.2% neutrophils, 7.0% lymphocytes, 1.8% monocytes. Blood cultures were incubated in BACT-Alert 3D (bioMérieux, France). Twenty-four hours later the growth from two bottles was identified as *C. testosteroni* and antibiotic susceptibility test was performed using the Mini API system (BioMerieux, France). The results were confirmed by conventional methods. The patient had been taken for laparotomy with suspicion of acute appendicitis and the diagnosis was perforated appendicitis. Before the operation cefazolin (3 × 1/day) was given to patient. Gram-negative bacilli and leucocytes were seen in Gram stain of intra-abdominal fluid taken during operation. In the culture of this fluid the microorganism was also identified as *C. testosteroni*.

Conclusions: For effective therapy; it is useful to know that *C. testosteroni* bacteraemia may accompany acute appendicitis and intra-abdominal perforation cases. It is important for clinicians to recognize the spectrum of infections that *C. testosteroni* is capable of causing, as well as similarities and differences between these infections. In these regards, our case report and review of the literature are helpful.

R2104

First serological evidence of spotted fever group rickettsioses in Taiwan

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Objectives: To investigate the sero-prevalence of spotted fever group (SFG) rickettsiae and provide the evidence of SFG rickettsioses existence in Taiwan, we conducted a retrospective serosurvey among 553 residents for antibodies against *Rickettsia*

conorii, *Rickettsia typhi*, and *Orientia tsutsugamushi* by using indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA).
Methods: A total of 653 sera (453 un-paired sera from healthy population and 200 paired sera from 100 febrile patients with clinical suspicion of rickettsial diseases) were tested. IFA and ELISA were used to detect IgG antibodies against *R. conorii* (strain Malish), *R. typhi* (strain Wilmington), and *O. tsutsugamushi* (strain Karp). The positive ELISA screening results were further confirmed by IFA. A serum titre ≥1:40 is considered as positive and serial dilutions are further tested only in febrile population to determine the end titre.
Results: Twenty, 26, and 33 out of 453 (4.4%, 5.7%, and 7.3%) sera in healthy group and 21, 8, and 24 of 100 febrile patients were positive for IgG antibodies against *R. conorii*, *R. typhi*, and *O. tsutsugamushi*, respectively. Three patients were demonstrated to have 4 folds or more elevation of anti-*R. conorii* IgG titres. There were 20 out of 553 (3.6%) objects had antibodies against at least 2 of the tested antigens.
Conclusion: Spotted fever group rickettsiosis (SFG) has been considered as a worldwide new emerging infectious disease. Seroepidemiologic data and confirmed cases have been reported in several countries in Asia. To our knowledge, there is no confirmed case of SFG rickettsiosis reported in Taiwan. The sero-prevalence rates were 4.4%, 5.7%, and 7.3% in healthy population and 21%, 8%, and 24% in patients with clinical suspicion of rickettsial diseases for SFG rickettsioses, murine typhus, and scrub typhus, respectively. These data suggest that rickettsial diseases are prevalent in Taiwan. We believe that these are first serological evidence of SFG rickettsoses in Taiwan. Because of significant overlapping in clinical aspects and serological cross-reactivity among these rickettsial diseases, we suggest routine testing of antibodies to SFG rickettsiae, in addition to *R. typhi* and *O. tsutsugamushi* for patients with clinical suspicion of rickettsial diseases in Taiwan. Further studies for detecting the prevalent species of SFG rickettsiae in Taiwan are warranted for more specific testing in future.

R2105

Staphylococcus aureus small colony variant in patients with cystic fibrosis in Genoa, Italy

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Background: small colony variants (SCV) are intracellular, auxotrophes, slow-growing subpopulations of *Staphylococcus aureus* (SA), with reduced antibiotic susceptibility, wich can cause persisting and relapsing infections in CF patients (pts). Aims Determine prevalence, pts clinical predisposing conditions, microbiological characters and antibiotic susceptibility of SASCVs strains in CF pts attending the Genoa Centre in 2004.
Methods: SA and SASCV isolates were recovered from sputa and throat swabs in Mannitol Salt Agar after 48 h at 35°C and identified by latex agglutination, coagulase test tube, API Staph system with 48 at 35°C and amplification of specific SA-nuc gene.Auxotrophism was assessed by testing thymidine (T) haemin and menadione disks. Methicillin susceptibility was assessed by cefoxitin disk diffusion in Mueller Hinton medium with 5% blood and confirmed by mecA gene. In pts colonised with SASCV antibiotic treatment courses before SASCV recovering, variation in FEV1 and BMI were determined.
Results: 25/199 pts had stable SASCV. SCV+ normal SA were recovered from 14 pts. 11 pts harboured only SCV. 21/25 pts with SASCV were colonised also by *P. aeruginosa*. 20/21 SCVs were T-dependent and only 1 was haemin-dependent; 36% of strains were methicillin resistant. Before first SASCV recovering, pts had therapy courses with: 11/25 nebulised aminoglicosides + varius, 8/25, nebulised aminoglicosides + cotrimossazole and 4/25 cotrimosazole + various. Pts receiving nebulised tobramycin were 60%. No significant differences in FEV1 and BMI before and after SASCV sputum recovering were found.
Conclusions: Our data show an increasing occurrence of SASCV in 2004 (12.5%) compared with previous years (7% in 2000); this finding could be related to the antibiotic pressure together with the lung predisposing condition in pts with long term SA colonisation. Moreover, SASCV could spread among pts, so accurate microbiological protocols for detection and antibiotic susceptibility testing are critical.

R2106

Salmonella typhi meningitis complicated with subdural empyema: a case report

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Objectives: Intracranial infections caused by Salmonella species are uncommon. The authors report a case of the rarely encountered *Salmonella typhi* subdural empyema following meningitis.
Case description: A 11-month-old boy was admitted to our hospital because of fever, despite one week antibiotherapy, and neurologic irritability. The patient had sign and symptoms of increased intracranial pressure and progressively change in mental status. *S. typhi* was isolated from consecutive two CSF culture, but haemocultures were negative. Radiologic examination demonstrated the development of subdural empyema. The children was discharged without neurologic deficit, after a 10 week intensive antibiotherapy with shunt operation.
Conclusions: *S. typhi* should be included in the differential diagnosis of meningitis, and even for subdural empyema, particularly in children from areas in which salmonellosis endemic. The reduce morbidity and mortality in such cases, prolonged antibiotherapy is essential with surgical intervention.

Infection control

R2107

Identification of *Streptococcus mutans* with antagonic effect

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Dental caries is an infectious process that ends destroying the tooth. *Streptococcus mutans* is considered the main agent causing this disease. The search for microorganisms with antagonic action on *S. mutans* can be an alternative, which might avoid or control this disease.
Objectives: To identify *S. mutans* strains with antagonic effect upon *S. mutans.*
Methods: Saliva samples were taken in 66 children and cultured on Blood agar and Mitis Salivarius Bacitracin agar. After incubation at 37°C in anaerobic atmosphere for 48 hours, a bacterial recount was made and the compatible colonies with *S. mutans* were subjected to biochemical tests. The determining of

the antagonic effect was made using the technique of double layer in agar.
Results: In children with and without caries, the frequency of *S. mutans* was, respectively, 91.7% and 96.7%. In the group of patients without caries, only two strains got no antagonic action, three strains got full antagonic action (100%), and the remaining presented different ways of inhibitory action. In the group of patients with caries only 5 strains didńt have any antagonic action, 32 strains had full antagonic action (100%), and the others strains had variable inhibitory action.
Conclusions: Were identified 112 *S. mutans* strains with great antagonic potential, which after accomplishing other requirements could be used in prevention or caries control strategies.

R2108

Infection control practice in forensic medicine

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Objectives: Forensic medicine workers have greater occupational risk for infectious diseases because of unique characteristics of forensic autopsy practice. In addition of needles and scalps, they are exposed to broken glass, bone shards and fragmented projectiles, as well as large amount of blood and open tissues on a daily basis. In this study, we aimed to evaluate the infection control measures and implementations in forensic medicine practice in Turkey.
Material and method: Between April–June, a questionnaire survey on infection control measures was mailed to Forensic Medicine doctors. The questionnaire consisted of 36 questions that were designed based on Universal Precautions and protective barriers for Forensic Medicine practice. The compliance with precautions and conditions during performance of death body examination, transportation and autopsy and vaccination status were evaluated. Data were analysed by using Epi-Info pragramme(version 5.0).
Results: Totally 111 doctors from 27 cities were responded the questionnaire. Their autopsy number average per month was 21.0 ± 27.7 (0–160), death body examination was 9.2 ± 11.8 (0–55). 43.2% of doctors were doing death body examination in somewhere. Percentage of wearing glove, mask, special clothes and use of handwashing/disinfectant were 81.5%, 63%, 54.3%, and 81.5 respectively in field. Percentage of wearing mask, protective eyewear, special gloves and boats were 59.6%, 25.5%, 72.3%, 61.7%. There was only two negative pressure room in between 27 institutes or department. 78.4% of doctors were vaccinated against hepatitis B and 44.1 % of applied tetanus booster dose.
Discussion: According to the results, we need national standards and implementations against infection risk in Forensic Medicine practice.

R2109

Surveillance of intensive care associated infections in Scotland

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Objectives: To evaluate the feasibility of carrying out surveillance of ICU acquired infection (ICUAI) in Scotland (i) using the HELICS surveillance protocol and definitions, and (ii) an existing electronic system for data collection.
Methods: Patient based surveillance comprising of HELICS Level 2 surveillance of Blood Stream Infections (BSI), Catheter Related Infections (CRI) and Ventilator Associated Pneumonia (VAP) was piloted for a minimum of two and a maximum of three months between 01/05/2005 and 31/08/2005 in five ICUs in Scotland. ICUAI surveillance data were collected through the existing Ward Watcher audit system currently in place in all ICUs throughout Scotland. Amongst the data collected in Ward Watcher are demographics, diagnoses and severity of illness scores. Ward Watcher has been developed to facilitate the collection of the additional data items required for surveillance of ICUAI according to the HELICS protocol. The surveillance methods were evaluated by means of several questionnaires that were sent to key contacts at all pilot sites in order to determine their opinion and experience with various aspects of the study. These included surveillance methodology, applicability of the HELICS protocol and definitions and the data collection system. Daily defined dose (DDD) data for all antibiotics dispensed to all ICUs in Scotland between April 2004 and March 2005 were collated to provide a measure of antibiotic use in the ICU.
Results: During the pilot period a total of 386 patients were admitted to the 5 pilot sites that participated in the pilot study, 199 patients stayed for two days or more. A total of 32 (16%) patients developed 44 episodes of infection according to the HELICS infection definitions. Data collection using the existing electronic data collection system was simple and straightforward and the quality of the data was deemed to be acceptable. The HELICS infection definitions for pneumonia, BSI and some CRIs were applicable in all pilot sites. Local and General CVCRI criteria were not applicable in all hospitals due to the diagnostic testing procedures carried out in some laboratories.
Conclusion: Surveillance of infections acquired in ICU in Scotland using electronic data capture and the HELICS definitions for infection is a feasible process with a minimal requirement for additional staff resources. Collection of dispensed DDD for all hospitals in Scotland is feasible and could contribute to our understanding of antimicrobial resistance.

R2110

Diving suit: sterile, ambulatory, personal device for protective and preventive isolation of nosocomial invasive fungal infections in neutropenic patients hospitalised in laminar air flow room who need a disruption of isolation

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Background: Patients with long neutropenia present high risk to develop invasive pulmonary aspergillosis (API) of poor prognosis. Pulmonary computed tomographic Scan (CT scan) allows earlier diagnosis of API and improve management in these patients (D. Caillot, JCO, 1997). However, CT scan need an isolation breaking which is potentially dangerous. We propose a diving suit to maintain the preventive isolation.
Materials and methods: The diving suit is sterile, personal, ambulatory and transparent to permit monitoring, visual and conversational contact. It is supplied with air with a self-contained station of ventilation (12 hours). The air contamination is controlled with 2 HEPA filters. Fifteen patients treated for haematologic malignancies like acute leukaemia have tested it after giving inform consent.
Results: The diving suit has been validated for air contamination, physiological (CO2), CT scan feasibility and patients acceptation. All patients (5 male, 10 females) presented a severe neutropenia (PMN <0.5 G/l during more than 10 days) at time of CT scan. Three patients have had already pulmonary CT scan before using diving suit and 2 claimed to be claustrophobic. No patient describe dyspnoea, neither pain nor discomfort. All patients felt reassured and agreed for a new CT scan with this

clothing. Two patients have had another CT scan with diving suit for suspicion of pulmonary aspergillosis. Diving suit was very easy to manipulate and did not disrupt monitoring or treatments administration. Diving suit was also compatible with Doppler and Radio Magnetic Nuclear. For one of these patients with fever resistant to antibacterial treatment, 3 thoracic CT scan were performed (1 per week) without any sign of API. The fourth CT scan allowed the diagnosis of API with halo sign. This diagnosis has been demonstrated by histology after surgery. In this case report, contamination has not been possible during first CT scan because of the diving suit, this information is very important to explain to the patient and his family.
Conclusion: Pulmonary CT scan needs very often to be performed in neutropenic patients to assess API diagnosis. An ambulatory, personal protective clothing allows no disruption of isolation for immunocompromized patients during a CT scan. It can also be proposed for medical staff protection when treating patients with SARS or other highly contagious agents.

R2111

Prevention of infections caused by showers

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Introduction: In clinical settings with immunocompromized patients infections caused by shower water, not only *Legionella* spp. but also *Pseudomonas*-like water contaminants, is evident.
Material and methods: Different methods for water decontamination (heat, chemical, filtration) were investigated for the effect of contamination of water. Shower heads with filters were installed in two departments to investigate the effect on the incidence of infections caused by water-related bacteria one year before and during the use of filter shower heads. Water samples were concentrated by filtration and examined for total germ count, identification of pathogenic bacteria and *Legionella* count regularly (time-scedule depend on the experiment).
Results: Shower heads and shower tubes were highly contaminated 48 hours after heat decontamination as well as chemical decontamination. Shower with filter reduced the bacteria from water significantly, but became increasingly contaminated with skin bacteria followed by water bacteria after one week. By adding decontamination of the shower heads with alcohol before use this contamination was almost reduced completely for the first two weeks. The number of infections related to water bacteria were low both before and after use of filters and even though the number of infections decreased in the departments it was not significant.
Conclusion: Ordinary shower heads and tubes should be heat decontaminated daily to keep an acceptable low number of bacteria in shower water. Alternatively shower heads with filters can be used at least two weeks if they are decontaminated with alcohol before use.

R2112

How to decrease device-associated urinary tract infections in adult intensive care units?

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Introduction: In year 2003, we found that our device-asssociated urinary tract infection (UTI) rates were considerably higher than those reported by intensive care units (ICUs) participating in the National Nosocomial Infections Surveillance System (NNIS). We aimed to decrease the high device-associated UTI rates.
Methods: We performed a prospective NI surveillance study in three adult ICUs, total 15 bed capacity, in Baskent University Alanya Hospital from January 2003 through October 2005. Nosocomial infections were identified using the NNIS definitions. The ICU staff was trained for the prevention of UTI. Infection control strategies with device insertion and maintenance practice were also checked. To facilitate hand washing we replaced the old water taps with the new ones with electronic taps. Manuspray was used more effectively.
Results: From 2003 to 2005, the urinary catheter utilization rates were 0.76; 0.76; 0.74 and the urinary tract catheter-associated infection rates were 19.1;13.6;4.8 per 1000 catheter days, respectively.
Conclusion: Hand washing, gloves, and universal precautions are extremely important to minimize device-associated UTI and clusters.

R2113

The effect of air ionisation on microbial content of the air

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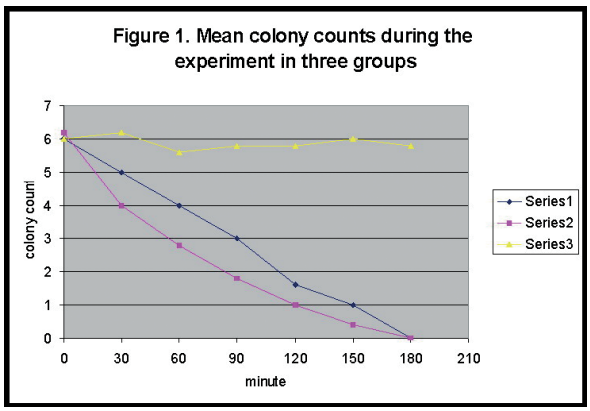
Objective: To evaluate whether air ionization reduces the microbial content of air.
Methods: Two air ionizers were placed in two boxes, and the third space was considered as control group. Negative air ion generation was performed in first two boxes for three hours. Air sampling was done at the beginning of experiment and every 30 minutes up to three hours in three groups. Colony counts and bacteriologic studies were performed and compared in three groups.
Results: In all 30 minutes intervals (except at the beginning of experiment), colony counts in AI groups were lower than control group. Coagulase negative staphylococcus (CNS) was the most common bacteria isolated, followed by *Bacillus* spp, *Acinetobacter* and *Escherichia coli*. The two latter microorganisms were not detected in AI groups.



Conclusion: Our findings qualitatively indicate that AI can reduce the microbial content of the air. Regarding the type of microbial air pollution and the amount of air cleaning needed, this method can be used solely or in combination with other air cleaning methods.

R2114

Sexually transmitted infections among registered female sex workers grouped according to age

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Objectives: 1. To assess the prevalence of ST pathogens among different age groups of asymptomatic sex workers.2. To compare patterns of sexually transmitted diseases (STDs) among Greek and immigrant sex workers.
Material and methods: A number of 258 asymptomatic female sex workers, aged 19–30, 31–0, 41–5, were examined for STIs, as well as a control group of 320 asymptomatic women of the same age who attended the out patients Gynaecology dpt of our hospital, during two years period (from October 2003 to October 2005). Vaginal and endocervical samples were examined microscopically for *Trichomonas vaginalis*, yeast, clue cells and *N. gonorrhoeae* and cultured for aerobic and anaerobic bacteria. *Chlamydia trachomatis* was detected with DIF (Cellabs Pty Ltd, Australia).
Results: Among sex workers aged 19–0, Bacterial vaginosis (BV) was found in 14% of immigrants and 7% of Greeks. *Candida albicans* was isolated in 22% and 7% respectively. In sex workers aged 31–0, *C. albicans* was found in 17% of Greeks and 5% of immigrants. The prevalence rate of *C. lbicans* in Greek sex workers aged 19–0 was 7% and in those of control group was 15%. The prevalence rate of *C. lbicans* among immigrant sex workers aged 19–0 and women of control group was similar (22% and 21% respectively).

	Sex workers					
	Age (years)					
	19-30		31-40		41-55	
Microorganisms	Greeks (n = 30)	Immigrants (n = 100)	Greeks (n = 36)	Immigrants (n = 40)	Greeks (n = 32)	Immigrants (n = 20)
S. agalactiae	2 (7%)	6 (6%)	2 (6%)	4 (10%)	0 (0%)	0 (0%)
BV	2 (7%)	14 (14%)	4 (11%)	4 (10%)	4 (9%)	2 (7%)
C. albicans	2 (7%)	22 (22%)	6 (17%)	2 (5%)	0 (0%)	6 (19%)
T. vaginalis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normal Flora	24 (79%)	58 (58%)	24 (66%)	30 (75%)	28 (91%)	12 (74%)

Conclusions: 1. The prevalence of BV in immigrant sex workers aged 19–0 was higher (14%) than in Greeks (7%). 2. *C. albicans* was detected in higher rate (22%) in immigrant sex workers aged 19–0 than in Greeks (7%). 3. *C. albicans* in Greek sex workers aged 19–0 was isolated in lower rate (7%) than in women of control group (15%). 4. The prevalence rate of *C. albicans* was found higher among Greek prostitutes aged 31–0 (17%) than in immigrants (5%). 5. *C. trachomatis* was detected in higher rate in immigrant sex workers (6%) than in Greeks (1%). 6. *T. vaginalis* was not detected in sex workers (immigrant and Greek), while in immigrants of control group was found in 5%.

R2115

Vaginal infections in women undergoing *in vitro* fertilization

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Objective: The purpose of this study was to investigate the prevalence rate of pathogens bacteria in vaginal and cervical samples from women undergoing *in vitro* fertilization.
Material and methods: Vaginal and cervical smears were examined from 99 women aged 18–50, who visited our hospital for pre-IVF screening (Group A) and from 320 women of the same age who attended the out patient Gynaecology dpt (Group B) during the last three years. Vaginal and cervical samples were examined microscopically for *Trichomonas vaginalis*, yeast, clue cells and cultured for aerobic and anaerobic bacteria. *Chlamydia trachomatis* was detected with DIF (Cellabs Pty Ltd, Australia). Urogenital mycoplasmas were identified by biochemical method (Mycoplasma system, Liofilchem s.r.l., Italy). In Group A, 69% were Greeks and 31% immigrants.
Results: No significant difference was found between Group A and Group B referring the prevalence of *Bacterial vaginosis* (BV), *Candida albicans* and *Streptococcus agalactiae*. *T. vaginalis* was found only in Group B (3%). In Group A the prevalence of BV was higher in Greeks (10%) than in immigrants (3%), while in Group B was 8% and 11% respectively. In Group A, *C. trachomatis* was found in 7 women (4% Greeks and 13% immigrants), while in Group B in 8% and 11% respectively. The incidence of *Ureoplasma urealyticum* in Group A was 10% in Greeks and 19% in immigrants, while in Group B was 23% and 29% respectively.

	Group A		
	Greeks (n = 68)	Immigrants (n = 31)	Greeks + Immigrants (n = 99)
Age (years)	N (%)	N (%)	N (%)
18-30	9 (13)	9 (29)	18 (21)
31-40	42 (62)	18 (58)	60 (60)
41-50	17 (25)	4 (13)	21 (19)

Conclusions: 1. There was no significant difference in the prevalence of BV, *C. albicans* and *S. agalactiae* between Group A and Group B. 2. The incidence of BV in Greeks of Group A was significantly higher than in immigrants. In Group B the incidence of BV in immigrants was higher in comparison to Greeks. 3. The prevalence of *C. trachomatis* and *U. urealiticum* was considerably higher among immigrants than Greeks. 4. *C. trachomatis* was found more frequently in Greeks of Group B than those of Group A. 5. *U. urealiticum* was detected in higher rate (26%) in Group B than in Group A (14%).

R2116

Incidence of candidaemia as an indirect marker for nosocomial infection control

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Objectives: To know the incidence of candidemia in Hospital de Manacor within the last four years, its meaning and case analysis.
Methods: A prospective analysis of candidemia episodes in a 207 beds hospital in Mallorca was made through the years 2002, 2003, 2004, and 2005, as a part of the hospital infection control strategy. The incidence of cases was computed in relation to the total of positive blood cultures, to 1000 hospital admissions, and to 10^5 stays. The data was adjusted to the annual complexity index. Blood cultures were processed in Bactec 9120 and 9050 systems. All the clinical cases were studied, and the results were interpreted in relation to other nosocomial control parameters.
Results: 12 cases were recorded (2 in 2002, 1 in 2003, 7 in 2004, and 2 until October 2005), resulting in a 0.57%, 0.41%, 2.53%, and 0.95% incidence in relation to the total number of blood positive cultures. It disclosed a 0.1%, 0.09%, 0.72% and 0.24% for 1000 hospital admissions, and 1.8%, 1.5%, 11.8% and 3.62% for 105 stays, respectively. The annual complexity index adjustment did not change the incidence values. Ten patients were male, and the median age was 75.5 years. The 91.6% of the cases were

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hospital-acquired infection: two in the intensive care unit, four in the medical acute area, and five in the surgical area. Seven of the candidemias were catheter-related (four central vein catheter), two had an urinary focus, two an abdominal origin, and one was related to a skin ulcer. All the isolated yeasts were identified as *Candida albicans*, except one *Candida glabrata* and one *Candida parapsilosis*. Global mortality was 58.3%, and directly attributed mortality 41.6%, with just one therapeutic failure. The transient increase of candidemia incidence related to the increase of MRSA infection during 2004 (11, 19, and 51 in 2002, 2003 and 2004 respectively) led to introduce new strategies in nosocomial control policies. The increase of candidemia incidence was also related to the increase of wide spectrum antibiotics use.
Conclusion: The characteristics of the analysed cases are similar to other studies. An increase of candidemia incidence can help to identify the necessity of improving nosocomial infection control policies.

R2117

Microbial keratitis in health area of Santiago de Compostela (Galicia, Spain)

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Objective: The aim of this study is to analyse the microbiological and clinical characteristics of microbial keratitis in the Health Care Area of Santiago de Compostela (459.180 inhabitants), to detemine how patients with suspected microbial keratitis should be empirically treated.
Methods: The results of 152 corneal scrapes performed in the Ophthalmology Service of Clinical Universitary Hospital of Santiago between November 1999 and November 2005 were reviewed. All samples were collected by the Ophthalmologist and processed immediately at the "bedside", in proteose-agar and saboraud-dextrose-agar. Medical records were reviewed for all patients. Risk factors and microbial isolations were analysed.
Results: 152 samples were evaluated from 137 patients with suspected microbial keratitis (72 males, 65 females). Mean age was 58.3 years (<1–99). 16% of the patients were contact-lens user, 8% had a trauma contaminated with vegetable matter and 3% had a corneal transplant. There was a positive culture in 77.7% (118/152) of scrapes. The 10.2% of the samples had polimicrobial culture. The 83.1% were bacterial keratitis and the most frequent isolate were *Staphylococcus aureus* (18.5%), followed by Gram-negative rods (17.6%), and *Streptococcus pneumoniae* (7.7%). The 16.9% were fungal keratitis, and the commonest isolate were *Candida* spp. and *Fusarium* spp.
Conclusions: 1. In ocular injury contaminated with vegetable matter we isolate coagulase negative Staphilococci predominantly, probably due to patients manipulation, and they are part of the saprophytic flora. 2. *Pseudomonas aeruginosa* and *Serratia marcescens* were the most frequent Gram-negative rods isolated. When *Pseudomonas aeruginosa* is isolated, there is a polimicrobial keratitis. 3. All *Staphylococcus aureus* isolated were methicillin susceptible. 4. Early diagnosis and prompt treatment with vancomycin/ciprofloxacin or vancomycin/ceftacidime are important for successful management, when bacterial keratitis is suspected.

R2118

Relationship between pathogenicity, susceptibility to antibiotics and serotyping of 335 Tunisian strains of *Streptococcus agalactiae*

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Objectives: Group B Streptococci (GBS) are an important cause of serious infections among newborns and adults out side perinatology. Interrelationship between serotypes and the pathogenicity orients toward the vaccinal prevention and the survey serotypes-sensitivity to antibiotics provides epidemiological enlightenments on the resistance to antibiotics.
Methods: Strains of GBS isolated from all specimens in a teaching hospital were collected during a period of 21 months from 01/01/04 to 30/09/05. Strains were identified according to the conventional characters in particular by a positive hippurate test and by group B specific latex agglutination (biomérieux). Serotypes were identified by serum agglutination test with type-specific antiserum (strep-B-latex-statens Serum Institute, Copenhagen, Denmark). The susceptibility to antibiotics was determined according to the CA- SFM recommendations.
Results: 335 strains of group B Streptococcus (GBS) were kept. GBS is the main Streptococcus isolated (with more of 80% of the total Streptococcus) essentially from vaginal specimens (48%) and urine samples (32%). Thirty three percent of strains were isolated from non-pregnant adults who are essentially diabetics with a median age of 57 years and a sex-ratio of 0.22. Serotypes III, V and Ia were being most common from all clinical isolate sources. All strain isolated from blood (n = 16) came from newborns. Serotype III prevailed among GBS vaginal isolates (50%). All isolates were susceptible to penicillin, and to glycopeptides. Resistance to erythromycin was found in 30%, to lincomycin in 32% and to tetracycline in 93%. Only one strain expressed a high-level resistance (RHN) to aminoglycosids. GBS isolates resistant to macrolid belong to serotypes V and III.
Conclusion: 2/3 of GBS were isolated in perinatology and 1/3 remaining concern essentially diabetic adult with a predominance of serotype III. The main antimicrobial resistances were noted for tetracycline (veterinary use antibiotics), macrolid and chloramphenicol. Study the relationship between serotyping and pathogenicity give possibilities of prevention by vaccination.

R2119

Mumps cases in Minsk, Belarus, 2001–2003

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Objectives: In Belarus, a mandatory single dose of mumps vaccine was instituted in 1981, and a two dose regimen – in 1995. The absence of information on mumps virus genotypes circulating in the Republic of Belarus has made it difficult to assess the situation. Moreover, by now no serological data is available on mumps outbreaks in Belarus. Thus, the purpose of this study was to isolate and sequence mumps virus strains from clinical mumps cases in Minsk, Belarus, and to estimate the role of vaccine failure in vaccinated patients.
Methods: During 2001–2003 fifteen mumps case were enrolled in our study. All mumps cases were clinically, laboratory and phylogenetically characterized here. Detection of mumps antibodies and IgG avidity testing was performed using commercial test kits. Plague reduction neutralization assay was performed with the wild-type measles viruses of the genotypes A, C and H, as well as with the Leningrad-3 mumps vaccine virus.

Results: The diagnosis of mumps infection was confirmed laboratory (by RT-PCR) in all subjects. The nucleotide sequence of RT-PCR material isolated from all patients was identical and belonged to the genotype H group of MuV. The majority of those fifteen patients had low AI, and only two (13%) had high AI. Thus, according to the IgG specific avidity 87% of all patients developed PIR and 13 % patients developed SIR. The level of VF was well confirmed by the PRN titres. The PRN assay with L-3 mumps vaccine strain helped to determine the history vaccination in four cases with the earlier unknown status. Thus, mumps in the previously vaccinated patients presented here were mostly (87%) attributed to primary vaccine failure

Conclusions: For the first time, MuVs were genetically characterized in Belarus. Phylogenetic analysis revealed the presence of genotype H within 2001–2003 years in the city of Minsk, Belarus. Our results here support the notion that total specific IgG antibody levels may not be an accurate reflection of level of protection. For estimating the vaccine failure (VF) the IgM and IgG evaluation seems to be insufficient. In the current study, we mostly took into account IgG avidity and the presence of neutralizing antibodies to evaluate the immune response to MuV. The PRN assay data was very helpful in the confirmation vaccination status and level of VF. Mumps in the previously vaccinated patients presented here were mostly attributed to primary vaccine failure.

Clinical epidemiology of nosocomial infections (POWI, VAP, UTI, BSI, …)

R2120

Airborne fungal contamination in the operating rooms of medical university hospitals, Yazd

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Objectives: Skin is one of the natural defence mechanisms of the human body act as an important barrier to prevent the penetration of pathogen microorganisms. Damaging of the skin in the patient, who is under operation and as a result of the post operating treatment usually using wide spectrum antibiotics, the opportunistic nosocomial agents especially the airborne fungal spores are a danger for patients. The general objective of the present study was to evaluate the types and frequency of filamentous fungi and yeast, present in the air of the operating rooms in the Yazd three major hospitals.
Methods: In the current cross sectional descriptive study, we used the contact plating method for air sampling collecting and culturing. We used 921 plates containing Sabouraud dextrose agar for air sampling of 7 operating rooms in 3 hospitals. The fungi, which isolated were diagnosed by microscopic and macroscopic methods and the results were analysed using SPSS software.
Results: Totally 1115 colonies from 13 genus's of fungi were isolated that *Penicillium* (22.4 %), yeast (19.4 %), *Clodosporium* (18.2 %) and *Aspergillus niger* (13.8%) were the most frequently isolated fungi. Operating rooms of hospital B had the most contaminated rooms in this study. There was seen a statistical significant differential between operating room of 3 hospitals (F = 43.7, Pval = 0). The average isolated fungal spores was 2 in surgery operating room of hospital B known as most contaminated room but in women's operating room of hospital A and the open heart surgery this was 0.6 c.f.u in each sample (F = 17.6%, Pval = 0). However Wednesdays show more contamination in compare with Monday and Saturday but there wasn't seen any statistically significant differences between weekly days (F = 0.45, P val = 0.665).
Conclusion: It seems that the operating rooms in the current study are highly contaminated with fungal spores and it is necessary to have more attention especially in open-heart surgery operating room. It seems that in hospital B the cooler system can act as the important source of contamination and also using the fungal anti-septic is necessary for all rooms.

R2121

Morganella morganii infections in a general tertiary hospital

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Objectives: *Morganella morganii* is a commensal Gram-negative bacillus of the intestinal tract of humans and other mammals and reptiles. Few reports exist in the literature regarding infections caused by this organism.
Methods: A retrospective study at the 650-bed University Hospital of Heraklion, Crete, Greece was performed during a 4-year period (2001–2004) to identify and analyse infections caused by *M. morganii*. Patients in whom the organism was isolated from clinical specimens were identified from the records of the Microbiology laboratory, while their clinical data were collected from the medical records. Definitions of the Centers for Disease Control and Prevention (CDC) were used to identify the infections, while the outcome was recorded as cure or failure.
Results: Twenty-four patients had *M. morganii* isolated from clinical specimens during the study period. Thirteen patients (54%) suffered from skin and soft tissue infections, 5 from pyelonephritis, 3 from female genital tract infections, 1 from pneumonia, 1 from gangrenous appendicitis, and 1 from tonsillitis. *M. morganii* was a constituent of polymicrobial infections in 14 patients (58%). The patients received various antibiotics, i.e., 6 patients received ciprofloxacin, 6 piperacillin/tazobactam, 1 ticarcillin/clavulanic acid, 1 ceftriaxone, 1 imipenem, and 1 cefuroxime monotherapy, whereas the remaining 8 received antibiotic combinations. Two of 24 patients (8%) died, despite antibiotic treatment.
Conclusion: Skin and soft tissue infection was the commonest type of infection due to *M. morganii* in our series. *M. morganii* is commonly a part of polymicrobial infections and can rarely cause fatalities in debilitated patients.

R2122

Investigation of an outbreak caused by multi-resistant *Acinetobacter baumannii* isolates in the ICU of a regional hospital

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Objective: *Acinetobacter baumannii* isolates show increasing relevance in nosocomial infections. Since they can easily develop resistance to several antimicrobials, such infections may be

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difficult to treat even with combination therapy. In our regional hospital *A. baumannii* isolates are scarcely isolated and usually exhibit susceptibility to several antimicrobial classes. In this study we present an outbreak of multi-resistant *A. baumannii* isolates that was undertaken in our ICU during a short period of time.
Methods: Over a period of 10 days in April 2005, 5 *A. baumannii* isolates were recovered from various clinical specimens (blood, bronchial secretions and surgical wounds) obtained from separate patients hospitalized in different wards. Identification of the isolates and antimicrobial susceptibility testing was performed by Microscan system (Dade Behring). Two of the patients were hospitalized in the ICU, 2 in surgical wards and one in a medical ward. However, medical records revealed that the latter 3 patients had previously been admitted to the ICU and subsequently transferred to other wards.
Results: All isolates were identified as *A. baumannii* that exhibited resistance to all carbapenems, fluorocinolones, aminoglycosides, expanded spectrum cephalosporins, as well as to aztreonam and piperacillin/tazobactam. They showed intermediate susceptibility to ampicillin/sulbactam while all were susceptible to colistin. E-test MBL (AB Biodisk, Sweden) did not show production of a metallo-b-lactamase in any of the isolates. In spite of the therapeutic treatment 3 of the patients died from septicaemia. The identical biochemical identification profiles and antimicrobial resistance patterns of the isolates in combination with the fact that all patients were previously admitted in the ICU suggested the hypothesis of nosocomial infection from a common *A. baumannii* strain. An epidemiological investigation was undertaken and an *A. baumannii* isolate showing the same biochemical and antimicrobial susceptibility profiles was isolated from the floor of the ICU. Thereafter, all patients were isolated, strict infection control measures and antiseptic techniques were implemented. No other episodes of multi-resistant *A. baumannii* were noticed.

R2123

The analysis of diagnostics of hospital-acquired pneumonia in the intensive care unit

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Aims: To estimate the recognizing of the fatal hospital-acquired pneumonia (HAP) in ICU.
Material and methods: We analysed hospital charts and autopsy reports of 278 patients (145 male and 133 female) that died during 2001–2004 year in ICU of the city hospital. The presence of the clinic symptoms HAP, the coverage by X-ray examination of chest, microbiology examination of sputum, the diagnostics HAP during life, coincidence of clinical and pathologoanatomic diagnoses, adequacy of antimicrobial therapy, average terms of medical treatment was evaluated.
Results: HAP was diagnosed at 49.3% of patients during life. Average age of the patients was 63.7 ± 2.3 years. Stroke (63.8%), ischaemic heart disease (77.0%), hypertonic disease (59.5%), chronic obstructive pulmonary diseases (49.2%), surgical operation (15.0%), diabetes mellitus (12.8%) were prevailed in the structure of patients morbidity. Clinic symptoms of HAP were observed at 57% of patients. The X-ray examination of chest was conducted 40.5% of patients. Bacteriological examinations were provided 18% of patients. Causal pathogens were obtained at 3.6 % of patients. There were *S. aureus* (7 strains), *S. pneumoniae* (1 strains), *P. vulgaris* (1 strains), *P. aeruginosa* (2 strains). During life HAP was diagnosed at 51.4% of patients, from them in the day of death at 18.9%, is exposed only autopsy at 49.6%. HAP by mistake was determined at 13.0%. The antimicrobial therapy was prescribed by 68%, however it was appointed in 2 days from the origin of the HAP signs. Average duration of stay in ICU was 7.6 ± 3.2 day.
Conclusion: Underestimation of the clinic symptoms HAP, the tardy X-ray examination of chest results in the late diagnostics HAP at severe patients in ICU, to the postponement of prescription of the proper antibacterial treatment and death of patient.

R2124

Antimicrobial resistance of *Escherichia coli* and *Proteus mirabilis* isolated in bacteraemia in a tertiary care hospital

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Objectives: The aim of this study was to determine the antimicrobial resistance in *Escherichia coli* and *Proteus mirabilis* isolated from blood cultures.
Methods: The study period covered four years from January 2001 to December 2004. The blood cultures were performed using the BacT/Alert 30 automated system. Identification of microorganisms and susceptibility testing were performed using the VITEK 2 automated system (bioMerieux France). Confirmation of extended spectrum beta-lactamases (ESBL) production was performed by double-disk-synergy test (DDST).The three dimensional test was employed to detect AmpC-like beta-lactamases.
Results: During the study period a total of 96 *E. coli* and 23 *P. mirabilis* were isolated from blood cultures sent in our laboratory. *E. coli* isolates were obtained from patients hospitalized in internal medicine department (n = 40; 42%), pediatrics units (n = 28; 29.2%), surgical units (n = 14; 14.4%), nephrology department (n = 14;14.4%). *P. mirabilis* isolates were recovered from patients hospitalized mostly in intensive care unit (n = 14; 61%), internal medicine department (n = 5; 19.5%),surgical department (n = 5; 19.5%).Resistance rates to commonly used antimicrobials like ampicillin (AMP), amoxicillin/clavulanate (AMC), amikacin (AN), ciprofloxacin (CIP), co-trimoxazole (STX) and third generation cephalosporins were 57%, 21%, 4%, 17% a, 30% and 23% respectively for *E. coli* . *P. mirabilis* isolates presented 96% resistance to AMP and AMC, 96% to CIP and STX, 30% to AN and 91% resistance to third generation cephalosporins. All isolates of *E. coli* and *P.mirabilis* were susceptible to imipenem. Out of the 22 *E. coli* resistant to third generation cephalosporins 20(91%) were found to be ESBL producers. For *P. mirabilis* 21 out of 23 isolates were resistant to third generation cephalosporins and cefoxitin. All strains gave positive the three dimensional test suggesting an AmpC type beta lactamase production.
Conclusion: Highly resistant *P. mirabilis* and *E. coli* were responsible for bacteraemias in our hospital. The high incidence of resistance to third generation cephalosporins was mainly due to AmpC-type beta-lactamases in *P. mirabilis* and ESBL production in *E. coli*. Continuous surveillance studies in order to observe changes in resistance, which might affect therapeutic choices are necessary.

R2125

Peritonitis due to *Corynebacterium striatum* in a CAPD patient

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Coryneforms are occasionally pathogenic microorganisms implicated in several infections like sepsis in immunocompromised patients, endocarditis in patients with prosthetic valves,

post-traumatic meningitis and peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). A case of severe peritonitis from *Corynebacterium striatum* in a CAPD patient is investigated. It regards a 58-year-old woman with chronic renal failure who has been under CAPD therapy since 1993. The patient developed several peritonitis episodes mainly caused by *Staphylococcus* spp. In March 2003 the patient presented obvious peritonitis accompanied by abdomen pain, fever, tachycardia, and turbid peritoneal fluid with 4.500 cells/mm mainly neutrophils (95%)and lymphocytes 5%. Initially the patient was administered with empiric therapy with cefuroxime and amikacin (according to the CAPD Unit's protocol) but without significant improvement. After a transient decrease in the number of cells in the peritoneal fluid, the cells increased up to 1050/mm despite the continuation of the antibiotic therapy. From the culture of the peritoneal fluid (two bags and exit-site of peritoneal catheter) a coryneform bacterium was isolated, which was identified as *C. striatum*. The antibiotic susceptibility test indicated that the bacterium was sensitive to vancomycin, teicoplanin, chlorampenicol, rifampicin, levofloxacin, cefotaxime but resistant to ampicillin, cephalothin, cefuroxime and amikacin. This suggested the discontinuation of the empiric therapy, and the patient was subsequently administered with vancomycin and rifampicin resulting to the successful treatment of the peritonitis. The culture and the isolation of the bacterium was performed according to the common methods of the laboratory and the bacterium was identified from the morphology of the colonies, Gram stain and API Coryne. The antibiotic susceptibility test was performed using the agar diffusion disk method. Peritonitis is a frequent complication in CAPD patients and is usually caused by *Staphylococcus* spp. Coryneforms are an uncommon cause of peritonitis, they are usually sensitive to the antibiotics of the empiric therapy of CAPD peritonitis and may generally induce mild infections. This case was unusual considering the severity of the infection as well as the increased resistance of the bacterium to the antibiotics.

R2126

A six-year prospective surveillance study for nosocomial infections in neurology unit

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Objectives: The aim of this study was to assess the epidemiology of nosocomial infection (NI) s in neurology unit in a university hospital.
Methods: The study was carried out at the Dicle University Hospital, Diyarbakir, Turkey (1050-bed). This study was performed prospectively, from 1st January 1999 to 31st December 2004. Active surveillance for NIs were performed by infection control team, using the criteria proposed by the Centers for Diseases Control and prevention (CDC) and National Nasocomial Infections Suveillance (NNIS) methodology.
Results: During six-year follow up period, 219 nosocomial infection episodes were detected in 203 patients out of 3323 inpatients. The overall incidence rates (NI/100) and incidence densities (NI/1000 days at risk) of nosocomial infections were 6.6% and 4.4/1000 patients-days, respectively. The most common nosocomial infections by primary site were urinary tract infection (Table). The most prevalent microorganisms were *Escherichia coli* (27%), *Klebsiella* spp. (14%), *Pseudomanas aeruginosa* (13%), *Enterobacter* spp (12%), coagulase-negative staphylococci (10%) and *Staphylococcus aureus* (7%).
Conclusion: The results may contribute to observe the magnitude and characteristics of NIs and to plan and evaluate policies and guidelines of infection control in neurology units.

Table. Nosocomial infections in neurology unit: site-specific incidence rates and incidence densities.

Type of NI	Number of infections	Percent of total infections	Incidence rates	Incidence densities
Urinary tract infection	96	44,2	2,9	1,9
Infection of skin	66	30,4	2,0	1,3
Pneumonia	39	18,0	1,2	0,8
Bloodstream infection	11	5,1	0,3	0,2
Others*	7	2,3	0,2	0,1
Total	219	100,0	6,5	4,3

*Catheter related local infection, diarrhea, sepsis, wound infection.

R2127

Septicaemia in paediatric surgery in Kuwait: a four-year prospective study

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Introduction: Blood stream infections account for most of the noscomial infections in the neonatal and paediatric intensive care units. The objectives of this study were to determine the incidence, risk factors, microbiological aetiology and antimicrobial susceptibility pattern of septicaemia in paediatric surgery patients.
Methods: Over a period of four years (2001–2004), all the documented cases of septicaemia were investigated. Risk factors such as low birth weight, prematurity, ICU stay, use of ventilators underlying disease and surgical interventions were determined. Blood and other cultures were incubated. All positive cultures were subjected to identification and antimicrobial susceptibility testing. Outcome of septicaemia was then analysed.
Results: 58 patients who developed 80 episodes of microbiologically documented bacterial and fungal sepsis were analysed. 23 were neonates and 35 were infants. Half of the patients were admitted to the ICU. Twenty-five patients had gastrointestional problems with surgical interventions. The 31 Gram-negative septic episodes included *Pseudomonas aeruginosa*, *Enterobacteriacae*, *Acinetobacter baumanii* and *Stenotrophomonas maltophilia*. The 39 Gram-positive septic episodes included coagulase negative Staphylococcus, *Staphylococcus aureus*, *Enterococcus faecalis* and *Viridans streptococcus*. Candidemia accounted for 10 episodes. All Gram-negative isolates were uniformly susceptible to third generation cephalosporins, aminoglycosides, piperacillin/tazobactam and carbapenems. All Gram-positive isolates were uniformly susceptible to Glycopeptides.
Conclusions: The main risk factors for septicaemia in pediatric surgery includes prematurity, ICU stay and underlying disease. The main etiological pathogens included coagulase negative staphylococcus, *Pseudomonas aeruginosa* and *Enterobacteriaceae*; empirical therapy with piperacillin/tazobactam for Gram-negative sepsis and glycopeptides for Gram-positive sepsis proved to be effective.

R2128

Increasing *Candida* spp. as a cause of nosocomial urinary tract infections: a 6-month survey in Iran

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(Tehran, IR)

Objectives: To determine the causative agents of hospital acquired urinary tract infections and susceptibility testing of isolated urinary pathogens.
Methods: Between April to October 2005 all urine isolates from hospitalized patients in Milad hospital of Tehran were

collected. Cultures of urine samples were performed by conventional methods. Antimicrobial susceptibility testing was performed by using disk diffusion methods as recommended by NCCLS.
Results: 83 isolates from urine culture were analyzed. There were 3 groups of causative agents: Gram-negative rods including *E. coli* – 28 isolates, *Klebsiella pneumoniae* -6, *K. oxytoca* 1, *K. planticola* -1, *Pseudomonas aeruginosa* –5, *Acinetobacter baumnnii* – 6 and other. Gram-negative rods-5. *Candida* spp. including *C. albicans* with 9 isolates and other *Candida* spp. with 11 isolates in total comprised 23% of all causative agents. Enterococci including *E. faecalis* with 9 isolates and *E. faecium* with 3 isolates were the only Gram-positive cocci isolates. Drug resistant was prevalent among all isolated organism. For example 96% of isolated *E. cole* were resistant to ampicillin. Resitance of this organism to nalidixic acid co-trimoxazol ofloxacin, ceftizoxim, cefotaxim were 70%, 51%, 62%, 62% and 7% respectively. All isolates of *Acinetobacter baumannii* and *Klebsiella* spp. and enterococci were multidrug resistant.
Conclusion: This study reveals that *Candida* spp. after *E. coli* were the predominant organism isolated from urinary tract infection in our hospital. Catheterization was the predominant predisposing factor.

R2129

Asymptomatic bacteriuria prevalence among pregnant women in a university hospital, Aydin, Turkey

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Objective: Asymptomatic bacteriuria is a risk factor for development of pyelohephritis, which is a cause of the morbidity and mortality of foetus and mother. In the present study, asymptomatic bacteriuria was screened among the pregnant women who presented themselves to Adnan Menderes University Hospital for antenatal care.
Methods: Urine samples were collected from 102 women during each trimester of pregnancy. Patients with urinary symptoms, active vaginal bleeding, or who were previously on antibiotics therapy, were excluded from the study. Bacteriuria considered positive if the urine cultures grew $>10^5$ colony-forming units of a single uropathogen.
Results: The average age of the asymptomatic bacteriuria positive women were 28.2 year old which was not significantly different from the average for all pregnant women included in the study (27.7 year-old). Among 102 women, 12 had asymptomatic bacteriuria in 1st trimester, 1 in second and 3 in third trimester. A total of 14 pregnant women had asymptomatic bacteriuria during at least one of the trimesters. *Escherichia coli* was the commonest bacteria isolated (6 isolates) during 1st trimester followed by group B Streptococci (3 isolates). One coagulase (-) *Staphylococci*, one *Enterococci* spp, and one *Proteus mirabilis* were also isolated during the first trimester. One pregnant woman had *E. coli* during all of the three trimesters despite treatment and 2 women had *S. aureus* and *E. coli* only during third trimester.
Conclusion: The prevalence of asymptomatic bacteriuria found to be 11.7%, 0.9%, and 2.9% during first, second and third trimester, respectively, among pregnant women who attended to antenatal care unit of Adnan Menderes University Hospital, Turkey. Pregnant women should be screened for asymptomatic bacteriuria and treated to avoid serious complications for both mother and foetus.

R2130

Infective endocarditis: results of a 5-year microbiological survey in the cardiology centre, Iasi, Romania

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Objectives: To characterize the epidemiological and microbiological spectrum of infective endocarditis in the Cardiology Center of Iasi, Romania.
Methods: We reviewed all episodes of infective endocarditis from April 2001 to September 2005 in the Cardiology Center of Iasi. Infective endocarditis was diagnosed using Duke's criteria. Classification of infective endocarditis based on the etiological agents was related to epidemiological characteristics including age, gender, nature of the injured valve, mortality rates and antibiotic treatment before blood culture was drawn.
Results: During the 5 years that were investigated, there were 60 definite infective endocarditis 3978 admissions. There were 44 (73.33%) episodes of endocarditis with positive blood cultures and 16 (26.66%) with negative blood culture, involving 36 males and 24 females (mean age = 47.93 years old). The most frequently involved sites of infection were the native valves 46 (76.66%), especially the aortic ones 39 (65%). In descending order of frequency, we isolated: 43.18% gram-positive cocci (19 strains), 5.90% gram-positive bacilli (7), 13.63% stable cell-wall deficient forms (6), HACEK-group 11.36% (5), fungi 6.81% (3), anaerobes 6.81% (3), gram-negative bacilli 2.27% (1). From the total 16 strains of cell-wall deficient forms (36.36%), 10 returned to their typical morphology (5 gram-positive cocci, 2 Gram-positive bacilli, 2 anaerobes, 1 gram-positive bacillus) and 6 were L-stable.
Conclusion: 1. Our study found an increased frequency of cell-wall deficient forms. With only one exception all these forms were isolated from patients under beta-lactams therapy ($p = 0.002$). 2. The lack of serological tests for Coxiella and Brucella may explain, in part, the high percentage of negative blood cultures, however, we report cell wall deficient forms as another possible cause for negative blood cultures. 3. Not significant correlations were observed between causative agents and age, gender or valve type. Only HACEK group was associated with male gender ($p < 0.05$). 4. Microscopic detection of L forms appears early enough and may be improved in order to adjust the therapy. The right treatment protocols remain to be established.

R2131

Postsurgical meningitis: the role of the infectious diseases specialist

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Introduction: Postsurgical meningitis is a rare complication of neurosurgical procedures with relevant morbidity and mortality. Highly resistant bacteria are often implicated, and it is difficult to get adequate CSF concentrations of some antimicrobial agents.
Methods: Chart review of postsurical meningitis treated by neurosurgeons in collaboration with an infectious diseases physician since 2003.
Results: 15 patient were analysed, 9 were males and 6 females. Median age was 40 ± 21.8 years, (20–87). 10 patients (66.6%) had history of brain tumor, 2 (13.3%) cerebral cyst, 2 (13.3%) had Arnold Chiari Malformation and 1 patient (6.6%) had vertebral

fracture. Most common symptoms: fever 14 cases (93.3%), meningismus in 6 (40%), headache in 5 (33.3%) and CSF fistula in 5 (33.3%). CSF was obtained by lumbar puncture in 9 cases (60%) and by intraventricular drainage system in 4 (26.7%). CSF findings were consistent with bacterial meningitis. Aetiologies: Coagulase negative *Staphylococci* (3), *Enterobacteriaceae* (5), Non-fermenters (4) and no isolation (3). 12 patients received antimicrobials agents previously, most of them amoxicillin-clavulanate (10). Dosages of antimicrobial agents of initial therapy were corrected in 13 of 15 patients by the infectious diseases physician. Most visual empiric therapy was ceftazidime + vancomycin (10). Most frequent consolidation therapies were cephalosporin + quinolone (4) and rifampin + vancomycin (3). Duration of therapy was 18.4 ± 3.2 days (14–21). One patient died and another relapsed successfully treated.
Conclusions: Several factors make difficult the management of patients with postsurgical meningitis. Cooperation between neurosurgeons and infectious diseases specialists optimizes the treatment of these patients. The infectious diseases physician improves initial empirical antimicrobial choice and dosages, adjust the antibiotic therapy once a pathogen is recovered, and establishes the proper duration of treatment and clinical controls.

R2132

Efficacy of doxycycline in treatment of bone and prosthetic joint infections

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Objectives: Few data exist on the usefulness of doxycycline (DOX) in osteoarticular infections associated or not to the presence of a biomaterial. The aim of this study was to evaluate the efficacy and safety of DOX for the treatment of patients with bone and prosthetic joint infections (BPJI).
Methods: Patients diagnosed of BPJI treated with DOX from January 2001 to December 2004 were assessed for the followings variables: age, gender, underlying diagnosis, microbiological isolates, treatment and outcome. Only patients with more than 10 days of DOX treatment were included. Finally, the medical records of 14 patients were reviewed. All patients had proven BPJI and underlying diseases included: Prosthetic joint infections (7), osteomyelitis (4) and chronic osteomyelitis associated with orthopaedic hardware (3). Patients were monitored for relapsing infection for as long as possible after the end of treatment.
Results: Monomicrobial infections were the most common (78%). Isolated microorganisms included: Coagulase negative staphylococci (CNS) (7), methicillin resistant *Staphylococcus aureus* (4), methicillin susceptible *S. aureus* (3), *Corynebacterium* spp. (2), and *Streptococcus viridans* (1). Four patients received initially DOX and ten patients received DOX after several courses of ineffective antibiotherapy (DOX was used as ''rescue'' therapy). Successful outcome was observed in 64% (9 of 14) of patients, failure of treatment was observed in 28% (4 of 14) of the patients, and 1 episode required change of the treatment because of antibiotic resistance. The infections in patients with failure were caused by the following microorganisms (3 CNS and 1 CNS + *S. aureus*). Patients received DOX for a median of 120 days (range 30 to 240). No serious side effects were observed. Successful outcome was observed in all the patients diagnosed of osteomyelitis. Clinical cure was achieved in 60% of patients in which biomaterial withdrawal was performed. This rate of success was superior to that seen in patients in which biomaterial was retained (40%).
Conclusions: Although the number of subjects in this analysis is small, these data suggest that doxycycline may be a reasonable treatment alternative for patients with certain types of osteoarticular infections.

R2133

Bacterial colonisation of vascular prostheses in re-operated patients

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Objectives: The aim of our study was to estimate bacterial colonisation of the vascular prostheses in re-operated patients depending on the number of thrombectomies performed after primary reconstruction.
Methods: 18 patients (15 M, 3 F; mean age 62 years) without clinical symptoms of graft infection, on whom graft thrombectomies were done as an emergency operations took part in our research. Unsealed Dacron prostheses were used in all patients for primary reconstruction. Swabs obtained from outer and inner surfaces of the prostheses during operation were inoculated on the bacteriological media and incubated in aerobic conditions. Species of bacteria were identified on the ground of their biochemical characteristics. Antimicrobial susceptibility of isolated strains was evaluated by disc-diffusion method.
Results (in table):

Consecutive thrombectomy	Number of cases	Time from primary reconstruction	Isolated bacterial strains	
			Outer surface of prosthesis	Inner surface of prosthesis
1st	7	5 months	-	-
		6 months	-	-
		8 months	-	-
		10 months	-	-
		11 months	-	-
		17 months	-	-
		9 years	-	*S. epidermidis*
2nd	3	5 months	-	*S. aureus MRSA, MLS_B* *S. epidermidis*
		20 months	-	-
		25 months	-	-
3rd	2	15 months	-	-
		12 years	*S. aureus*	-
4th	2	3 years	*S. epidermidis MRCNS*	*S. epidermidis MRCNS*
		4 years	*S. epidermidis MRCNS*	*S. epidermidis MRCNS*
5th	1	3 years	*Corynebacterium amycolatum*	-
6th	1	2 months	*S. aureus MRSA, MLS_B*	*S. aureus MRSA, MLS_B*
7th	1	7 years	*S. aureus MRSA*	*S. aureus MRSA*
8th	1	10 years	*S. aureus*	*S. aureus*

(-) no growth detected

Conclusions: 1. Bacterial colonisation of prosthesis occurred in 50% of examined patients (without clinical signs of infection). 2. Most frequently isolated species proved to be *S. aureus* and *S. epidermidis*. 3. Every consecutive thrombectomy performed on a given graft increases the probability of its bacterial colonisation.

R2134

Pseudomonas aeruginosa bacteraemia: analysis of 262 episodes

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Objective: The aim of this study is to evaluate the trend in incidence of *Pseudomonas aeruginosa* bacteraemia (PAB), underlying diseases, source of infection, antibiotic treatment and mortality.
Methods: Prospective study of all patients with PAB. Study period Nov 93–Jun 05. Blood cultures were performed by means of BACTEC 9240. Te infection control team studies every patient with positive blood cultures. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, source of bacteremia, nosocomial/community acquired, antibiotic susceptibility, treatment and outcome.
Results: 262 episodes, 177 males (67.56%), age 67.17% >60years, Underlying illnesses: Neoplasia 41.92%, Diabetes 16.79%, COPD 12.6%, HIV 8.4%. Hospital acquired 49.62% ranging over time from 38.7% to 57.6%. Source of PAB: Primary 29.0%, Urinary tract 21%%, Respiratory 19.47%, Abdominal 10.31%. Predisposing conditions: mechanical ventilation 8.8%, ICU 9.9%, surgery 13%, urinary catheter 25.19%, antibiotic 42.37%, intravascular catheter 48.5%, immunosuppressive therapy 27.1%, neutropenia: 16.41%. Inappropriate antibiotic therapy 20.23%. Crude mortality 32.06. Time to positivity of blood cultures 80.92% less than 48 h. Polymicrobial bacteremia: 20.23%. Sensitivity to antibiotics: Amikacin 94.7%, Imipenem 90.91%, Ceftazidime 89.02%, Gentamicin 86.74%, and Ciprofloxacin 77.65%. Nosocomial bacteremia: 130 cases, 63.85% males, 56.1% older than 60 y. Underlying illnesses: neoplasia 44.6%, VIH 7.7%. Source of bacteraemia: primary 33%, urinary 20.7%, respiratory 14.6%, catheter related 10%, surgical site 5.3%. Crude mortality nosocomial acquired: 35.66%.
Conclusions: The incidence of PAB in our hospital remained stable over the last 11 years with a peak incidence in 1997 and 2002. Neoplasia was the most common underlying disease. Sensitivity to antibiotics didn't change over time and the most active antibiotics are Amikacin, Imipenem and Ceftazidime.

R2135

Bacteriology of burn wound infections in a burn care centre, Mashhad, Iran and determination of the antibiotic susceptibility patterns of isolated bacteria

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Objectives: Burn site infections, as a nosocomial infection, are a major cause of mortality and morbidity in burn units. It is crucial for every burn institution to determine the pattern of microbial colonization in burn wound and the antimicrobial susceptibility profiles. This study was carried out on patients admitted to the Burn Unit, Emam Reza University Hospital, Mashhad, with the aim to verify the pattern of microbial colonization of burn wounds and determine the susceptibility of isolated bacteria against antibiotics.
Methods: Throughout the study period 344 samples were obtained from the 126 burn patients. After isolation and identification of bacteria, their susceptibility patterns against 14 common antibiotics were studied utilizing agar diffusion method.
Results: The results show that 27.7% of wounds were sterile at first dressing, but by passing the time the rate of contamination increase in the way that only 5% of wound were sterile at the third week. *Pseudomonas aeroginosa* was the most common isolated bacterium (31.7%). Most of bacteria were resistant against amoxicillin but ciprofloxacin and imipenem was the most effective antibiotics among tested antibiotics.
Conclusion: The obtained results about causative agents and their antibiotic susceptibility pattern particularly about gram negative bacilli and *Staphylococcus aureus* emphasis that we not only aren't in suitable position but also are very far from eradication of these infections, so implementation of improved infection control practices and policies is urgently required.

R2136

Unusual anaerobic Gram-negative bacterium from an abscess

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Bilophila wadsworthia is a Gram-negative anaerobic bacterium, which has been recently described (1989) and can rarely be isolated from infections. These infections usually involve gangrenous appendicitis and less frequently absesses of the peritoneal cavity and bacteraemia. This study presents the case of a 74-year-old man who was admitted to the Urologic clinic with an absesse of the left semiscrotum, reporting symptoms of high fever and diabetes mellitus. The absesse was opened and drained and the collected pus was send to the laboratory. The sample was cultured using the common culture media. In the aerobic conditions culture *Escherichia coli* and *Morganella morganii* were isolated. Under anaerobic contitions small, confluence, transparent, gray colonies were grown, after four days of incubations. These colonies appeared, in the Gram stain, as a Gram (-) negative bacterium of a medium size. The bacterium was strongly catalase positive, slightly urease positive, asaccharolytic and nitrate-nitrite reducing. Using the API 20A system the microorganism was identified as a *Bacteroides corrodens*, which was not in agreement with the biochemical properties of the isolated bacterium (catalase positive, urease slightly positive). After subcultures in bacteroides bile esculin agar, small, transparent colonies, with black pigment in the centre were isolated which were considered as *Bilophila wadsworthia*. After the antibiotic susceptibility tests of all the isolated bacteria, (*E. coli*, *M. morganii*, *B. wadsworthia*) the patient was administered with triple antibiotic therapy containing metronidazole, amikacin and cefuroxim. Although *B. wadsworthia* is part of the normal flora of the intestine, it is not commonly isolated in inflammations and absesses of the appendicitis, or of the peritoneal cavity and in cases of bacteraeimia. This is due to its growth difficulties in the common culture media, as a slowly growing bacterium, as well as to the identifications difficulties when using the different identification microsystems for anaerobic bacteria.

R2137

Investigating an *Acinetobacter baumannii* outbreak in a Portuguese hospital

J. Mapril *(Carnaxide, PT)*

Objectives: To describe the epidemiology, antimicrobial susceptibility ang genomic profile of an outbreak of multiresistent *Acinetobacter baumannii* in a Portuguese hospital.
Methods: During 3 months, from May to July, 12 patients were detected with multiresistent *Acinetobacter baumannii* mainly in respiratory secretions (7), but also in blood (1), urine (4),

exudates (2) and catheter (2). Patients were cared in two units: Internal Medicine/Nephrology Department and Cardiothoracic Surgery Unit. Species identification was done by using the Vitek system (bioMerieux, France). Antimicrobial susceptibility testing was done by using the Vitek automated microdilution system and by disk diffusion method. Six strains were studied by RAPD and PFGE (5 from the Internal Medicine/Nephrology Department and 1 from the Cardiothoracic Surgery Unit).
Results: 12 patients had clinical relevant multiresistent *Acinetobacter baumannii* with similar profiles on TSA. For those 6 strains studied 4 proved to belong to the same cluster ''A'' (all patients from the Internal Medicine/Nephrology Department) and the other 2 to different clusters ''B'' and ''C'' (1 from the Internal Medicine/Nephrology Department and 1 from the Cardiothoracic Surgery Unit).
Conclusions: The examined isolates from the Internal Medicine/Nephrology Department were compatible with the spread of clone (A) between the studied patients. Nevertheless, other clones were also circulating in the hospital at the same time.

R2138

Preliminary study on colonisation of *A. baumannii* in paediatric intensive care unit

H. Guducuoglu, K. Aygul, G. Yaman, H. Izci, M. Berktas *(Van, TR)*

Objectives: In recent months, there have been several outbreaks in various Pediatric Intensive Care Units (PICU) in our country with high mortality rates. For this reason, we planned to investigate the possible colonisations in PICU of our hospital and evaluate the clonal relatedness of the clinical samples. And we also planned to eradicate the pathogen microorganisms from this unit to prevent an outbreak.
Methods: For this reason many samples were collected from PICU of our hospital and then the isolated microorganisms were identified by PhoenixTM NMIC/ID, Becton Dickinson, USA kits. The results were also confirmed by conventional methods.
Results: As a result, a total of 23 microorganisms were isolated from the samples of the screening. Twenty of them were *A. baumannii* and *A. baumannii/calcoaceticus* complex, three of them were *Escherichia coli*, *Enterobacter cloacae* and *Acinetobacter lwoffii/haemolyticus*. *A. baumannii* and *A.baumannii/calcoaceticus* complex strains were isolated from different samples like ventilation device, entubation sets, medicine pumps of some patients, naked hand of mothers and staffs, and head, neck and abdominal sites of some patients. These microorganisms were evaluated as colonisation. The antimicrobial resistance rates of *A. baumannii* and *A. baumannii/calcoaceticus* complex strains were as followed: amikacin 60%, aztreonam 100%, cefepime 100%, ceftazidime 100%, chloramphenicol 100%, ciprofloxacin 15%, gentamicin 100%, imipenem 5%, levofloxacin 15%, meropenem 10%, piperacillin 100%, trimethoprim/sulphamethoxazole 20%.
Conclusion: As a result of this study, we detected a colonisation of *A. baumannii* and *A. baumannii/calcoaceticus* complex in our PICU and it was found out that these microorganisms have gained high resistance to multiple antimicrobial agents. The clonal relatedness and the source of origin of these strains will be investigated by pulsed field gel electrophoresis. Meanwhile if any new *Acinetobacter* strains will be isolated from clinical samples from PICU of our hospital, the new isolates will be compared and the clonal relatedness will be investigated with the colonized bacteria. We gave advices for the eradication of *Acinetobacter*, education of the employees and taking serious preventions on controlling colonisations.

R2139

One-year infection survey in a cardiac surgery unit

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Objective: Aim of this study was to evaluate infection rate and type after cardiac surgery, including both those typical of ICUs and those strictly related to surgery, in order to implement infection control programmes based on epidemiology and risk factors.
Methods: Between April 2004 and March 2005 the number and type of surgical procedures performed in the Cardiac Surgery Division of Policlinico San Matteo, Pavia, was matched with clinical and microbiological data from our Institute. Infection was defined according to CDC criteria.
Results: A total of 885 patients who had undergone cardiac surgery with either traditional or a minimally invasive approach, including heart transplant patients and left ventricular assistance device users, were included. 100/885 patients (11.3%), 67 male and 33 female, presented at least one infection and 57/100 suffered from more than one infection, for a total of 261 infectious episodes. The most common complications were bloodstream infections (42.5%), UTI (21.8%), VAP (11.1%) and surgical site infections (8%). 20/111 bloodstream infections were line sepsis and in 10 cases we observed recurrent bacteraemia from the same microorganism, probably due to indwelling catheters or misdiagnosed infectious foci. We observed 3 cases of invasive fungal infections. Incidence of infections was higher in patients undergoing combined procedures. *Staphylococcus aureus* with a high prevalence of methicillin-resistant strain (62.9%) and *Pseudomonas aeruginosa* were the most common pathogens. We observed contained outbreaks of vancomycin-resistant enterococci and *Enterobacteriaceae* producing ESBL. A large number of episodes were polymicrobial, especially sepsis and VAP. The mortality attributable to infections was higher than overall (32 vs. 7.9%), with death occurring mainly during the first 30 days after aortic surgery and re-operations.
Conclusion: In the literature only few data on post-cardiac surgery infections are available but for surgical site infections. Our survey documented that the incidence of infections was 11%, mainly consisting of life-threatening infections. They had a significant effect on mortality and their incidence increased with a greater length of stay in the ICU. This knowledge could be used for optimising empiric antimicrobial treatments and implementing preventive strategies.

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Travel medicine, tropical and parasitic diseases

R2140

Characteristics in laboratory analysis of leptospirosis

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Leptospirosis in an animal-disease, which is caused by leptospira. The person who is contaminated with leptospira, according to the kind of the bacteria, is likely to stay: asymptomatic, to have a mild self-containing, non-icteric version of the disease or finally to sustain an acute often lethal condition (Weil–syndrome). Responsible for contamination to the human are domestic or wild animals, mostly rats and mice. The target is to analyse the laboratories profile of patients with leptospirosis who have been hospitalized in the pathological clinic of our hospital.

Material: In a two-year period of time from 2002–2004, 5 five patients have been hospitalized, one woman and four men aged from 42 to 78 with symptoms compatible to leptospirosis. Two of them developed Weil syndrome and were transferred to special center in third grade hospital.

Results: From the laboratory profile: anaemia 5/5 (Ht-average 29%), leucocitopenia – 2/5 (average 3500), leucocytosis 3/5 (average 17000) thrombocytopenia 5/5 (average 65000). Liver dysfunction 5/5 (SGOT av. 99/ SGPT av.84/ TBILL av.1.6/ GGT av.288/ ALP av. 456), CPK av.725. Renal dysfunction –5/5 (creatinine av. 3.5). Two of the five patients developed Weil-syndrome (acute renal failure, haemmoragic signs and severe liver dysfunction) and were transferred to third grade hospitals.

Conclusion: The laboratory profile of patients with leptospirosis, apart from the multi-organic dysfunction is not characteristic. This makes the taking of the patient's history of fundamental significance. According to the centre of information related to the Weil disease, if a patient has a clinical history, which is likely to refer to the disease, he must be considered as being ill until this has been excluded. Because of the rising incidence, the severance of the clinical symptoms, leptospirosis is being included to the obligatory declared cases to the national epidemiological center of observation and intervention.

R2141

The role of the veterinary laboratory agency: confirmation of brucellosis from human isolates

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Infection with *Brucella* primarily presents as 'undulant fever' in man. Clinical diagnosis is often difficult; fever, night sweats, headache and arthralgia are debilitating and similar to other zoonotic diseases. There are no vaccines suitable for humans, complications such as osteoarthritis may occur and the chronic stage of the illness is difficult to treat. Brucellosis is sometimes misdiagnosed, especially if a full history of the patient is unknown. Those at most risk are from countries where the disease is endemic (much of the world) and are farmers, abattoir workers, butchers, veterinarians and those who consume unpasteurised milk or milk products. To assist in diagnosis, consideration must be given regarding the possibility that the patient may have travelled or lived abroad, exposure from a low infectious dose can lead to these symptoms. Laboratory workers are at risk, at VLA we have Containment (biosafety) Level 3 facilities that enable us to handle the organism safely. The laboratory criteria for diagnosis isolation of the organism from a clinical specimen and/or rise in serum antibody level.

We are OIE FAO WHO reference laboratory for brucellosis, engaged on surveillance and research on the disease primarily in animals. Due to our expertise and facilities we are able to confirm isolates referred to us from our Health Protection Agency. We perform 'classical' and more rapid molecular diagnosis and can identify isolates to biovar level, including information that can be used to help trace the route of infection. These methods are described in the presentation.

R2142

Pyrexia of unknown origin complicated by dengue fever and pseudo-rupture of the gallbladder: a case report

S.K. Surrun, C.K. Thomas-Golbanov *(Singapore, SG)*

Introduction: Pyrexia of unknown origin is one of the most difficult clinical problems to elucidate. We present a patient with pyrexia of unknown origin complicated by dengue fever and pseudo-rupture of the gallbladder.

Case report: A 64-year-old Chinese lady, without any previous significant medical history, was admitted with fever of two weeks' duration. The clinical examination was unremarkable. The blood investigations revealed thrombocytopenia and a raised ESR. The initial laboratory work-up was normal but the paired dengue serology was positive. The full autoimmune serology was negative and complement levels were normal. The chest radiograph was normal. During the hospital stay she developed right upper quadrant abdominal pain and a CT scan of the chest and abdomen showed fluid collection around the gallbladder suggestive of a perforation. A cholecystetomy was performed but the gallbladder looked normal and the histopathology was also normal. After the operation the patient improved but the fever persisted. The clinical examination did not reveal any focus of infection. Repeat laboratory work-up was normal except for a raised ESR and hypoalbuminaemia. Repeat CT scan of the chest and abdomen showed minimal pleural effusion. The bone marrow trephine biopsy was normal.

Discussion: Our patient had pyrexia of unknown origin and the episode of dengue fever seemed to have been superimposed on it. However the CT-scan findings of a possible rupture of the gallbladder and the fact that dengue fever is associated with cholecystitis prompted us to do a cholecystectomy. The operative and histological findings revealed a normal gallbladder confirming that the CT-scan findings were that of a pseudo-rupture of the gallbladder. After the operation the patient improved but the fever persisted and the clinical examination was unremarkable. As the repeat laboratory investigations was normal except for a raised ESR and hypoalbuminaemia, the CT-scan of the thorax and abdomen unremarkable, the trephine bone marrow biopsy normal and the auto-immune serology negative, the problem of pyrexia of unknown origin was still present and unresolved.

Conclusion: Pyrexia of unknown origin can be even more challenging when associated with dengue fever and its complications.

R2143

Sternoclavicular arthritis as first manifestation of brucellosis: a case report

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Objectives: In this report we aimed to attract attention to this rare complication of brucellosis. Sternoclavicular arthritis is uncommon for brucellosis. We report a 44-year old male patient presenting with one month history of fever, night sweat, left sternoclavicular swelling and pain over the sternum. Physical examination revealed a fluctuant mass over the body of sternum extending to the left parasternal region. Ultrasound examination showed focal collection with internal echoes representing abscess in the left parasternal region. Pus material obtained by US- guided needle aspiration yielded *Brucella melitensis*. The patient was cured with medical therapy.
Conclusion: Brucellosis should be considered in the differential diagnosis of sternoclavicular arthritis even in the patients without immunosuppression and in intravenous drug users particularly living in endemic areas.

R2144

Myiasis on the scalp by *Chrysomya bezziana*

H. Davami *(Jahrom, IR)*

Objectives: Myiasis is the invasion of tissue of animal by the larval stage of flies (diptera). It is an accidental infestation in human. *Chrysomya bezziana* is the agent of myiasis. Introducing the case: In this report a fifty six years old patient with myiasis on the scalp caused by *Chrysomya bezziana* is introduced. He is a rural man referred to clinic with a mass on tempero-occipital area. He did not mentioned about any trauma or ulcer on his scalp. In physical examination an ulcer sized 2×3 centimetre full of larva was observed. The diagnosis was myiasis and the parasites were removed and the tetanus vaccine was inoculated and the oral antibiotics were prescribed.
Conclusion: Parasitological examination was determined the agent as *Chrysomya bezziana*.

R2145

A *Strongyloides stercoralis* gastrointestinal infection burdened by an extraordinary treatment resistance, probably supported by an underlying Sjogren's syndrome which required long-term corticosteroid treatment

R. Manfredi, S. Sabbatani, P. Baccarini, G. Marinacci, F. Chiodo *(Bologna, IT)*

Introduction: In the majority of otherwise healthy patients (p), S*trongyloidiasis* (S) remains underestimated: only when some primary-secondary immunodeficiency occurs, *S* may involve multiple organs, outside the more obvious gut localization, towards disseminated-relapsing manifestations.
Case report: A female p who suffered from Sjogren syndrome treated since over 20 y with cyclic oral steroids, due to dyspeptic signs-symptoms underwent an endoscopy which detected a histopathology-confirmed gastroduodenal *S*. After a 3-day albendazole a repeated endoscopy-histology showed a persisting *S*, so that a further albendazole cycle was administered, but 2 subsequent controls (4 and 10 months after) demonstrated the persistence of *S. stercoralis* infection. Upon Hospital admission (9 months later), our p suffered from weight loss and anaemia. Stool, sputum, and urine search for *S. sterocoralis* tested negative, as well as HIV and HTLV-1 serology, and tumoural markers. A 2-day ivermectine at 12 g/day, preceded a 3-day albendazole course performed the subsequent week, and a 3-day mebendazole one week later. Repeated endoscopy was performed one and 2 months after discharge, confirming a sustained cure of *S*, associated with improved general conditions and a body weight regain. During the entire evaluation-treatment period, the concurrent steroid therapy (prednisone, 25 mg/day), deemed necessary for the concurrent Sjogren disease, was never interrupted.
Discussion: *S* is endemic in tropical-subtropical regions, where severe associations were demonstrated in HTLV-1-infected p. In developed countries, p with a broad spectrum of underlying diseases, or receiving immunosuppressive drugs, may suffer from *S* refractory to first-line therapy, due the frequency of re-infestation. Although anecdotal reports regarded p with chronic disorders and collagen vascular disease, no p with Sjogren syndrome was reported to date. The described report is therefore an unique association of a difficult-to treat *S* occurring in a p with a steroid-controlled Sjogren syndrome. Since controlled trials and definite recommendations are lacking, either albendazole, thiabendazole, ivermectin or mebendazole at different dosages-schedules were used, while the management of relapses lacks of evidence-based guidelines. Clinicians should also consider that *S* is expected to increase its frequency, based on environmental changes (increased temperature-humidity), just in countries where an increasing number of p become at risk for opportunism.

Resistance & mechanisms of action of antifungals

R2146

Antifungal resistance patterns among oral *Candida* species from patients receiving head and neck radiotherapy

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Objectives: Patients receiving radiotherapy treatment for head and neck cancer are highly susceptible to oral candidiasis. This infection is a common source of discomfort and a potential source of systemic infection. This study was conducted to understand the current status of resistance to antifungal agents among patients hospitalized in Clinical hospital Rijeka.
Methods: Oral swabs were collected from 32 hospitalized patients receiving head and neck radiotherapy treatment for malignant disease. Yeasts isolates were tested for their susceptibilities to two antifungal agents (amphotericin B and flucon-

azole) by using commercially available Etest (AB Biodisk, Solna, Sweden).
Results: At the time of sampling, 14 (43.75%) patients were found to be colonized with yeasts. All the isolates were *Candida albicans*. Antifungal susceptibility patterns showed that 100% of isolates were susceptible to amphotericin B (mean MIC – 0.1045 microg/ml), while 92.8% were susceptible to fluconazole (mean MIC – 0.11 microg/ml).
Conclusion: The frequence of resistant *Candida* is low in patients with cancer receiving head and neck radiotherapy treatment. However, it is important to continuously determine the distribution and susceptibility patterns of yeasts which should assist in developing optimal prophylactic strategies that can reduce clinically detectable oral candidiasis in this group of patients.

R2147

In vitro Candida albicans model for evaluating the inhibitory effect of known and potential antifungal agents on germ tube formation

A. Igloi, F. Hernadi, F. Peles, P. Kereszturi, A. Szabo *(Debrecen, HU)*

Objectives: *C. albicans* can differentiate from spherical yeast cell to a filamentous hypha form through the development of a germ tube. This ability of *C. albicans* can be considered an important pathogenic factor. This *in vivo* morphological transformation can also be brought about *in vitro*. However, the above-mentioned transition either takes place in conventional media only to a minor degree or the yeast cells multiply by budding alone. Previously we were able to develop a *C. albicans* cell differentiation model in which this morphological transformation can be detected in a significant part of the cells (87%). Our aim was to establish if our *in vitro* model was suitable for a quantitative examination of the effect of known and potential antifungal agents on fungal cell differentiation.
Methods: The investigation of inhibitory effect of known antifungal agents was performed by using ATB FUNGUS strip (bioMerieux). The new antifungal compounds were investigated by macrodilution method. The effect of antifungal agents on morphological transformation was examined by counting the differentiated forms and spherical cells in Buerker-chamber under light microscope.
Results: The morphological transformation was inhibited almost up to 100% by polyenes (amphotericin B and nystatin) and 64–92.3% by imidazoles (econazole, ketoconazole and miconazole). The 5-fluorocytosine did not show serious inhibitory effect. 9 new piridazine-azole compounds out of the 22 that we examined inhibited germ tube formation (30.3–100%). An inhibitory effect was noticed in 4 compounds out of the 5 examined benzylidene-benzosuberones (42.9-57.1%).
Conclusion: It can be stated that we were able to develop a *C. albicans* cell differentiation model that can be used to examine the quantitative effects on morphological transformation of antifungal agents used in clinical practice and potential agents. It can be claimed from our examinations that polyenes that bind to the ergosterol of cell membrane and imidazoles that inhibit the ergosterol synthesis produced an inhibitory effect on the morphological transformation, while 5-fluorocytosine that inhibits the fungus nucleic acid synthesis does not. It seems that the inhibition of morphological transformation is connected with the destruction of cell membrane. However, to be able to state this with certainty further experiments are needed.

Fungal infections

R2148

Second-line combination antifungal therapy with caspofungin plus low-dose amphotericin B deoxycholate in patients with invasive fungal infections failing primary treatment

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Objectives: Combination antifungal treatment (CAT) appears an appealing option in patients (pts) with invasive fungal infections (IFI), especially in those experiencing failure to primary therapy.
Methods: From June 2004 to April 2005, 14 pts affected by IFI (7 with candidaemia due to *C. albicans*, 3 due to *C. glabrata*, and 4 with pulmonary aspergillosis), who had failed primary antifungal therapy, were consecutively treated with Caspofungin (CAS) 70 mg on 1st day, and 50 mg thereafter, plus low dose (LD) Amphotericin B deoxycholate (dAmB) 0.5 mg/kg/d. All pts had high-risk underlying conditions (4 acute myelogenous leukaemias, 4 solid tumours, 4 prolonged ICU stays, and 2 major abdominal surgical interventions). Failure to prior therapy was determined by: 1) fever and worsening of clinical conditions, and 2) persistent candidaemia, or 3) worsening of lung CT scan together with increase of *Aspergillus galactomannan antigenaemia* (AGA), after 96 hours (hrs) from the start of antifungal therapy.
Results: All 14 pts, who were critically ill at the time of switching therapy, survived; within 72–96 hrs from the beginning of CAT, clinical stability and fever clearance, together with negative blood culture, or negative AGA were observed, and confirmed thereafter. LD dAmB did not require any premedication, but none of the pts suffered from side effects, nor treatment discontinuation was needed. Mean CAT duration was 26 days. None of the pts relapsed within a follow up period of at least 60 days from the end of treatment.
Conclusion: CAT including new drugs, such as CAS, is an appealing option supported by promising data. CAT with CAS and LD dAmB appears effective in critically ill pts with IFI failing primary treatment. The synergistic activity of dAmB, even at LD, and CAS seems to be clinically relevant; considering the safety demonstrated, LD dAmB allows also remarkable cost sparing in comparison with lipid formulations. Wider studies are warranted in this setting.

R2149

Oral candidiasis in antineoplastic chemotherapy-treated patients

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Objective: The incidence of *Candida* species and the sensitivity to antifungal agents in chemotherapy-associated oral mucositis were determined in an analysis of nosocomial patients with various solid tumours and haematologic malignancies.

Methods: Smears were taken from the lesions when oral candidiasis was clinically diagnosed and were plated out in Sabouraud Dextrose Agar (SDA). *Candida* spp. were identified by assessing germ tube and chlamydospore formation and by examining Api 20C sugar assimilation patterns. For in vitro susceptibility of isolated yeasts to antifungal agents we used a commercial method (the Fungitest panel), which allows susceptibility testing for 5 antifungal drugs at two different concentrations in modified RPMI 1640 (amphotericin B, 5-fluorocytosine, ketoconazole, itraconazole, miconazole and fluconazole). During the study all the patients received antineoplastic chemotherapy.
Results: Fifty cases of oral candidiasis were diagnosed from 193 patients (25.8%). *Candida albicans* was isolated from 28 patients followed by *C. tropicalis* (14 patients) and *C. krusei* (4 patients). Fluconazole is frequently used for prophylaxis and treatment of fungal infections in the immuonocompromised patients of our hospital. The findings from in vitro antifungal susceptibility testing confirm that the antifungals tested are effected against *C. albicans* strains except itraconazole in two isolates and also confirm the innate resistance to fluconazole of the four *C. krusei* strains isolated.
Conclusion: The results of this study denote the need for constant surveillance of the oral mucosa in cancer patients under antineoplastic chemotherapy. The good response of the patients to antimycotic treatment underlines the importance early diagnosis, which is expected to reduce the risk of haematogenous systemic candidiasis.

R2150

Invasive fungal infections and antimycotic resistance – a 7-year-surveillance

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Objectives: We conducted a 7-year surveillance to assess frequency, distribution and antimycotic resistance of different yeasts and moulds causing invasive mycoses in our University hospital.
Methods: All fungal isolates cultured from BALs, blood cultures, ascites fluids and wound swabs from 1998 to 2004 were analysed. Considering clinical data only probable agents of invasive mycosis were taken into account.
Results: From 1998 to 2000 the total number of patients with candidiasis was increasing from 408 to 508. In 2001 481, in 2002 412, in 2003 480, and in 2004 437 cases were detected. *C. albicans* was identified in 58.8% of all yeast isolates in 1998 and in 65.9% in 2004. *C. glabrata* followed by *C. krusei* were the most frequently isolated non-albicans species (for 2004: *C. glabrata* 16.5 %, *C. krusei* 6.4 %). In case of fungaemia *C. albicans* was identified in approx. 50% of cases in the period from 1998 to 2002, but an increase up to 82.6% was observed in 2004. The total number of candida septicaemias varied from 16 to 28 per year. Pneumonia was the most frequent invasive candidiasis recorded (1998: 285 cases = 69.9 % of all candidiasis, 2004: 297 = 68.0% respectively). Rates of antimycotic resistance for fluconazol (FLU) and itraconazol (ITR) were as follows: *C. albicans*: FLU: 1999 = 1.5%, 2004 = 2.5%; ITR: 1999 = 2.3%, 2004 = 1.3%; *C. glabrata*: FLU: 1999 = 23.1%, 2004 = 20.8%; ITR: 1999 = 23.9%, 2004 = 22.4%. All strains of yeasts tested against voriconazol were sensitive with exception of 2 strains of *C. glabrata* and one isolate of *C. krusei*. Almost all cases of systemic mould infections were lung infections with *Aspergillus* ssp. In the 7-year period a total of 193 systemic mould infections were observed.
Conclusion: *C. albicans* is still today the leading causative agent for invasive candidiasis in our setting. The number of non-albicans species was not increasing. The in vitro-sensitivity of *C. albicans* against fluconazol was only slightly decreasing and against itraconazol increasing in the observation period. Almost all yeast strains were sensitive to voriconazol.

R2151

Isolation of *Rhizopus microsporus* varietas microsporus from urine in patient with renal mucormycosis

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Isolated renal mucormycosis is an uncommon kidney infection affecting usually immunocompromised patients. Primary renal mucormycosis is a rare infection that occasionally causes acute illness with sepsis. We report a unique case of isolation fungus *Rhizopus microsporus* var. microsporus from urine in patient with renal mucormycosis in the microbiological literature.
A 37-year-old man with diabetes mellitus, with gastric resection and with defect of calprotectin metabolism was admitted with a five-day history of scrotal pain and weight loss. The preliminary diagnosis was sepsis at orchiepididimitis. Urine and blood culture were negative. Necrotic and nephrotic syndrome developed during hospitalization. Renal biopsy showed membranoproliferative glomerulonephritis; renal insufficiency developed; CT demonstrated an enlarged left kidney. The urine specimen was opaque with fluff. Fluff was examinated in 20% KOH preparations by direct microscopy and revealed numerous nonseptate hyphae. Culture grew moulds on Sabouraud agar. This mould grew as a floccose aerial mycelium which was brown to grey in surface coloration; it did not grow at 50°C. Microscopically, the colonies were composed of broad hyphae with stolons which bore rhizoids and fascicles of unbranched sporangiophores. Our identification was *Rhizopus* sp. The species identification was confirmed by M. vanova as *Rhizopus microsporus*, var. microsporus. *Rhizopus microsporus* was repeatedly isolated from urine. Treatment was changed to amphotericin B lipid complex. A simple nephrectomy was performed. Examination of the enlarged kidney showed presence of identical mould. Postoperatively the patient was continued on systemic amphotericin B, he started to urinate. He died of heart failure 3 months after nephrectomy.
Renal mucormycosis is usually not clinically suspected and are diagnosed only through surgical, pathological or postmortem examinations. Identification of the genus and species requires culture of tissue and assessment of the morphology of the fungal growth. They usually become nonviable when their walls are damaged by tissue homogenization. The availability of lipid formulation of amphotericin B within the past few years has provided an attractive alternative for the management of zygomycosis. The mortality rate of primary renal mucormycosis is high, with an overall survival rate of only 36%.

R2152

Isolation of keratinophilic fungi from sample soils of yards farms and forest in Khorasan and Golestan provinces of Iran

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Objectives: We have studied a small scale method for Isolating and of keratinophilic fungi from sample soils of yard farms and

forests and determination of dominanants species in Khorasan and Golestan provinces of Iran.
Methods: 290 soil samples were collected from various areas of Khorasan and Golestan provinces of Iran to determine the prevalence of keratinophilic fungi and dominant species.
Results: A total of 1503 species of fungi from 290 sample soils including 25 genera and 31 species were isolated viz. *Annixiopsis stercoraria* (23.355%), *Fusarium* spp. (13.37%), *Chrtysosporium* spp. (13.03%), *Aspergillus* spp. (12.97%), *Paecilomyces lilacinus* (6.98%), *Penicillium* spp. (5.23%), and other fungi (25.07%). McNemar's Test showed that non-keratinolytic fungi are dominant in this investigation. ($P < 0.05$, $\chi^2 = 29.24$) and also showed that in whole studied area *Anixiopsis stercoraria* (23.35%) and *Fuzarium oxysparum* (11.97%) are dominant ($P < 0.05$, $\chi^2 = 4$).
Conclusion: *Anixiopsis stercoraria* is the most prevalent keratinophilc fungi and also dominant species that isolated from 290 soil samples of three different geographical regions included mountain states, forest states and desert states. The distribution pattern of the different keratinophilic fungi and their significance are discussed.

R2153

Candidaemias in intensive care units of a tertiary hospital in Spain

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Introduction: Invasive fungal infections are an important cause of morbidity and mortality in intensive care unit (ICUs) patients. The distribution of species *Candida* causing nosocomial candidaemia is changing due to the use of antifungal drugs mainly.
Objectives: Know the incidence of candidaemia and species distribution comparing the groups *Candida albicans* versus other non-albicans *Candida* species in ICUs patients, from 1 September 1999 to 31 December 2004.
Methods: An incident case was defined by the first isolation during the study period of any *Candida* species from blood of a patient in ICUs. The incidence rates of candidaemia per number of admissions in UCIs were determined for *C. albicans* and other species of *Candida* no albicans.
Results: During 64-month-period a total of 206 episodes of fungaemia were collected in a tertiary hospital in Spain. Seventy-eight consecutive episodes of candidaemia concerning to ICUs. *C. albicans* was the most prevalent isolated species (n = 35, 44.9%); the remaining strains (n = 43, 55.1%) were non-albicans *Candida* species mostly represented by *C. parapsilosis* and lesser by *C. glabrata*. The distribution by year and species was: in 1999 non-albicans *Candida* species (n = 1) of a total 1 candidaemia. In 2000 there were 10 episodes of candidaemia, *Candida albicans* (n = 5, 50%) versus non-albicans *Candida* species (n = 5, 50%). In 2001 candidaemia total was 16 episodes and the distribution was *C. albicans* (n = 6, 37.5%), versus non-albicans *Candida* species (n = 10, 62.5%). In 2002 were 15 fungaemias; *C. albicans* (n = 7, 46.7%) and non-albicans *Candida* species (n = 8, 53.3%). In 2003 16 fungaemias was detected and the distribution was *C. albicans* (n = 7, 43.8%) and non-albicans *Candida* species (n = 9, 56.2%). And finally in 2004, 20 fungaemias being of *C. albicans* (n = 10, 50%); non-albicans *Candida* species (n = 10, 50%). The overall incidence rate per 1000 admissions was 7.07, ranging from 4.78 in 2000 to 11.2 in 2004 (7.22 in 2001; 6.16 in 2002 and 5.99 in 2003). In *C. albicans* the incidence rose from 2.39 in 2000 to 5.6 in 2004 (2.71 in 2001; 2.87 in 2002; 2.62 in 2003). The incidence of non albicans *Candida* species showed tendency rose too and were 2.39 in 2000, 4.51 in 2001, 3.29 in 2002, 3.37 in 2003 and in 2004 5.6.
Conclusions: Although the global incidence rose and most studies show an increased shift incidence of candidaemia from *C. albicans* to non-albicans *Candida* species, our group observed no shift despite a significant increase in the use of fluconazole.

R2154

Disseminated infection due to *Candida guilliermondii* in a patient with acute myelogenous leukaemia. A case study

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Objective: To evaluate the origin of candidaemia in a patient with acute myeloid leukaemia
Methods: We used biphasic media (Haemoline Performance Duo: bioMerieux-France) for blood cultures. Isolates were identified using ID 32C (API yeast identification system, bioMerieux) and susceptibility tests were performed with ATB FUNGUS (bioMerieux). The strains were considered clinically significant based on standard definitions established by the European Organization for Research and Treatment of Cancer/ Invasive Fungal Infections (2002).
Results: A 43-years-old patient admitted with acute myeloid leukaemia, developed bronchopneumonia and sepsis during profound neutropenia. Fever and pulmonary infiltrates did not improve by using empiric antibacterial therapy (cefoperazone-sulbactam, sulphametoxazol-trimethoprim). Blood and sputum cultures were performed and the patient received voriconazole. Both cultures, from blood and sputum, yielded *Candida guilliermondii* after 48 hours of incubation. The isolates had the same biochemical and antimicrobial spectrum, and were susceptible to amphotericin B and fluconazole. After a few days of therapy with voriconazole, fever disappeared and the clinical state of patient improved. A culture from pharyngeal swab, performed after 11 days, yielded the same microorganism.
Conclusion: 1. The emergence of less common but medically important fungal pathogens, including *Candida guilliermondii*, contributes to the rate of morbidity and mortality, especially in the increasingly expanding population of immunocompromised patients. 2. We consider that the oropharyngeal colonization with *Candida guilliermondii* and profound neutropenia predisposed our patient to develop bronchopneumonia and candidaemia.

R2155

Fungaemia caused by yeasts in patients of a university hospital, Cracow: epidemiology and risk factors profile over a 9-year period

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Objectives: Fungaemia is reported as the 4th cause of septicaemia in the international literature, with inherent serious morbidity and significant mortality. No local epidemiological data on this problem are yet available. Aim of the study was to evaluate local epidemiology of systemic fungal infections in adults considering potential risk factors, during the last decade.
Methods: 28 704 samples of peripheral venous blood collected from 6031 patients with symptoms of systemic infection were analysed. Patients were treated in the Departments of: Haematology (HAEM), Neurotraumatology and Neurosurgery (NT&N), General and Gastroenterological Surgery (G&GES), Infectious Diseases, Internal Medicine and Metabolic Diseases. Blood samples were incubated using automatic BactT/Alert

system. Identification of fungi was performed using commercially available ID 32C kit.
Results: (1) 242 cases of fungaemia were documented (median age: 51.5 ± 18.7; average hospitalisation: 53.6 ± 37.9 days); (2) highest no. of fungaemia episodes were found in patients with non-neoplastic gastrointestinal tract disease (30.5%), solid tumours (24.1%) and haematopoietic system neoplasms (16.4%); (3) a rising tendency of fungaemia episodes was observed: from 0.6 to 2.5 episodes/1000 treated (mean 2.0/1000); (4) highest incidence was confirmed in the G&GES and NT&N Departments: a rise from 0.2 to 4.5 episodes/1000 ($p < 0.002$) and from 0.8 to 4.0 episodes/1000 ($p < 0.02$), in the HAEM Department it decreased systematically; (5) main risk factors for developing fungaemia were: preceding antibiotic therapy (92.1% cases), central catheterization (81.1%), stay at the ICU (66.7%), surgical procedures (66.5%); mechanical ventilation (66.2%), immunodeficiency involved only 1/5 of patients (20.0%); (6) total mortality was over 30%; highest percentage of deaths was registered in the G&GES (41%) and NT&N (35%) Departments; (7) a total of 255 yeast strains were identified in blood samples, representing 4.0% of all positive blood cultures; (8) general aetiology: *C. parapsilosis* (33.9%), *C. albicans* (30.7%), *C. glabrata* (10.0%) and *C. tropicalis* (8.8%); (9) in onco-haematological patients *Candida* non-albicans (71%) and non-Candidal strains (21%) dominated.
Conclusion: Results obtained by us confirm current worldwide trends relating to the epidemiology of hospital acquired systemic fungal infections: their incidence, aetiology as well as the risk factors involved.

R2156

Scopulariopsis brevicaulis fungaemia: case report and implications for the diagnostic microbiology laboratory

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Objectives: To describe an unusual case of fungal infection by *Scopulariopsis brevicaulis* in a paediatric patient with acute lymphoblastic leukaemia (ALL) and to discuss the challenges in laboratory detection of this organism from blood culture.
Case: A 10-year-old girl receiving maintenance chemotherapy after relapse of ALL following bone marrow transplantation was admitted with fever and a dry cough. Her CRP level was 141 mg/L. Blood cultures at this time were negative. A CT scan revealed multiple pulmonary lesions suggestive of fungal pneumonia. AmBisome was added to empirical antibiotic therapy. Bronchial lavage was not performed. Blood cultures collected 10 days later yielded *S. brevicaulis*. Voriconazole was added but blood cultures remained intermittently positive. Microbiological response and complete resolution of fever was finally achieved after the addition of oral terbinafine. AmBisome was later discontinued and the patient discharged. To date, she remains asymptomatic and apyrexial on oral voriconazole and terbinafine. However, some pulmonary lesions remain.
Methods: Inoculated blood culture bottles were processed using the BacTAlert automated system (BioMerieux), which detects growth due to microbial production of CO2. Antifungal MICs were determined at the HPA Mycology Reference Laboratory, Bristol, UK.
Results: *S. brevicaulis* was cultured from blood specimens on 5 occasions (over a period of 4 weeks). However, on 3 of these occasions the inoculated blood culture bottles failed to signal positive. Fortunately, manual observation of the bottles prior to discard revealed several small cream-coloured spherical structures (fungal balls). Culture of the intact fungal balls and the blood culture fluid yielded no growth. However, culture of the disrupted fungal balls yielded *S. brevicaulis*. Antifungal MICs were: amphotericin 1 mg/L, voriconazole 8 mg/L, itraconazole 16 mg/L, caspofungin 4 mg/L and terbinafine 0.5 mg/L.
Conclusions: *S. brevicaulis* fungaemia is rare and this report reveals that its detection by automated blood culture systems may be problematic. In the present case, laboratory diagnosis relied significantly upon manual examination of the inoculated blood culture bottles. The presence of antifungal agents may reduce the sensitivity of detection of the automated system. In addition, the compact structure of the *S. brevicaulis* fungal balls may represent a micro-environment from which CO_2 is less readily released into the culture medium.

R2157

Risk factors for invasive aspergillosis in haematologic malignancy patients in a tertiary teaching hospital in Korea

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Introduction: Invasive aspergillosis (IA) is an important cause of morbidity and mortality in patients with haematologic malignancies (HM). The changes in the population at risk for IA due to intensified and new treatment strategies require a continuous re-evaluation of risk factors for IA. We conducted a retrospective case control study at a tertiary teaching hospital in order to identify risk factors for IA among patients with HM during the hospital construction period.
Methods: In this study, we evaluated the risks among 41 case patients who developed proven, probable, or possible IA according to standardized definitions in HM patients between July 2000 and May 2005. Control patients with no signs and symptoms of IA were matched for the time and place. The clinical data analysed included demographic characteristics; Charlson comorbidity score at the time of diagnosis of IA, the type of haematologic neoplasm risk factors for IA present at or within 1 month before the diagnosis of infection and previous antifungal use within 3 months of diagnosis of infection. Risk factors associated with IA were identified in a multivariable Cox regression model.
Results: In total, 41 IA cases (eight proven, ten probable, 23 possible), and 170 controls were analysed. Forty-three patients (20.4%) had AML, 27 (12.8%) had ALL, 85 (40.3%) had lymphoma, 33 (15.6%) had myeloma, 7 (3.3%) had myelodysplastic syndrome, and 18 (8.5%) had other disease. Anatomic sites of infection included lungs (n = 31), sinus or nose (n = 6), skin (n = 2), spine (n = 1), and skeletal muscle (n = 1). Variables that increased the risk for IA included duration of hospitalization (HR, 1.022; 95% CI, 1.002–1.042; p, 0.031), AML (HR, 0.031; 95% CI, 0.001–0.762; p, 0.033), malnutrition (HR, 4.812; 95% CI, 1.309–17.689; p, 0.018), and high iron level (HR, 1.001; 95% CI, 1.000–1.001; p, 0.017). Twenty patients died (48.8%) among IA and death was attributed to IA.
Conclusion: Results of risk factor analysis indicated that important variables predictive of IA included longer duration of hospitalization, non-AML, malnutrition, and high iron level. These results contribute to the further characterization of patients groups at high risk of IA and may help to target costly prophylactic measures against IA.

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R2158

Fungaemia in paediatric patients

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Objective: To define the epidemiological pattern of paediatric fungaemia in our hospital during the last 10 years.
Methods: Retrospective study of all fungaemia episodes from La Fe University Children's Hospital. All yeasts were isolated from blood cultures from January 1995 to December 2004. Data such as demographical aspects, hospital department and concomitant diseases were collected. Isolates were identified by the Vitek 2 system (BioMerieux, France).
Results: During the study period, 154 episodes of fungaemia were diagnosed. The more frequent species isolated were *C. parapsilosis* (46.1%), followed by *C. albicans* (42.86%), *C. guilliermondii* (3.25%), *C. tropicalis* (1.95%), *C. glabrata* (1.30%), *C. lusitaniae* (1.30%), *Saccaromyces cerevisiae* (1.30%), *C. krusei* (0.65%), *Trichosporum asahii* (0.65%) and *Pichia ohmeri* (0.65%). The majority (67.32%) of fungaemia episodes occurred in Neonatal, Paediatric, Reanimation and Burns Intensive Care Units; 27.45% were from Medical Departments and 5.88 % from Surgical Departments. *C. parapsilosis* was the most frequent species isolated in Intensive Care Units and Medical Departments (49.52% and 40.48%, respectively). However, *C. albicans* was more frequent in Surgical Departments (44.4%) than *C. parapsilosis* (33.3%). Fungaemia was more frequent in neonates (58.44%) and in boys (59.09%).
Conclusions: *C. parapsilosis* is the most prevalent species causing fungaemia in our Children's Hospital. Fungaemia is more frequent in Intensive Care Units, in boys, and in neonates. This study has been partially financed by a Grant (2/2005/0140) of Fundació per a la Investigació Hospital La Fe.

R2159

Antifungal susceptibility of *Candida guilliermondii* isolated from blood cultures

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Background: *C. guilliermondii* is isolated with a low frequency from blood cultures (1–5%). Fungaemias due to this species have been reported in cancer, surgical and intensive care unit patients. Risk factors for acquiring a fungaemia by this species are treatment for malignancy, neutropenia and bone marrow transplantation.
Objective: To study the incidence and susceptibility patterns of *C. guilliermondii* isolated from blood cultures in La Fe University Hospital during the last ten years.
Methods: Retrospective study of all candidaemia episodes by *C. guilliermondii* diagnosed from January 1995 to date in our hospital. Mycology, laboratory and clinical records were reviewed. The susceptibility study was carried out using a microdilution method according to CLSI guidelines (document M 27-A2).
Results: In the period of study we found 584 episodes of candidaemia, seven of them were due to *C. guilliermondii* (1.19%). Five cases (patients no. 1, 2, 3, 4 and 5) were children. Prematurity, intravenous catheter and previous antibiotic use were the most important risk factors associated in children. Most of them survived the episode after antifungal treatment. In adults (patients 6 and 7), the risk factors were the same as reported by other authors and all died in spite of antifungal treatment. The antifungal MICs (mg/ml), antifungal treatment received and outcome of patients are shown in the Table.

Strain & Patient # /Age	AmB	Fz	Itr	Fc	Vor	Cas	AF treatment	Outcome
1/7yr	0.5	8	0.5	0.25	<0.004	2	AmB→AmB+Itr(5d)	Died
2/10d	nd	0.25	0.25	2	0.03	1	AmB	Survived
3/6m	1	2	0.25	0.45	0.06	1	AmB	Survived
4/2yr	0.5	4	0.5	0.25	0.12	1	Fz→AmB+Fz	Survived
5/11d	0.5	nd	0.06	1	1	1	AmB	Survived
6/59yr	0.12	32	0.5	0.03	0.03	2	Cas→Cas+Fz	Died
7/77yr	0.25	2	<0.08	0.12	0.016	2	Fz→Vori+Fz	Died

AmB, amphotericin B; Fz, fluconazole; Itr, itraconazole; Fc, flucytosine; Vor, voriconazole; Cas, caspofungin; AF, antifungal; nd, non determinate.

Conclusions: *C. guilliermondii* is a very unusual cause of candidaemia in our hospital. During the ten-year period, the majority of cases are found in children. In one of cases with fatal outcome, good correlation between clinical outcome and the in vitro activity of the antifungal agents is observed.This study has been partially financed by a Grant (2/2005/0140) of Fundació per a la Investigació Hospital La Fe.

R2160

Oral colonisation by *Candida* and *Staphylococcus* in denture wearers

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Objective: To investigate the microbial oral colonization in Spanish denture wearers.
Patients and methods: Forty-six persons carrying dentures were randomly recruited from our Odontology Clinics, 28 with different types of denture stomatitis and 18 without stomatitis. Swabs were taken from the denture and the underlying mucous. Microbiological culture and isolation were performed following standard procedures in Columbia agar, and *Candida* ID2 and *S. aureus* ID chromogenic agar plates (bioMérieux, France). Yeasts were identified by conventional mycological methods, as the germ tube induction test in serum, microscopical morphology and chlamydospore production in corn meal agar with Tween 80, and by ID 32C (bioMérieux). Identification of *Candida dubliniensis* was confirmed by PCR with specific primers. Staphylococci were identified by catalase reaction, differential growth in Mannitol-Salt agar, Slidex Staph Plus latex reagent and ID 32 STAPH (bioMérieux). Slidex MRSA detection test (bioMérieux) was used for the evaluation of methicillin resistance.
Results: Thirty-six patients were carriers of yeasts (19 with different types of denture stomatitis and 16 without stomatitis) and 85 yeast isolates have been obtained: 42 of them from dentures and 43 from oral mucosa. *Candida albicans* was the most commonly yeast from the 67.4% of patients (63.5% of isolates). Other species isolated were: *Candida tropicalis* (19.6% of patients and 17.6% of isolates), *Candida glabrata* (10.9% of patients and 11.8% of isolates), *Candida guilliermondii* (4.3% of patients and 2.4% of isolates), and *C. dubliniensis* and *Candida krusei* (both 2.2% of patients and 1.2% of isolates). Forty percent of denture wearers were colonized by Staphylococci, being *Staphylococcus aureus* in three patients (6.5%). MRSA was not isolated. Staphylococcus epidermidis was isolated from five patients (10.9%), *Staphylococcus warneri* from eight patients (17.4%) and *Staphylococcus saprophyticus* from two patients (4.3%). In 12 patients there was a mixed colonization by *Candida* and *Staphylococcus*. One patient was colonized by *C. dubliniensis*, *C. krusei* and *S. aureus*.
Conclusion: We have found a high incidence of colonization with *Candida albicans* and *Staphylococci* in denture wearers with independence of the presence of denture stomatitis.[This study was in part supported by grant from the Fondo de Investigación Sanitaria del Ministerio de Sanidad de España (PI030662/2003)].

R2161

Scedosporium apiospermum lung infection with fatal outcome in an immunocompetent host

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Introduction: Invasive infection by *Scedosporium apiospermum*, a common soil fungus with an ubiquitary distribution, have been largely reported in immunosuppressed patients (haematological malignancy, organ transplantation, AIDS, steroids treatments). Scientific literature reports only few description of invasive infections of the immunocompetent host. In this report, we describe a case of *S. apiospermum* invasive lung infection with histological confirmation in a immunocompetent patient.
Case report: A 68-year-old man was admitted in our Department complaining of dyspnoea, haemoptysis, weight loss, asthenia and anorexia of 6 months' duration. He also reported night sweat and pyrexia. On admission, his physical examination showed cachexia, hyperpyrexia and purulent sputum. He had an inflammatory anaemia and biological inflammatory syndrome without leucocytosis. The patient had history of lung tuberculosis 40 years before. Chest X-ray and computed tomography (CT) showed a huge cavity with declivous necrotic material affecting the upper right lobe. Bronchoscopy evidenced bronchial distortion (tuberculosis' sequelae). *M. tuberculosis* and non-specific bacteria did not grow from any specimens of bronchial washing and sputum and no malignant cells were found. Mantoux test and *Aspergillus* spp. serology and antigenemia were negative. A three-week culture of sputum and bronchial washing grew *S. apiospermum;* serology was strongly positive. An antifungal treatment with voriconazole was started, but one month later, in reason of no amelioration on CT control and persistence of fever and weight loss, a surgical approach was decided. He underwent an upper and medium lobectomy of the right lung. Subsequent nosocomial pneumonia with respiratory failure caused patient's death few days later. Histology of surgical specimen confirmed *S. apiospermum* infection.
Discussion: Invasive pseudoallescheriasis of the immunocompetent host is poorly reported in literature. This case report highlights importance of differential diagnosis in infection of pre-existing lung cavities in immunocompetent patients, including tuberculosis, common bacterial infections, *Aspergillus* spp., mycetoma and rare fungal infection like *Scedosporium* spp., for which a delayed diagnosis potentially worsen prognosis. Indications of an early medical and surgical treatment needs to be defined, such as value of antifungal associations (ex. voriconazole and terbinafine).

R2162

Aspergillus fumigatus infections in patients with chronic granulomatous disease hospitalised in a children's memorial health institute, Warsaw, Poland

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Objectives: The aim of this study was to assess serological and molecular methods usefulness for diagnosis of invasive aspergilosis in patients with chronic granulomatous disease (CGD) presenting at the Children's Memorial Health Institute, Warsaw, Poland.
Methods: Samples were obtained from 3 patients hospitalized in our institute with clinically proven or suspected active fungal infection. For all patients galactomannan detection (PLATELIA ASPERGILLUS TEST, BioRad) and polymerase chain reaction (nested-PCR) were performed in serum samples. Simultaneously, various clinical materials (blood, urine, BAL, CSF, sputum wound and mucosal swabs) were taken for routine, mycological diagnosis. All clinical samples were inoculated on Sabouraud plates and cultivated for 14 days at 28–30°C under aerobic conditions. Isolates were identified by microscopic and biochemical methods. Antifungal susceptibility testing was performed according to NCCLS recommendations.
Results: For all patients, antigen detection results in serum samples were negative. In serum samples from two patients during active infection, *Aspergillus* DNA was detected by PCR method. During this time, *Aspergillus fumigatus* was also detectable in bronchoalveolar lavage fluid (BAL) of both patients and in cerebrospinal fluid (CSF) of single patient. In 1 of 3 patients with symptomatic pulmonary and sinusal aspergilosis, PCR result for *Aspergillus* sp. was negative. In both patients PCR results became negative after two months of antifungal treatment with voriconazole (VZ).
Conclusions: 1. Our results indicate high usefulness of PCR method for *Aspergillus* sp. detection and empirical antifungal therapy monitoring in CGD patients.2. Sandwich ELISA test for galactomannan detection in serum is not a reliable method for invasive aspergilosis diagnosis because of false negative results.

R2163

Susceptibility of clinical isolates of *Cryptococcus neoformans* and *Cryptococcus gattii* to voriconazole

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Cryptococcosis is an important human infection generally associated with high mortality. At the moment, clinical data favours the combination of amphotericin B desoxycholate and 5-flucytosine as first-line therapy for serious Cryptococcus infections such as meningoencephalitis. However, this regimen is associated with a considerable toxicity. Voriconazole is a recently available triazole antifungal drug with broad-spectrum activity, including activity against *Cryptococcus* species. Although several studies have been published in the last years revealing the *in vitro* activity of voriconazole against *Cryptococcus neoformans*, little information is available for *Cryptococcus gattii*. The purpose of this study was to analyse the in vitro susceptibility of clinical isolates of Cryptococcus against voriconazole. Susceptibility tests were performed according to the protocol M27A-2, Clinical and Laboratory Standards Institute (CLSI, former NCCLS). *Cryptococcus* was identified by standard mycological techniques based on characteristics of growth appearance on Sabouraud dextrous agar and Niger agar. Species identification was performed based on the production of urease and using canavanine-glycine-bromthymol blue medium. Minimal inhibitory concentrations (MICs) for voriconazole were determined for 37 isolates of *C. neoformans* var. grubii and 7 isolates of *C. gattii*. The MIC for 95.5% of these isolates was 0.03 μg/ml, and a MIC of 0.62 μg/ml was found for a single isolate of *C. gattii*. No resistant strain was found. Since previous studies have reported that *C. gattii* is less susceptible than *C. neoformans* to many antifungal drugs, including voriconazole, close monitoring is required. The results of this study reinforce the potent in vitro of voriconazole against *Cryptococcus* species.

R2164

Chronic mucocutaneous candidiasis with secondary adrenal insufficiency: a case report

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Objective: Chronic mucocutaneous candidiasis is a complex of disorders in which patients have chronic and recurrent *Candida albicans* infections of skin, nails and mucous membranes and several other disorders such as other infectious diseases, autoimmune disorders, endocrinopathies, thymoma, dental enamel dysplasia, vitiligo, alopecia totalis and interstitial keratitis can be seen. Chronic mucocutaneous candidiasis can be familial or sporadic and is generally seen in childhood but also there are some cases reported in adults. In our case chronic mucocutaneous candidiasis with secondary adrenal insufficiency is reported.
Case: A 34-year- old woman was admitted with the complaint of oral and oesophageal candidiasis. The patient was suffering from oral and oesophageal candidiasis for one year period and *Candida albicans* which was sensitive to Amphotericin B, intermediate sensitive to Fluconazole and resistant to itraconazole was isolated from the cultures of oral and oesophageal biopsy specimens and although proper antifungal therapy was given four times, recurrent lesions were appeared within one month after the antifungal therapy. When the patient was admitted except oropharengeal and oesophageal mucous membranes there was no other tissue involvement and from the cultures of oral plaques *Candida albicans* was isolated and the antifungal sensitivity was same. The patient was diagnosed with cell mediated immune deficiency in which T cell activation markers of CD69 and CD25 expressions were 20–30% (half of the control of 50–60%). This test is done with various dilutions 1/50 concentration of Candida antigen. The candidin skin test with 1/400 concentration of Candida antigen was positive at 48 hours. Anti-HIV was negative and CD4, CD8 counts were normal. Caspofungine 50 mg/day intravenously was given for 14 days as antifungal therapy and the lesions were disappeared on fifth day of the therapy. Beta glucan 10 mg/day therapy was given to support the immunity. Because endocrinopathy is seen 50% of the patients with chronic mucocutaneous candidiasis the hormone levels were examined. Cortisol level was 2.6 μg/dl and the cortisol levels increased by insulin-hypoglycaemia test and synacthen test so secondary adrenal insufficiency was confirmed and deltacortril was given 5 mg/day.

R2165

Species distribution and antifungal susceptibility of *Candida* isolates collected from hospitalised patients

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Background: Human candidiasis is increasing now in incidence and in clinical manifestation, due to the high frequency of prolonged antibiotic therapy, the invasive procedures and immune deficiencies. The immunosuppression by transplant, the drug therapy for cancer and AIDS favour the disseminated infections. Knowledge of clinical manifestations and predisposing factors is profitable in order to establish the proper prophylaxis and treatment.
Objectives: Patients hospitalized for clinical assumption of candidiasis were assessed. The aim of this study was to evaluate species distribution and antifungal susceptibility of *Candida* isolates recovered from pathological products, at some hospitals in Cluj-Napoca.
Methods: In a period of 2 years, 338 *Candida* isolates were collected. All isolates of *Candida* spp. were identified by the germ tube test and the API test (bioMerieux). Identification of species was followed by examination with broth the microdilution method, as described in NCCLS M27-A2, of the antifungal susceptibility to six agents, with readings after 24 and 48 h of incubation. MIC of antifungal agents was determined by the E test.
Results: The candidiase aetiology was established in 338 patients. 70% of the candidiasis were nosocomial infections, 5% occurred in patients hospitalized in an intensive care unit, 25% in patients hospitalized for cancer therapy. Mucosal candidiasis prevails (63.1%) followed by cutaneous candidiasis (25.2%) and disseminated candidiasis (10.8%). The overall species distribution was:41% *C.albicans*, 22.3% *C. parapsilosis*, 12.4% *Candida glabrata*, 12% *Candida tropicalis*, 2% *C. krusei*, 0.7% *C. guilliermondii*, and 5.8% *Candida* spp.
Conclusions: An important issue in candidiasis therapy is the occurrence of the resistant strains, both to common antifungal drugs and to those recently discovered. Our data suggest that the *Candida* species distribution and the antifungals resistance rate are similar to those reported previously in USA and Europe, but disparities in the species distribution and in the antifungal susceptibility of *Candida* isolates from other studies and countries are described. Our future goal is to study the susceptibility of *Candida* to antifungal drugs in order to struggle against these wide spread infections. The findings emphasize the need for continuous surveillance and further clinical investigational studies.

AIDS and HIV infection

R2166

Predicting factors of loss to follow-up after HIV-positive testing in a hospital in Paris, France in 2004

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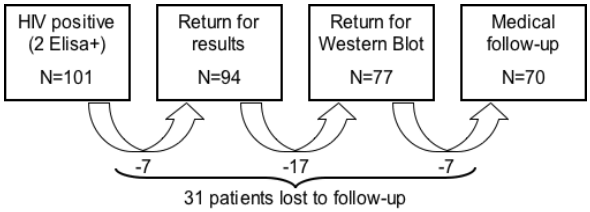
Objective: Defining predictors of loss to follow-up in an anonymous and free of charge HIV voluntary counselling and testing (VCT) centre in Paris.
Methods: Bichat's hospital VCT is an important HIV testing centre with a positive rate around 2%. Our study is retrospective concerning all HIV positive screened patients from 01 January 2004 to 31 December 2004. Information was collected by reviewing patients files using a standardized questionnaire. Our cohort was divided in two groups (loss of follow up versus followed up), and compared for each variable. The entering variables are age group, gender, sexual orientation, partner HIV positive, contamination factor, condom systematic use, employment status, medical care coverage, residence status and ethnical origin. Data was analysed using Splus software, in addition to descriptive data analyses, associations between ''lost to follow-up'' status and variables were assessed for statistical

significance using Fisher exact test for discrete variables and Wilcoxon test for the age.
Results: During the study, 5634 persons applied for HIV testing, 114 were positive. 101 are included in our study (13 were withdrawn). 65% men, 35% women. 62% heterosexuals. 89% sexual contamination. Mostly migrants from Africa 61.4%. 29% Africans lost to follow-up for 9% French. The only significant predictor of loss to follow-up found by a univariate model is the ethnical origin ($p = 0.04$). Interactions between the variables selected in the final multivariate model were also considered and will be presented at the congress.



Conclusion: In our study the HIV positive patients are mostly immigrants in a difficult social status. The observed rate of 31% of loss to follow up raises the issue of finding alternative approaches to insure that persons undergoing testing for HIV, return for the results and then have efficient access and linkage to care. We propose additional emphasis in patients counselling, regarding the predictor found in order to target the messages for persons with higher risk of loss to follow-up. At Bichat hospital, trained navigators are now present at the VCT to help the immigrant population. Their collaboration needs to be emphasized and its impact on the rate of loss to follow up, measured. Our results and the many missing data, emphasize the need to collect complete information during pretest counselling.

R2167

Detection of HIV sequences in respiratory tract bacteria of Cambodia and Kenya AIDS-positive patients

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Objectives: The hypothesis whether bacteria from respiratory tract of HIV-1 positive patients carry HIV-like sequences was examined.
Material and methods: Bacteria and Patients. Respiratory tract (nose, pharyngeal svabs) bacteria were isolated from 38 Cambodian and 24 Kenyan HIV-1 positive patients.
PCR amplification. Polymerase chain reaction specific for HIV sequences was carried out using these primers:
P38F: ATAATCCACCTATCCCAGTAGGAGAAT
P39R: TTTGGTCCTTGTCTTATGTCCAGAATG
P1F: CATTTGGAAAGGACCAGCAAAACTACT
E1R: TCATATGCTTTAGCATCTGATGCACAA
E68F: AGCAGCAGGAAGCACTATGG
E69R: CCAGACTGTGAGTTGCAACAG
G3F: TTGGACATAAGACAAGGGCCAAAA
G4R: GTCGTTGCCAAAGAGTGATTTGAG
Hybridization. 32P-labeled probes used were following: 38-39; 68-69, P1-E1 and G3-G4. DNA sequencing. The sequencing reaction was performed using Big Dye Terminator kit and sequences were resolved on 310 Genetic Analyzer.
Results: Bacteria were diluted to concentration 10^{-10} and single colonies were analysed. Around 34% of bacteria isolated from Cambodian and 33% from Kenyan AIDS patients were found to be positive in colony hybridization with HIV-specific PCR probes prepared using mentioned primers on pHB10 template. Bacterial DNA of all patients was isolated and 200–300 ng were analysed in dot blot using the same HIV specific probes. Approximately 31% of DNA isolated from Cambodian and 31.5% of Kenyan HIV-positive patients were positive in dot blot assay. Reproducibility of results between dot blot and colony hybridization was more than 90%. Bacterial DNA of positive isolates was amplified in PCR reaction with all four sets of primers. Sequencing of these products revealed high homology with the reference HIV sequence, while these sequences were not present in samples isolated from healthy individuals. Bacteria bearing HIV-like sequences were classified as *Klebsiella pneumoniae* (48%), *Staphylococcus aureus* (13%), *Staphylococcus pyogenes* (9%), *Escherichia coli* (4%), *Proteus mirabilis* (4%) and *Candida albicans* (17%), *Candida tropicalis* (9%) respectively.
Conclusions: 1. HIV-1 sequences were detected in respiratory tract bacteria of Cambodian (34%) and Kenyan (33%) AIDS patients by colony and dot blot hybridization (31; 31.5%) using HIV-1 specific probes. 2. PCR amplification and subsequent sequencing of positive bacterial DNA samples revealed high homology with HIV-1.

R2168

Trends of HIV infection in Giurgiu county, Romania

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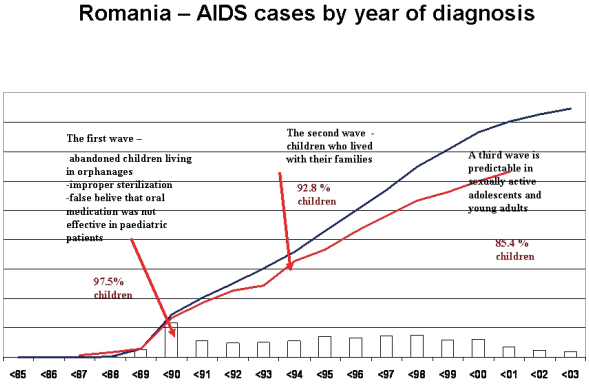
Background: Romania encountered three major waves of HIV infection: first occurred between 1987–1991, in children from institutions. The second occurred between 1994–1995 in children from families. Since then a third wave of epidemic is predictable in sexually active adolescents and young adults.
Objective: To analyse the existing information concerning the extent and the dynamic of the HIV/AIDS epidemic in Giurgiu county during 1995–2005.
Methods: Virological, immunological and clinical status of all new HIV diagnosed patients were evaluated from 1995 till present in a prospective study.
Results: We observed a decrease in the number of new HIV infected individuals, in 1995 there were 62 new HIV diagnosed patients in 1998, 28; in 2000, 20; in 2003, 6 and in 2005 only 3 new HIV infected. The median age at diagnoses increased year by year from 8.9 ± 6.24 years old in 1995, to 14.2 ± 8.78 years old in 1997, 17.47 ± 8.80 in 2000 and then in 2003 to 27.5 ± 14.30 years



old. We also observed a decrease in the rate of AIDS to HIV in new infected persons from 1.27 in 1995 to 0.5 in 2003 and 0.1 in 2005. No difference in gender incidence of HIV infection was noticed. The vertical transmission is still in the low range (<5%). The percentage of new diagnosed HIV individuals that started antiretroviral therapy increased from 43.5% in 1995 to 83.3% in 2003.

Conclusion: The weigh of paediatric AIDS in Romanian epidemic is balanced after 1996 by continuous spread of the disease in adult population. The number of new HIV infected individuals by year of diagnose is decreasing in Giurgiu County and the trend is to diagnose patients in early stages. The epidemic, driven initially by a nosocomial tragedy in Romania now risks spreading more widely through increased sexual risk behaviour together with sexually transmitted infections.

R2169

Unilateral lipomasty in a cohort of HIV (+) individuals

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Purpose: To describe a series of HIV (+) male individuals that presented in a tertiary care HIV clinic with unilateral lipomasty and to document the possible risk factors.

Methods: A population of 110 HIV (+) male patients (pts) was studied. All pts initiating HAART were prospectively followed according to a structured clinical protocol that included regular visits to the clinic with complete clinical and laboratory evaluation. Any signs and/or symptoms of breast enlargement and other morphological body shape changes were carefully recorded. An ultrasound test documented lipomasty and excluded gynaecomasty or neoplasia. A 2:1 age and gender matched control group of seropositive pts was used for comparison with lipomasty patients.

Results: 9 out of 110 (8%) pts (median age of 48 yrs) presented with a unilateral painless breast enlargement and were compared with 18 controls. The two groups did not differ with regards to immunological and virological parameters. All lipomasty pts showed other signs of lipodystrophy whereas none of the controls did. There were no significant differences between the two groups regarding current HAART regimens or ever use of NRTIs or NNRTIs. However 8/9 (88.9 %) lipomasty pts were PI experienced compared to 8/18 (44.4 %) controls ($p < 0.05$). Lipomasty patients were more likely than controls to a) have prior D4T exposure ($p < 0.01$), b) greater cumulative NNRTI use ($p < 0.05$) and c) greater duration of the current HAART regimen ($p < 0.01$). Lipomasty was significantly associated with hypercholesterolemia ($p < 0.05$) and hypertriglyceridaemia ($p < 0.01$).

Conclusion: Unilateral lipomasty is occasionally detected in HIV pts. In the current study it was frequently observed in association with other morphological body shape changes as well as with dyslipidaemia. Prior exposure to PIs, D4T and greater duration of the HAART regimen may be associated with the development of unilateral lipomasty.

R2170

Wet cupping associated with transmission of HIV infection: case report

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Background: Wet Cupping (Hijama) is a common traditional alternative therapy in Saudi society. Due to the presence of contaminated instruments, it may be a route of transmission to various viral infections including HIV. According to the medical literature, there has been no previous report on the association between wet cupping and transmission of HIV.

Case report: A 39-year-old man presented with a high fever, progressive shortness of breath and easy fatigability. The patient was diagnosed to have PCP. HIV serology was ordered and it was positive as well as the western-blot. The patient denied any previous sexual relationship, except with his two wives. No past history of blood transfusion or any other IV drug use. However, he had at least six (6) times when he had wet cupping ''hijama'' episodes at local barbers in Riyadh, 6 years ago. The local barbers are known to use contaminated instruments between customers.

Discussion: Wet cupping may be associated with the transmission of several viral infections (similar to tattooing) in contaminated setting. We believe in our case, the mode of HIV transmission was through wet cupping ''Hijama'' by using contaminated instruments. Due to lack of other risk factors to transmit HIV and due to the literature on tattooing in transmission of other viral infections including HIV, we think that wet cupping can likely be associated with transmission of viral infection between customers if contaminated instrument is used.

R2171

Hyperlactataemia during highly active antiretroviral therapy. Frequency, possible pathogenetic pathways, and clinical significance

L. Calza, R. Manfredi, F. Chiodo *(Bologna, IT)*

Introduction: Despite the increasing evidence of hyperlactatemia as an emerging side effect of HAART, its frequency, pathogenesis, management, outcome, and prevention are still under active investigation.

Methods: A case-control study has been performed on >1000 HIV-infected patients (p) in the year 2004. When evaluating HAART-treated p with adherence levels >90%, p with hyperlactacidemia were compared with p with normal lactate levels in relation with a broad spectrum of variables.

Results: Of the 755 evaluable p, 272 (36%) experienced at least one evidence of hyperlactacidemia (mean value 24.7 ± 8.8 mg/mL). Only 56 p of the study group (7.4%) experienced two or more subsequent alterations, with progressively increasing levels in 73.2% of p, while a grade 4 hyperlactatemia (>39.6 mg/dL) was found in 5 p only (0.7%). When comparing the 272 p with elevated lactacidemia with the 483 controls, no significant difference was seen as to age, gender, type of risk for HIV infection, duration and stage of HIV disease, CD4 + lymphocyte count, HIV plasma viral load, and type and duration of use of single and combined antiretroviral drugs (HAART). In an univariate analysis, p with hyperlactacidemia showed a significantly longer anti-HIV therapy ($p < 0.004$), a more frequent lipodystrophy syndrome ($p < 0.005$), hypertriglyceridemia ($p < 0.02$), and raised serum creatinphosphokinase ($p < 0.02$) and aldolase ($p < 0.001$) levels. The prevalence of signs-symptoms referable to lactic acidosis (nausea, vomiting, abdominal pain, fatigue, myalgia) was low (2–3%) and comparable between p and controls. Hyperlactacidemia never determined HAART interruption or modification in our single centre cohort.

Conclusions: Hyperlactacidaemia emerged as a proportionally frequent HAART complication, although laboratory abnormalities greatly exceed in frequency the occurrence of a symptomatic metabolic acidosis. No correlations were found with use and duration of specific anti-HIV drugs, although an association

with a longer exposure to antiretrovirals, and other concurrent HAART-associated adverse events, was found.

R2172

An observational study of subcutaneous multiple lipomatosis in patients receiving highly active antiretroviral therapy. Possible correlations with metabolic abnormalities and pathogenetic insights

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Introduction: As a consequence of HAART administration, the lipodystrophy syndrome and a broad spectrum of metabolic abnormalities emerged. Localized fat accumulation may occur as visceral adipositiy, increased breast size, gynaecomastia, lipomastia, and the so-called buffalo hump, although very limited reports are to date available on the role lipomatosis during HIV infection.
Methods: Sixteen patients (p) of >1000 treated with HAART as of end of August, 2005 (>1.5%) (13 males and 3 females aged 36–58 years) experienced multiple ultrasonography-confirmed subcutaneous lipomas (3 to >20 lesions), predominantly involving the trunk and upper and lower limbs, usually associated with local discomfort.
Results: Among involved p, the duration of HIV seropositivity at lipomatosis onset varied between 32 and 116 months, and no p had developed full-blown AIDS. Our 16 p experienced 5–14 different anti-HIV therapeutic lines: almost all available protease inhibitors (PI) and nucleoside analogues had been used previously or during the occurrence of lipomatosis, while a NNRTI was used by in 4 p only. All p were given a PI-based HAART since 16–74 (mean 28.2 ± 13.9) months. While the virological-immunological situation of HIV disease was favourable, a broad spectrum of concurrent lipodistrophy syndrome and dysmetabolic-related alterations were found. In detail, an evident lipoatrophy was present in 13 p out of 16, associated with central adiposity in 10 p. Hypertriglyceridaemia, hypercholesterolaemia, and hyperglycaemia were detected at in 12, 8, and 2 p, respectively. The subsequent follow-up (8–66 months) was characterized by the occurrence of further lesions in 8 p (with 3 p undergoing plastic surgery with satisfactory results) and a substantially stable disease in the remaining 8 p, in absence of spontaneous regression, even when the initial HAART regimen was changed.
Conclusion: Sucutaneous lipomas have not been reported with increased frequency during HIV infection, including the HAART era. An accurate diagnostic workup (ultrasonography and eventually fine-needle biopsy) is needed to prevent the rare occurrence of malignant degeneration. The frequent association of lipomatosis with other clinical-metabolic disturbances related to HAART should deserve further epidemiological and pathogenetic studies to better investigate their eventual, mutual relationship and to identify eventual prevention strategies.

R2173

Efavirenz versus nevirapine, their significantly different profile on lipid metabolism and its correlates

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Introduction: Altered metabolism represents an emerging feature of HIV-infected patients (p) treated with HAART, but limited informations are avilable regarding the two non-nucleoside reverse transcriptase inhibitor (NNRTI): efavirenz (E) and nevirapine (N).
Methods: Among over 1000 p treated with HAART for >12 months, the metabolic pattern of NNRTI was assessed according to three different backgrounds. The first one included antiretroviral-naïve p starting a NNRTI-based regimen; the second included a large spectrum of p experienced with 2 to 10 therapeutic lines (but still NNRTI-naïve); the third group included p who added for the first time a NNRTI only on late rescue therapies with ≥4 drugs (and including protease inhibitors).
Results: 386 p treated with E were compared with 334 p taking N in our prospective observational survey lasting 12 to 30 months, by a multivariate analysis of serum lipid-glucose levels, and other metabolic abnormalities. Among the 213 p naïve to antiretrovirals, an altered triglyceridemia was more common ($p<.001$) in the E versus the N group. Considering the 344 antiretroviral-experienced p who introduced a NNRTI for the first time, the frequency of hypertriglyceridaemia appeared greater in the E group ($p < 0.0001$), with earlier development of this feature in p on E versus N ($p < 0.0001$). Also in the 163 p receiving salvage HAART, the rate of hypertriglyceridemia-hypercholesterolemia-hyperglycemia tested greater among p treated with E versus N ($p < 0.04$ to $p < 0.005$), and the time to peak alterations was more rapid in the E group ($p < 0.04$). Comparing the 386 p receiving E with the 334 p on N, the frequency of elevated triglyceride, cholesterol, and glucose levels was greater in E-treated p ($p < 0.0001$ to <0.005). Some grade of lipodystrophy was present in 276 pre-treated p, but an appreciable ameliorement occurred after NNRTI introduction in 14 p of the E group, versus with 39 p on N ($p < 0.005$).
Conclusion: A sufficiently prolonged follow-up shows that E may not resolve dysmetabolism, but might also prompt metabolic abnormalities with more frequency and intensity compared with N. The two available NNRTI have a significantly different dysmetabolic profile, which leads to an increased interest in pathogenetic and prevention studies.

R2174

Eighteen years of research on AIDS: contribution of and collaborations between different world regions

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Objectives: The scientific community invests significant resources on HIV/AIDS research to confront the current epidemic. We reviewed the medical literature, in order to evaluate the contribution of different world regions on HIV/AIDS research during the past 18 years.
Methods: We retrieved articles, using an elaborative methodology, from 3 journals focusing on HIV/AIDS between 1986 and 2003, indexed in the Journal Citation Reports (JCR) and the Web of Science databases of the Institute for Scientific Information (ISI). Comparisons were made by dividing the world either into 9 geographic regions, and by using the human development index (HDI) categorization.
Results: A total of 9.502 articles on HIV/AIDS were retrieved from 3 AIDS journals over an 18-year study period (Table). USA and Western Europe together and 5 developed out of 9 world regions made up a striking 83% and 92% of the world's research production on HIV/AIDS, respectively. Researchers from countries included in the high, medium, and low HDI category produced 2, 240, 9, and 15 articles per billion population, respectively. About half of articles originating in Latin America

and the Caribbean and half in Asia were produced in collaboration with the USA. On the contrary, 40% of articles from Africa and 58% from Eastern Europe were produced in cooperation with Western Europe. Collaboration between researchers within developing regions was negligible.
Conclusion: The vast majority of the world's research on AIDS is produced in the developed world. International collaborations are limited and in general follow traditional cultural and political lines of international relationships. Cooperation of scientists among the developing world is minimal.

R2175

Evolution of antiretroviral drug resistance and resistance mutations in multi-treated HIV-1 infected patients during the last three years

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Objectives: Drug resistance mutations in the human immunodeficiency virus (HIV-1) reverse transcriptase (RT) and protease genes is a major cause of antiviral therapy failure. Therefore, we evaluated the evolution of mutations and antiretroviral resistance in multi-treated patients over a three-year period.
Methods: The plasma of 326 HIV-1 infected patients (94 in 2002, 119 in 2003 and 113 in 2004) was analysed. Most patients were males (75%), mean age 41 years (range 20–73). Drug resistance was genotyped by sequencing RT and protease genes using the ViroSeq HIV Genotyping System (Abbott). The Stanford database was used to interpret resistance data (http:/hivdb.stanford.edu/hiv/).
Results: On the HIV-1 RT region 955 mutations were detected, 277 of which were associated with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). On the protease gene 865 were found, 722 of these were secondary mutations. Changes at codons 184, 215 and 103 were most frequent in the RT region, but at position 184 they decreased over the time by 21% in 2002, 15% in 2003 and 8% in 2004. L63P was always the most prevalent protease mutation. Mutations at new codons 65, 116 and 238 (2003) and 33, 43, 68, 211 and 228 (2004) in the RT region were observed. Multi-drug resistant mutation Q151M was found in 4 cases (two in 2003). The resistance associated with protease inhibitors and NRTIs increased in 2003 and in 2004, except for Lamivudine and Abacavir. NNRTIs had similar percentages of resistance during the study period. From 2002 to 2004 we notice a statistically significant ($p < 0.001$) increase in susceptibility to Lamivudine. Wild type sequence was detected in 9 cases, and genotyping was not available because not enough cDNA was obtained in 72 patients.
Conclusion: Resistance frequently limits the efficacy of antiretroviral drugs in the treatment of HIV-1. The detection of key mutations associated with antiretroviral resistance can be of use in the clinical management of these patients.

R2176

Nasal carriage and antibiotic resistance of *Staphylococcus aureus* in HIV-infected patients

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Objectives: To evaluate the rate and risk factors of nasal *S. aureus* carriage in asymptomatic HIV patients; to assess antibiotic resistance of yielded *S. aureus* strains.
Methods: In this prospective case controlled study anterior nares swabs/cultures were obtained from 91 asymptomatic adult HIV patients attending out-patient department of AIDS and immunodeficiency of Tbilisi, Georgia and 60 HIV negative persons. Antibiotic resistance was testing by using disk-diffusion method. To determine the susceptibility to methicillin, oxacillin was used as a marker. HIV patients undergo investigation about their past medical history and behaviour.
Results: A total 39 swabs (42.8%) yielded *S. aureus* from HIV positive patients and 14 swabs (23.3%) from HIV negative persons. Antimicrobial resistance was determined for isolates from HIV positive patients. Yielded *S. aureus* strains were resistant to: clindamicin – in 30.7% of cases, chloramphenicol – 41%, oxacyllin – 53.8%, amoxicillin-clavulan acid- 64.1%, ampicillin – 97.4%, penicillin G – 100%, ciprofloxacin – 23.08%, trimetoprim – 48.7%, erythromycin – 41.02%, amycacin – 28.2%, tetracycline – 46.15%. All isolated *S. aureus* strains were susceptible to vancomycin.
Conclusions: High rate of *S. aureus* carriage was detected in studied HIV population. The *S. aureus* carriage was not correlated to CD4+ T-cell count or granulocytopenia, but was influenced by previous hospitalisation, skin infection, antibiotic use and IDU practice. High rate of antimicrobial resistance may be related to uncontrolled use of cheaper oral antibiotics in Georgia. The prevalence of methicillin (oxacyllin) and ciprofloxacin resistant *S. aureus* colonization was higher in HIV infected then in general population of Georgia.

R2177

An epidemiological study regarding knowledge, attitude and source of information on HIV-AIDS among the teachers of secondary and higher secondary schools of Ahmedabad City, India

R. Patel *(Ahmedabad, IN)*

Background: Apart from parents, teachers are the most important source of information for students. Assessment of teachers for AIDS gives baseline information regarding their knowledge and attitude which can be utilized in the future to decide the strategy for health education. Keeping this in mind, present study was done with an objective to determine the teachers knowledge regarding nature, mode of transmission, source of information and attitude toward people leaving with AIDS (PLWA).
Method: Study design: Cross-sectional study.Setting: Meeting of 182 teachers of secondary and higher secondary schools of Ahmedabad. A pretested, semi structured, self administered proforma was given to all teachers attending the meeting Analysis was done with statistical software Epi-info for calculating various indicators like Mean, Standard deviation, Proportion, Chi-square test.
Results: Out of the total, 68% were males and 32% were females.88% knew that AIDS could be transmitted by having multiple sex partner. 79% knew about risk of HIV by infected blood transfusion and use of contaminated needles. 19% of males and 8% of females believe that AIDS can be transmitted by kissing to an AIDS patient. 12% of females and 20% of male teachers believe that an HIV patient must not be allowed to do any job or business. Only 67% of respondents knew that AIDS could be prevented by proper use of condom.
Conclusion: Knowledge about other sexually transmitted diseases was comparatively lower than that of AIDS. Knowledge regarding multiple sex partner and use of contaminated needle, as mode of transmission of AIDS was comparatively higher in females. Responses like transmission of AIDS by mosquito bite and hand shaking with AIDS patient shows level of incorrect concept regarding AIDS transmission. 80 % of respondents had shown their readiness to receive information about HIV. Newspaper, banners, posters and television had come up as major source of information among teachers.

R2178

Epocal modification of re-imbursement facilities of all lipid-lowering drugs in Italy. Lack of consideration of HIV-infected patients with HAART-related dyslipidaemia, who lose their right to free access to statins, fibrates, and omega-3 derivatives

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Introduction: The significant HAART-prompted advances achieved in the management of HIV disease are at risk to be frustrated by the modified re-imbursement modalities of all lipid-lowering drugs (LLD) available in Italy. The remarkably increased life expectancy attained thanks to HAART, is burdened by significant risks to develop a diet- and exercise-uncontrolled hypercholesterolaemia-hypertriglyceridaemia, often concomitant with insulin resistance and visceral adiposity, factors which strongly predispose to cardiovascular events and stroke.
Methods: The novel prescribing rules of LLD based on a computer-generated score, were matched with the present situation of ~1000 HIV-infected patients (p) treated with HAART, in order to assess the frequency and type of dyslipidaemia, and the estimated rate of need of LLD prescriptions.
Results: The rate of hypertrigyceridaemia and hypercholesterolaemia exceeded 28% and 19% of p respectively, while around 22% of p had a mixed dyslipidaemia. Over 200 p were currently treated with statins and/or fibrates, with the eventual adjunct of omega-3 fatty polyunsaturated acids. When applying the risk score proposed for the general population, <10% of these p reached the threshold of a >20% risk of a major vascular event in the next decade (due to the proportionally lower mean age, the absence of familial dyslipidaemia, diabetes mellitus, elevated systolic pressure, and anti-hypertension therapy), while only very few p needed a secondary prophylaxis. As a result, >90% of HIV-infected p presently treated with LLD due to present antiretroviral therapy recommendations have lost all rights to LLD re-imbursement in Italy, and are at serious risk to give up LLD due to not sustainable linked costs.
Conclusion: The recent dispositions of the Italian Health Care System did not consider HIV-infected p, who are exposed to a frequent, severe, jatrogenic dyslipidaemia, and an elevated major vascular risk despite their lower mean age, and the lack of multiple generic risk factors. Most HAART benefits might be blunted by the sudden lack of LLD re-imbursement, which is estimated to regard the majority of treated HIV-infected p. A comparison with LLD re-imbursement facilities in other countries is also warranted, to draw some epidemiological and pharmacoeconomic elements suggesting a re-extension of re-imbursement facilities of these life-saving drugs to HIV-infected p.

R2179

Long-term statin use does not act on immune markers of HIV disease progression, in HIV-infected dyslipidaemic patients treated with a virologically effective HAART regimen. A prospective study, controlled versus fibrates, and lifestyle changes

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Introduction: Recently statins were hypothesized to act unfavourably on the immune system through an altered cytokine pattern and a Th1/Th2 imbalance, as shown in non-HIV-infected patients (p) [JAIDS 2005;39:503] while a small experience claimed a $p < 0.05$ loss of absolute CD4+ count of p treated with a protease inhibitor (PI)-based HAART in a 6–18-month period [HIV Clin Trials 2003;4:164] although the 4 compared p groups were small in size (5–11 p each) and not homogeneous (a group received PI only).
Methods and results: From >1000 HIV-infected p we identified all p on HAART since >12 months with compliance >90% and with altered fasting cholesterol (>200 mg/dL) and/or triglycerides (>250 mg/dL) of >6-month duration. When comparing the quarterly immunological trend of the 88 p prospectively taking statins (pravastatin, fluvastatin or rosuvastatin) for a predominant hypercholesterolemia, versus the 103 p receiving fibrates (bezafibrate, fenofibrate or gemfibrozil) for a prevailing hypertriglyceridaemia, versus the 76 p who underwent a diet/exercise program only, no significant difference in mean-median CD4+ count occurred among the three p groups during a mean follow-up which now reaches 15.3 ± 6.7 (range 6–26) months. All p remained evaluable when still on an HAART regimen ensuring virologic suppression and an unchanged hypolipidaemic therapy.
Discussion: Examining the broad-spectrum pleiotropic activities attributed to statins also the virologic course of HIV infection received limited evidences from a study claiming a direct anti-HIV activity of statins by down-regulating the small signaling Rho protein (which acts on cell viral entry) [*J Exp Med* 2004;200:541] while neither virologic failure nor viral blips occurred among 78 HAART-stable p who started a statin [*JAIDS* 2005;39:637]. To increase confusion, efavirenz increases statin metabolism [JAIDS 2005;39:307] while also the anti-triglyceride fibrates may have extensive pleiotropic effects [*Curr Vasc Pharmacol* 2005;3:87]. In fact, both fibrates and other molecules interacting with the peroxisome proliferator-activated receptors (PPAR) (eg risoglitazone-pioglitazone) may affect major T-lymphocyte cytokine mediators [*Circ Res* 2002;90:703; *J Immunol* 2004;172:5790]. Based on our experience and waiting for enlarged in vivo studies conducted on affordable p samples, we stay with the last statement of N. Shafiq about ''The Statinth wonder of the world: a panacea for all illnesses or a bubble about to burst'' [*J Negat Results Biomed* 2005;4:3]

R2180

HIV-associated malignant bladder carcinoma during the HAART era: an infrequent but intriguing finding

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Background: Most of AIDS-related malignancies declined after HAART introduction. The 1993 AIDS notification system reports AIDS-linked disorders only upon diagnosis, while pathologies occurring in patients (p) already diagnosed with AIDS are significantly underestimated. Increasing cancer may be caused by the prolonged p's life expectancy, the residual immune imbalance, and the late detection of HIV disease (so-called ''AIDS presenters''). Among solid tumours, bladder neoplasms are extremely infrequent with only three anecdotal episodes reported to date.
Methods: Since more confidence with HIV-cancer association is needed, we report two recent cases of relapsing HIV-associated bladder carcinoma.
Results: Our experience of two cases of transitional, papillary, stage G2 bladder carcinoma, shares some common points between involved p: it interested male p in their fifth decade

of life, specific risk factors were excluded and HIV infection lasted from around two years and was favourably controlled by HAART. While the single relapse of bladder carcinoma experienced by our first patient required endoscopic surgery, the multiple recurrences and the advancing disease (G3) stage led to a radical cystectomy in the second p, despite repeated endoscopic, surgical, and cytotoxic therapy, but cancer appeared under control in both p during the subsequent 10–14-month follow-up. The urologic support was valuable for early diagnosis and appropriate therapy and monitoring, which needed repeated endoscopic-surgical interventions, and intravesical chemotherapy, although only radical surgery proved effective in our second p.

Conclusions: Although the proportionally rare occurrence of HIV-associated bladder cancer and the lack of correlation with HIV disease progression, could result in a trivial pathogenetic association, this neoplasm is borne by an increasing incidence. Only three cases were presented by the international literature as single reports: two of them in the pre-HAART era. Clinicians facing HIV p with haematuria should consider a bladder carcinoma, to avoid a missed or delayed diagnosis.

R2181

Pulmonary tuberculosis in patients with and without HIV/AIDS

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Object: Pulmonary TB is still the most common form of the tuberculosis in HIV infected patients, with different presentations according to the degree of immunosuppression. The aim of this study was to investigate the impact of HIV infection on the clinical, laboratory and radiological presentation of tuberculosis.

Methods: We compared 80 HIV negative pulmonary TB patients with 40 HIV positive pulmonary TB patients during 1999–2005 in a teaching hospital of Tehran University of Medical Sciences, Iran.

Results: Tuberculosis was more common in men in both groups. The mean age of HIV positive patients was lower than HIV negatives (35/95 ± 10/4 versus 46/95 ± 20/39 p = 0.002). Weight loss and chronic cough were significantly more frequent in HIV negative patients (76/3% vs 45% p < 0/001 and 93/8% vs 80% p < 0.05 respectively), whereas fatigue were more prominent in HIV positive (45% vs 21/3% p < 0.001). Cavitations and infiltrations were reported in the CXR of HIV/TB patients less than HIV negative patients (OR = 0/21, 95% CI 0/05-0/97, P = 0.01 and OR = 0/27%, 95% CI 0/09–0/75%, P = 0.02 respectively). Primary involvement pattern was observed more than secondary involvement in HIV/TB group (OR = 3/95, 95% CI 1/73–9/03, P = 0.001). The laboratory findings in HIV/TB patients were as follows: more negative PPD skin (75% VS 50%) tests, higher ESR (86/5% VS 63/7%), lower mean Hb (10/6 VS 12/4) and lower mean leukocyte (6545 vs 9195) and lymphocyte count (1281 vs 1838). In all of the above mentioned findings, the differences between two groups were significant. 47.3% of patients had Lymphocyte count less than 1200, which means they were in AIDS stage.

Conclusion: By suppressing the immune system, HIV can alter the clinical, laboratory and radiological features of tuberculosis. So it is crucial to consider tuberculosis in differential diagnosis of every HIV patients with respiratory symptoms.

R2182

Human immunodeficiency virus type-1 associated lymphoma: Tunisian experience

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Background: Tunisia is one of African countries where seroprevalence of human immunodeficiency virus (HIV)infection is low. However, lymphoma is considered as the second AIDS-definig illness after Kaposi's sarcoma in our experience.

Objectives: We report clinical, epidemiological, and histopathological findings in patients with HIV–associated lymphoma.

Methods: We retrospectively reviewed all patients aged above 15 years and hospitalized for haematologic disorders from January 1985 and December 2004 in the department of infectious diseases of Rabta Hospital. Patients with a diagnosis of agressive lymphoma were included. The stage of lymphoma was based on Ann Arbor system.

Results: 10 patients were reported with HIV-associated lymphoma; 60% were heterosexual and 40% intravenous drug users. The mean age was 34 years (21–45 years) and sex ratio was 4.The mean interval between the diagnosis of HIV infection and lymphoma was 2.4 years. The lymphoma was the first AIDS-defining illness in 3 cases. CD4+ lymphocyte count below 200 cell/mm^3 are observed in 7 cases, and a mean plasma HIV viral load was 619750 copies/ml. 2 patients were under highly active antiretroviral therapy (HAART) before the diagnosis of lymphoma. All patients had B systemic symptoms (fever, night sweats, fatigue, or loss of body weight). 5 patients had HIV-associated non Hodgkin lymphoma (HIV-NHL): a diffuse large-cell lymphomas (3 cases), a Burkitt's lymphoma (1case) and immunoblastic lymphoma (1case). One patient had a probably primary cerebral lymphoma. 3 patients with HIV-HL were at stage III or IV. Extranodal involvement was observed in 6 cases. 6 patients were treated with chemotherapy and/or radiotherapy. From all patients, only one is still alive15 months after diagnosis.

Conclusion: In our experience, lymphoma remains the most lethal complication of AIDS, associated with a very poor prognosis. Survival may be improved by early diagnosis and restauring immune status with HAART.

R2183

HIV-asociated tuberculous meningitis: a descriptive study

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Background and objective: Human immunodeficiency virus (HIV) infected patients have extra-pulmonary involvement of tuberculosis infection more frequently than non HIV-infected patients. This study was undertaken to characterize the clinical manifestations and outcomes of tuberculous meningitis in HIV-infected patients and the potential role of the highly active antiretroviral therapy (HAART).

Methods: We reviewed the clinical charts of patients with HIV-infection and tuberculous meningitis (TM) during two periods: 1989–1995 and 1996-forward. TM was considered definite if mycobacterium tuberculosis (myt) was stained, amplified or cultured from cerebrospinal fluid (CSF). TM was considered probable if there were according features from CSF and cultured myt from other sites, clinical response to therapy and no other microorganism implicated.

Results: We recorded 25 patients, 13 with TM definite, 20 (80%) before HAART use. Twenty-two patients (88%) were males. The mean age was 35 ± 10.56 (21–62) years. The main risk practice for HIV infection was intravenous drug use, in 17 patients (68%). HIV infection diagnosis and tuberculous meningitis diagnosis was concomitant in 9 patients (36%). Seventeen patients (68%) did not have previous tuberculosis. According to the Medical Research Council criteria 9 patients (36%) had stage I disease, 11 (44%) had stage II disease and 5 (20%) had stage III disease. The median CD4+ cell count was 110 ± 127 (6–504). Adenosine deaminase was measured from CSF in 23 patients and was high in 15 (65.22%). Ziehl stain was positive in only 1 case (4%), and culture in 13 patients. Chest film was normal in 18 patients (72%). CT of the head in 22 patients were: meningeal enhancement in 4 (16%) cases, non-enhancing lesion in 4 and normal in 16 (48%). The treatment regimen was isoniazid, rifampicin, pyrazinamide and ethambutol in 21 patients (84%) with adjuvant corticosteroids in 14 patients (56%). Only 1 patient had ventricular-drainage procedure. Eight patients (33%) died, 5 of them (20.83%) directly attributable to the TM. We did not find differences in presenting features, imaging procedures or laboratory tests nor mortality between these two periods.
Conclusions: We observed a reduction in incidence of the TM in HIV-infected patients since HAART introduction without new cases in our institution since 2001, but we did not find differences in presenting features or the outcome of the disease.

R2184

HIV/AIDS in children: the disease taken too rarely into account

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Objectives: In Poland in the period 1987–2005 about 90 cases of HIV infection in children were diagnosed. During this time 12 children died because of AIDS. The aim of this study was the analysis of age of HIV diagnosis and suspected age of infection.
Methods: In our department about 100 children were diagnosed to HIV from 1996 to 2005. Among them this infection was found in 25 individuals. Diagnosis was based on various clinical symptoms and virological examinations.
Results: In 23 children maternal transmission was confirmed. The age of diagnosis was from 1 month to 10 years. Only 4 children were diagnosed in their first month of life. In 8 individuals HIV infection was showed between 3rd and 6th month of life, in 3 children between 8th and 12th month. Five patients were diagnosed at the age 2–4 years, and 3 patients between 5th and 10th year of life. In admission at our department 2 children was with clinical category N, none with category A, however 11 persons with category B and 5 persons with category C (symptomatic AIDS). In relation to maternal infection, the diagnosis of children preceded HIV diagnosis in mothers. In two cases HIV infection was hidden by mother and revealed barely after documented HIV in child. Twenty-one mothers were unaware of infection. In some of them the disease was advanced. In two children the source of infection remained unknown. These patients were with category B and C.
Conclusions: In Poland testing to HIV is not obligatory in pregnant women. Thus this infection is not too common in our country, we postulated to introduce such examination together with examination to syphilis before and during pregnancy. In children with recurrent infections the HIV aetiology ought to be considered.

R2185

Comparison of aetiology and resistance in respiratory isolates of AIDS patients vs. non-AIDS patients in South Sudan and Kenya

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Objectives: Immunodeficiency with reduced clearance of respiratory pathogens, multiple courses of antibiotic therapy in consequence of opportunistic infections and long-term rifampin therapy for tuberculosis (TB) and cotrimoxazol chemoprophylaxis have been associated with antibiotic resistance in HIV positive patient population.
Methods: In a small point prevalence survey, we compared aetiology and resistance phenotype in two groups of patients in Sub-Saharan Africa – 50 HIV positive patients in Nairobi and 182 HIV negative patients in OPD in Mapuordit in South Sudan. Samples were transferred to Slovakia by air and aetiology and resistance was determined from respiratory tract isolates.
Results: In comparison of aetiology among first group 79 HIV positive patients in Nairobi (A) (85 samples) and 105 samples from 182 patients from HIV population in South Sudan is different. *Moraxella catharralis* was significantly less frequent (28 vs 14%, $P < 0.05$) in patients in Nairobi. Vice-versa, *Streptococcus pyogenes* (9% vs 0%, $P < 0.02$) and *S. aureus* (21% vs 12%, $P < 0.05$) were more frequently detected in Sudan as well as *Enterobacteriaceae* (24% vs. 12.9%, $P < 0.05$).We observed following differences: in Sudan, there was no evidence of AMP-R H. influenzae (in Kenya 50% AMP-R) and MRSA (in Kenya 8%) as well as PEN-R pneumococci (in Kenya 6.6%). All 105 isolates from South Sudan were 100% sensitive to all antibiotics. We have observed that respiratory tract isolates from patients with HIV on HAART have significant different aetiology and resistance profile: HIV positive patients: *Moraxella catharalis* is among pathogens significant more frequent in HIV positive patients (24.7% vs 14%, $P < 0.04$) as in HIV negative patients. Vice-versa, *Str. pyogenes*, *S. aureus* and *Enterobacteriaceae* most often colonized respiratory tract in HIV negative patients ($P < 0.05$). Concerning resistance phenotypes, in South Sudan resistance phenotypes didn't occur (either 1 ERY-R *Str. pyogenes* or PEN-R pneumococcus, any MRSA – methicillin resistant staphylococcus and AMP-R *H. influenzae*). No resistance is apparently explainable due to reason of absolute inaccessibility and no exposition to antibiotics due to 21 years civil war and related therefore isolation.
Conclusion: Concerning HIV positive population, resistance phenotypes were narrower also in consequence antecedent exposition ATB, because HIV patients have more often opportunistic infections plus they received cotrimoxazol in prophylaxis.

R2186

HAART in HIV patients: analysis of first therapy regimen. MACOVI study (Málaga HIV cohort)

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Objectives: To analyse the first highly active antiretroviral therapy (HAART) in a study in the real life in patients naive HIV and to describe the causes by which this treatment is interrupted.
Methods: Multicentre, retrospective and observational cohort study from all the HIV naive patients, diagnosed from January-1997, starting a first HAART until December-2003 in any hospital of Málaga area (south of Spain).

Results: A total of 603 patients who started a first HAART regimen were included in analyses. 151 (26%) stay with the same pattern with a mean time of 22.8 ± 17.7 months. Switching before one year was observed in 36%. Causes were toxicity 22.1%, simplification 20.5%, virological failure 15%, structured treatment interruption 7.1% and other causes 7.2%. The main toxicities were: gastrointestinal effects (25.5%), principally vomit and diarrhoea by protease inhibitors (PIs); neuropsiquiatric (24.8%), overall efavirez related effects and stavudine-associated peripheral neuropathy; haematologic (15.7%), essentially zidovudine-associated anaemia; skin problems (15%) mostly non-nucleoside reverse transcriptase inhibitor (NNRTI) and abacavir associated hypersensitivity reactions and finally kidney diseases (9%) all by indinavir-associated lithiasis. PIs were associated significantly with gastrointestinal and kidney problems in comparation with NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) ($p > 0.001$); and the NNRTIs were linked with neuropsiquiatric and skin signs ($p > 0.001$). The triple NRTIs formulation was lesser toxic ($p > 0.001$). There were no differences in withdrawn ratio or virological failure rates by HAART regimens, even with CD4+ under 100/mm^3.
Conclusion: More than 33% of first HAART regimen were discontinued before one year and the main cause was toxicity followed by virological failure. There were no differences between PIs and NNRTIs in failure and toxicity rates as HAART discontinuation causes. The toxicities causing HAART modification or discontinuation were gastrointestinal, principally PIs linked, neuropsiquiatrics and cutaneous, both attached to NNRTIs. Three NRTIs co-formulation was the lesser toxicity-discontinue regimen.

Hepatitis

R2187

Hepatitis B virus genotyping, core promoter and precore/core mutations among Afghan hepatitis B infected individuals: a preliminary report

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Introduction: In spite of continuation of hepatitis B virus (HBV) vaccination, HBV infection remains as an important public health problem worldwide. Although the HBV genotype distribution has been determined in some parts of the South Central Asia, no survey has been conducted to clarify HBV genotype in Afghanistan.
Materials and methods: Twelve Afghan HBV infected patients living in Afghanistan were enrolled in this study. Partial HBsAg and basic core promoter, precore and core (BCP/preC/C) regions were amplified and subjected for direct sequencing. In parallel, precore G1896A mutation was also determined by an amplification-created restriction site method.
Results: Results revealed HBV genotype D (95% bootstrap value), sub-genotype D1 (98% bootstrap value) and subtype ayw2 in all Afghan isolates. Afghan isolates clustered in a separate branch in the D1 sub-genotype called D1′, supported by 82% bootstrap value. The percentage of intra-genotypic distance among Afghan isolates was 1.05% and inter-genotypic distance with the other genotype D was 2.87% and with other genotypes was 7.50–11.1%. The wild type, mixed infection and precore mutant were found in six, two and four HBV isolates, respectively. The A1762T/G1764A BCP dual mutation was found in one isolate. Three isolates presented single mutation in the BCP dual mutation region, whereas two of them showed a novel G1764T mutation.
Conclusion: In conclusion, this preliminary study revealed HBV genotype D, sub-genotype D1, and subtype ayw2 of HBV among hepatitis B infected patients from Afghanistan. Further investigation should be carried out for better understanding of HBV genotype distribution in other virgin parts of the South Central Asia.

R2188

Primary sclerosing cholangitis as a sole manifestation of hyper IgM syndrome

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Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by inflammation, destruction and fibrosis of the intrahepatic and extrahepatic bile ducts that leads to cirrhosis of the liver. PSC is often complicated by recurrent episodes of bacterial cholangitis .The cause of PSC is unknown but many investigators suspect that it is an autoimmune disease. Other aetiologies, such as infectious agents, toxins or recurrent infections of the bile ducts are also possible causes. About 30% of patients with PSC have elevated serum gamma-globulin concentrations and about half have elevated serum IgM concentrations. About half of patients have serum antibodies against a perinuclear antigen in neutrophil cytoplasm (ANCA). The most common symptoms are fatigue, jaundice, pruritus, and abdominal pain. PSC is said to progress relentlessly to cirrhosis, although a patient's condition may remain stable years. We report a 12 years old female diagnosed as a case of hyper-IgM syndrome who presented with recurrent infections and sclerosing cholangitis. She developed also Evans syndrome (autoimmune haemolytic anaemia and thrombocytopenia). Immunological evaluation showed decreased levels of serum IgG and IgA with elevated levels of IgM. Liver biopsy demonstrated the presence of idiopathic sclerosing cholangitis. The patient was started on monthly IVIG therapy at 400 mg/kg and also prophylactic antibiotics, prednisolone and vitamin E with normalization of her IgG and IgM levels and a decrease in the incidence of infections and normalization of liver function.

R2189

Evaluation of immunity to hepatitis A virus in patients with chronic viral hepatitis B and C

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Objectives: Our study was conducted to estimate the immunological status to hepatitis A virus (HAV) in patients with chronic hepatitis B and C and to identify the risk of hepatitis A superinfection and the need of vaccination against HAV in these patients.

Methods: There were examined a total of 630 patients of our hospital aged from 35 to 65 (65% men, 35% women). There were two (2) groups of patients. Group A: 420 patients with chronic hepatitis B and Group B: 210 patients with chronic hepatitis C. The diagnosis was confirmed with the help of clinical data, biochemical tests and serological markers. At the same time we also controlled 480 healthy individuals. Serum samples from the patients and the healthy individuals were tested for the detection of total and IgM antibodies against HAV. The tests were performed by immunoenzymatic methodology (MEIA, AXSYM - ABBOTT)

Results: In Group A were found to be positive for total anti-HAV 320 out of 420 (76.2%) patients with chronic hepatitis B. In Group B were detected positive total anti-HAV in 168 out of 210 (80%) patients with chronic hepatitis C. The presence of anti-HAV was related to the patients' age. There was a significant tendency towards higher prevalence of anti-HAV in older patients. In group of healthy individuals 226 out of 480 (47.1%) persons were positive for total anti-HAV. None was found to be positive for anti-HAV IgM.

Conclusions: This study showed that there is a significant prevalence of HAV in the patients with chronic hepatitis B and C: 76.2% and 80% respectively compared to 47.1% in healthy persons. It was demonstrated that HAV infection may have a more clinical course in patients with chronic liver disease particularly among older patients. Therefore, vaccination against HAV should be recommended, mainly in younger persons, with previously screening for anti-HAV because natural immunity is common.

R2190

Prevalence of HIV, hepatitis B and C among intravenous drug users on methadone maintenance treatment in Slovenia

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Objectives: Intravenous drug users (IDU) are known to be at high risk of acquiring blood-borne infections. The aim of our study was to determine the prevalence of HIV, hepatitis B (HBV) and hepatitis C (HCV) infections among IDU on methadone maintenance treatment (MMT) in Slovenia and to establish the differencies between 3 Slovenian regions.

Methods: A cross-sectional study among IDU on MMT from 3 regions (coastal region, Ljubljana city-capital, Slovene Styria) was carried out in the year 2000. 40 IUD were randomly selected from each region. The blood samples from 120 IUD on MMT were tested to HIV (anti-HIV 1/2 antibodies and p24 HIV antigen), to HBV (HbsAg antigen, antibodies anti-HBs and anti-HBc) and to HCV (anti-HCV antibodies, HCV RNA and HCV genotype).

Results: Of the 120 IUD, 86 (71.6%) were male and 34 (28.4%) female. The mean age was 26.2 ± 6.9 years (range 18–48). The mean length of self reported injecting drug use was 5.4 ± 4.0 years. The mean age of first injecting drugs being 21.2 ± 4.7 years (range 15–44). All tested persons were HIV negative. AntiHBc antibodies as a sign of HBV infection were detected in 14 (11.7%) IUD, 2 of them had also markers of active HBV infection (HBsAg positive). IUD with markers of HBV infection were older than those without markers (32.6 vs. 25.7 years; $p < 0.001$) and had a longer history of drug injection (9.2 vs. 5.0 years; $p < 0.001$). AntiHBs as a sole marker of HBV were found in 14 (11.7%) IUD. 92 (76.7%) of IUD had no markers of HBV infection and they were still susceptible to HBV infection. Anti-HCV antibodies were detected in 33 (27.5%) IUD, 28 (84.8%) of them were also HCV RNA positive, indicating an active HCV infection. The HCV prevalence were significantly different between regions ($p = 0.021$). The antiHCV positive IUD were older than those without HCV infection (31.1 vs. 24.6 years; $p < 0.001$) and had a longer history of drug injection (8.2 vs. 4.4 years; $p < 0.001$). Genotype 1 was found in 19 (67.9%) out of 28 HCV RNA positive IUD.

Conclusion: The prevalences of HIV (0%), HBV (11.7%) and HCV (27.5%) were among the lowest found to date among IUD. Only 11.7% of IUD on MTT were successfully vaccinated against HBV infection and 76.7% of IUD on MMT were still susceptible to HBV infection. Therefore, a more intense action concerning vaccination against HBV is needed.

R2191

Fulminant fungal peritonitis and ascites in a HIV-infected patient with HCV-related chronic hepatitis. A role for prolonged nimesulide self-administration?

R. Manfredi, S. Sabbatani, F. Chiodo *(Bologna, IT)*

Background: The mortality rate of HIV-infected patients (p) with liver disease is substantially increasing.

Methods: An exceedingly rare case of *Candida albicans* fulminant peritonitis and ascites in a p with HIV-HCV-coinfection and stable cirrhosis, and possibly related to exaggerated self-administered nimesulide, is reported.

Results: A 46-y-old p with HIV infection known since 14 years received isolated 3TC-d4T since six years with a favourable laboratory response: HIV-RNA 480 copies/mL, CD4+ lymphocyte count 428 cells/μL. Neither liver biopsy nor specific treatment was performed for a concurrent stable HCV liver cirrhosis. Two months before admission, our p had a shoulder fracture, and uncontrolled nimesulide self-medication was performed during six weeks. A rapidly worsening ascites and oliguria led to admission. Slightly increased ALT, amylase, and bilirubin were detected, but a rapidly increasing ascites and diffuse edema occurred, paracentesis, and diuretic-albumin administration failed, and the worsening ascites-anuria evolved into kidney failure. One day later our p deceased and necropsy examination showed a diffuse polyvisceritis and a micronodular hepatitis with abundant ascites, in absence of kidney-urinary tract anomalies and other signs of decompensated cirrhosis. After p's death multiple ascites cultures yielded isolated *Candida albicans*.

Conclusions: HIV-infected p have increased risks of liver toxicity. NSAID are implicated in severe, and possibly lethal hepatotoxicity. The exceedingly rapid and severe evolution towards a *Candida*-infected ascites associated with refractory anuria, in absence of decompensated cirrhosis, acute hepatotoxicity, and kidney involvement at autopsy, was never observed after NSAID/nimesulide use. Animal models showed a NSAID-induced increased enteric vascular permeability causing infectious peritonitis. Clinicians facing p with advanced chronic hepatitis but no decompensated cirrhosis should remind that NSAID may act on liver-bowel function and could prompt a liver-kidney damage, possibly complicated with infectious ascites.

R2192

Acute reactivation of a severe pulmonary tuberculosis during associated interferon pegylate-ribavirin treatment carried out for a known chronic hepatitis C

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Introduction: Tuberculosis (T) may be reactivated starting from a latent T infection, when immunodeficiency (usually jatrogenic), or other risk factors, become apparent. Post-primary T episodes were described also decades after a primary *Myc. tuberculosis* infection, in patients (p) who show apparently limited radiographic signs at chest X-ray. Some grade of immunodeficiency may also depend on associated IFN-ribavirin for an underlying chronic HCV hepatitis, as expressed by the frequent emerging of leuko-neutropenia, and altered cytokine network.
Case report: In a p aged >50 years with negative history of T, an occasional chest X-ray showed fibrous-calcific infiltrates at upper right lobe. After 11 years, due to a progressive chronic HCV hepatitis, IFN plus ribavirin were started with good tolerability for 7 months, until a sudden occurrence of cough and haemoptisis associated with a pulmonary lesion suggestive of T became apparent, in the same area where some reliquates were demonstrated 11 years before. A HRCT examination disclosed 2 excavated infiltrates. Both direct microscropy and culture of sputum-BAL proved positive for *Myc. tuberculosis* (susceptible to all tested compounds), while a positive Mantoux became evident. An absolute lymnphopenia (nadir 966 cells/μL), prompted a T-cell subset study, which showed an imbalance of the CD4/CD8 ratio and an absolute CD4+ count of 290 cells/μL. Notwithstanding 5 consecutive weeks of isoniazide-ethambutol-rifampicin-pyrazinamide administration, the sputum remained positive, thus confirming the role of immunodeficiency in prompting a difficult-to-treat T.
Discussion: Animal models demonstrated that an increased release of immunosuppressive cytokines (IL-10-TGF-ß), may prompt a T reactivation, while a maintained T-cell competence enhances T latency. Although a few cases of non-infectious lung involvement, interstitial pneumonia, and bronchiolitis obliterans were described during IFN therapy administered to transplant p, no episodes of reactivated T were reported. Although our disease occurrence seems unique, the increased use of IFN and potent agents for the management of chronic hepatitis or other diseases, might support the reactivation of latent T. A careful medical history, Mantoux reaction, and a chest X-ray, are mandatory before starting IFN. The immunosuppression related to IFN-ribavirin may go beyond the expected leuko-lymphopenia, and also act against the quantitative-functional role of T lymphocytes, thus playing a key role in latent T reactivation.

R2193

Immune response tp standard dose of hepatitis B vaccine in HIV clients of Kermanshah behavioural disease counselling centre in 2004

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Objectives: Because of HIV and Hepatitis B coinfection and due to having the same transmission ways among HIV positive cases, Hepatitis B has turned into a major health concern. The increasing number of HIV cases and their relevant problems, especially opportunistic infections, demands for Hepatitis B vaccination. This study, therefore was conducted to evaluate the immune response against Hepatitis B vaccine and related factors among HIV positive cases and the probable approaches to improve its level.
Methods: In this cross-sectional study, 169 HIV positive cases who were Kermanshah's Behavioural Diseases Counselling Center's clients, with negative HBs-Ag and HBc-Ab, were vaccinated against hepatitis B virus with a 20 μg of recombinant HBsAg at 0–1–6 month schedule in deltoid region. A month after the last shot, their HBs-Ab titre was measured. Titres higher than 10 μIU/ml were considered as a suitable immune response. Data included in this study were: age, gender, CD4 count, antiretroviral treatment history, hepatitis C coinfection and injecting drug abuse. Then these data were analysed through χ^2 test.
Results: Among 169 under study cases, immune response was totally 52.7% and then 51.9% and 66.7% for males and females respectively (PV = 0.313). Immune response was 54.3%, 44.3%, and 45.3% in CD4 count>500, 200–449, and < 200 respectively (PV = 0.039). In cases with antiretroviral treatment and without antiretroviral treatment the immune response was 81.8% and50.6% respectively (PV = 0.045); In IDUs and HCV co-infected cases, the immune response rate was 51% and 51.3% respectively (PV = 0.294).
Conclusion: In this study the CD4 count and the history of antiretroviral treatment correlation with immune response level was significant, but other factors like age, HCV co-infection, drug abusing, and gender were ineffective factors in immune response to hepatitis B vaccine. Therefore, early vaccination among cases with higher CD4 counts or cases under antiretroviral treatment seems necessary.

R2194

Interferon-alpha response to hepatitis B/C and B/D co-infections versus HBV infection alone

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In patients with chronic hepatitis B, estimates of the rates of HCV co-infection vary from 9% to 30%, depending on the geographic region. HCV infection can suppress HBV replication. But clinicians must exercise caution when treating coinfected patients with combination interferon plus ribavirin given this risk of HBV reactivation. The clinical course of chronic hepatitis D (CDH) is generally more severe than that of other forms of viral hepatitis. Although the prevalence of HDV infection has declined in the Mediterranean basin in the last decade, it is still an important public health problem. Until now, the only drug that has been shown to be effective for treatment of CDH is interferon-a (IFN-a). We have 231 HBV infected patients 8 of them have HCV and 12 HDV coinfection. From them 7 HBV + HCV, 9 HBV + HDV and 42 HBV patients were proper with liver biopsy (chronic hepatitis necroinflammatory score >4), DNA and ALT levels to begin the IFN-a therapy. They have now IFN-a at least 3 months. Our aim is to compare the IFN-a effects in patients who have co-infection C or D and who have hepatitis B infection alone. In patients who have co-infected, the decrease of DNA level before treatment and after 3 months was not significant (Wilcoxon signed ranks test p = 0.213). But the patients who have only HBV infection the decrease in 3 months is statistically significant (p = 0.0001). If we look at ALT levels in first group the decrease is not significant (but the p value found 0.07), in the second group statistically significant. In both groups platelet, haemoglobin and polymorphonuclear cell counts decreases statistically significant ($p < 0.05$). HBV infected individuals have the risk B and C or B and D co-infection. This effects the therapy. Dual infections present unique

management challenges and treating this patients is more difficult. We should know the propensity for developing more severe liver disease.

R2195

Quality assurance in HBV DNA and HCV RNA quantification

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Objectives: Assessment of accuracy and reproducibility of the Real-time PCR test for the HBV and HCV in diagnostic laboratory. Standardized molecular methods for the quantification of viral nucleic acids is needful to monitor chronically infected patients, allowing evaluation of therapy efficacy and antiviral drug dosing by clinicians.
Methods: We developed two ''in house'' rapid, single tube LightCycler Real Time PCR for accurate quantification of HBV and HCV viraemia with dual hybridization probes technology (FRET). For each virus a serial ten-fold dilution quantitative standard curve was performed and memorized, using international WHO HBV/HCV human reference standard plasma (HBV and HCV Accurun series, BBI Inc.). During each assay one reference standard was used and viral nucleic acids were quantified by interpolation with the memorized Accurun standard curve. To evaluate the test reproducibility the Coefficient of Variation (CV) and Standard Deviation (SD) of Accurun samples were monitored.
Results: A total of 1095 plasma samples for HBV DNA and 3068 for HCV RNA were quantified; 91 and 279 Real Time PCR assays were respectively performed from October 2002 to September 2005. 462 samples resulted HBV DNA positive, with a range from 85 to 5×10^{11} DNA copies/ml, the HBV reference standard crossing point displayed a CV of 6.75% and DS of 1.89.1739 samples were HCV RNA positive (range from 57 to 2.5×10^{9} IU/ml); two different HCV reference standard were utilized, the first (170.000 IU/ml) displayed a crossing point CV of 3.55% and DS of 1.08, and the second standard (910.000 IU/ml) displayed a crossing point CV of 4.15% and DS of 1.16.
Conclusions: The very low CV and DS values of the standard of this two ''in house developed'' Real Time PCR protocols demonstrate the good accuracy and reproducibility of the tests; therefore representing a valid diagnostic tool in monitoring HBV and HCV chronic infections. An External Quality Assessment (EQA) program for dosing viral nucleic acids would be a needed addiction.

R2196

Seroepidemiology of hepatitis A in patients with chronic hepatitis C

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Hepatitis A virus (HAV) rarely causes fulminant hepatitis in general population. Yet it is a cause of significant morbidity in patients with chronic liver disease in whom routine HAV vaccination is recommended. However, studies of HAV seroprevalence in population with chronic Hepatitis C virus (HCV) infection are scarce. A cohort of 185 patients with chronic Hepatitis C (CHC) were examined between January 2004 and February 2005.Patients were stratified in four groups on the basis of the mode of HCV transmission. The HAV seroprevalence in patients with CHC was compared with that in 72 blood donors, aged 18–50 years.Among patients with CHC, 126 were patients of hepatological clinic (HP), 37 were dialysis patients (DP), 9 were multitransfused adults with b-thalassaemia (BTH) and 13 were intavenous drug users (IDU). The patients of the first two groups were 50–70 years old, whereas the other two groups were composed of younger people aged 20–30.The majority of HP, DP and BTH patients received blood or blood products before the implementation of routine screening for HCV (1990). The dialysis treatment and other parenteral exposure during a hospital admission in the past, were different reasons of the CHC in the above groups. IDU were contaminated with HCV by injecting drugs. Sera of the above 185 CHC patients antiHCV (+) (MEIA AXSYME Abbott) and HCV RNA (+) (TMA VERSANT Bayer) were tested also for anti-HAV (MEIA AXSYME Abbott). The prevalence of anti-HAV was 77% in HP, BTH and IDU, whereas in DP was 70%. In the group of 72 antiHCV (-) blood donors, the prevalence of antiHAV was only 17%. The much higher prevalence of anti-HAV found in HP and DP, groups with older patients, is more likely to be related to HAV infection during infancy. In âÔÌ, a group with younger population the high rate of anti-HAV seems to be related to the multiple transfusions and to the passive transmission of anti-HAV, whereas in IDU, a group consisting of young men, the lifestyle as also the higher possibility of parenteral contamination with HAV, were the probable reasons of high seropositivity. Although the prevalence of anti-HAV in HP, DP, BTH and IDU was high, immunization of patients with chronic hepatitis C, without immunity to HAV, may be necessary.

R2197

Prevalence of HBV and HCV infection in a high-security prison

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Prisoners are a high-risk group for HBV and HCV infection, because of the increased frequency of intravenous drug use and its special demographic composition.
Objectives: The purpose of this study was to determine the prevalence of HBV and HCV infection in the prisoners of a high security prison in Greece.
Methods: A total of 150 out of 249 prisoners (60.2%) consented to serologic testing. Ninety-seven (64.5%) of them were foreigners and 53 (35.5%) were Greeks. The sera were tested for HBsAg, anti-HBs, anti-HBc and anti-HCV. All tests were performed using MEIA method (Axsym, Abbott Laboratories). Specimens positive for the above tests were double-checked using the same method.
Results: 1) The HBsAg carriers represented the 11.3% of this social group, with higher prevalence among foreigners (12.4%) in comparison with Greek prisoners (9.4%). Similar results have been described in studies from other Greek prisons (9.3–13.4%). 2) The prevalence of HBV infection is 45.4% and 37.7% among foreigners and Greek prisoners respectively (average 41.6%). 3) The prevalence of HCV infection is 17.3%. HCV infection appears oftener among Greeks than among foreigners 24.5% and 13.4% respectively – which is a relatively low ratio, compared to the results reported by other studies (24.5–68.5%). 4) 3.3% of the prisoners were carriers of both HBV and HCV. 12% of them have had natural exposure to these viruses.
Conclusions: Our results indicate the importance of policies to prevent transmission of HBV and HCV infection in prison population. The measures required should include hepatitis B vaccination, HCV testing and counselling, medical management of infected persons, and substance abuse treatment in incarcerated populations.

R2198

Depression and social support in asymptomatic hepatitis B carriers

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Objective: To understand the effects of chronic hepatitis B (CHB) infection on the psychological status of these patients. The second aim is to investigate the effect of perceived social support on depression in CHB patients.
Methods: Recently diagnosed (<6 months) 110 chronic hepatitis B (HBV) patients with normal liver enzymes level have been included to the study. Patients were investigated for depression, social support and attribution variables. Socio-demographic variables of the patients including gender, age, family status, having children, education level, income was recorded. their illness was discovered.
Results: In the total sample mean depression scores were 7.28 ± 7.93. 72.7% had scores less than 9. 20% of the patients had scores between 10 and 18 indicating mild to moderate depression; 4.6% of the patients had scores between 19 and 29 indicating moderate to severe depression; and 2.7% of the patients received scores of more than 30 indicating severe depression. Thus a total of 27.3% of the patients had depression ranging from mild to severe. There was no significant effect of Socio-demographic variables and age on depression scores. Patients who had mentioned that their families had pulled away from them had significantly higher depression scores than patients whose families had supported them (6.75 ± 6.96 vs. 21.67 ± 21.01; p = .002). Similar to this result was that patients, whose friends had pulled away, had significantly higher depression scores than patients whose friends had supported them (6.65 ± 6.57 vs. 18.17 ± 17.81; p = 0.001). Social support was a negative predictor of depression.
Conclusion: Although physical harm was not present in the patients, depression can be a common psychological symptom. Providing sufficient information about the disease to the patients and their families may help in changing their attributions, thus decreasing depressive symptomatology as well as increasing support provided to the patients by their families and friends. Social support mechanisms prove to be a positive aspect in relation to the psychological functioning of patients in coping with a chronic illness.

R2199

HBeAG negative serological status and low viral replication levels characterise chronic HBV-infected women at reproductive age in Greece. A two-year, prospective, single-centre study

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Objective: To evaluate the seroprevalence of HBsAg in 26746 women at reproductive age in Greece and the HBeAg/anti-HBe serological status of the HbsAg (+) ones as well as serum HBV-DNA levels at labour in a subgroup of 63 HbsAg (+) pregnant women.
Methods: Between August 2003 and September 2005 a total of 26746 women at reproductive age (range 16–45 years) were prospectively evaluated. Serological markers were detected using enzyme immunoassays. Serum HBV-DNA was calculated using a sensitive quantitative PCR assay.
Results: The majority of the study population came from Greece (67%) whereas 20.3% of them came from Albania, 6.9% from Eastern European countries, 3.8% from African countries, 0.9% from Asian countries, 0.5% from countries of Northwestern Europe, 0.2% from Australia and 0.2% from North American countries. Overall, 1.53% of the study population were HbsAg (+) and the majority of them (65%) were Albanian. Among Albanian women the mean prevalence of HBsAg was 4.9%, 5.57% among Asian women and 1.29% among women from Eastern European countries. The prevalence of HBsAg among African (0.29%) and Greek women (0.57%) was very low. Only 4.2% of HbsAg (+) women were also HbeAg (+) whereas the vast majority of them (>95%) were HbeAg (-)/antiHBe (+). None of the females from countries of North America, Northwestern Europe and Australia were HBsAg(+). Undetectable levels of viraemia (<200 copies/ml) were observed in 28.6% of pregnant women evaluated at labour and 15.9% exhibited extremely low levels of viral replication (<400 copies/ml). Only 12.7% of pregnant women evaluated at labour exhibited extremely high serum HBV-DNA levels (>10 000 000 copies/ml) whereas 48.5% of them exhibited HBV-DNA levels between 1500 and 40 000 copies/ml.
Conclusion: The overall prevalence of HBsAg is relatively low among women at reproductive age in Greece but is higher enough among specific populations (Asian, Albanian). The HBeAg(-)/antiHBe(+) serological status is a finding observed in the vast majority of HBsAg(+) women of our study population and a significant proportion of them (44.5%) exhibit extremely low or even undetectable viral replicative status at labour, suggesting possibly that only a proportion of HBsAg(+) women in Greece exhibit an extremely high risk of vertical transmission of the infection.

R2200

Comparison of anti-HCV and HCV RNA results by real-time reverse transcriptase quantitative polymerase chain reaction

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Objectives: In this study we compared HCV RNA results determined by Real-time Reverse Transcriptase quantitative PCR with anti-HCV results and investigated the relationship between these parameters with ALT/AST levels of patients.
Methods: 690 tests from 508 different patients of Medical Faculty of Osmangazi University were studied between January 2002 and December 2004. Anti-HCV (Abbott Axsym System HCV 3.0), HCV RNA (real time Taqman technology, Roboscreen kit and ABI Prism 7700 Perkin Elmer) results and ALT and AST levels of patients were examined.
Results: In our study group, 455 (65.9%) tests of 690 were found positive for anti-HCV and 235(34.1%) tests were negative. In 235 (51.6%) tests of 455 tests which were found to be positive for anti-HCV, HCV RNA was found to be positive and in 220 (48.4%) tests of them HCV RNA was negative. In 20 (8.5%) of 235 serum samples that were negative for anti-HCV, HCV RNA was found to be positive and in 215 (91.5%) of them HCV RNA was negative. When considering about liver enzyme levels, of 690 serum samples, 338 showed normal enzyme levels; in 272 both ALT and AST were elevated, in 23 only AST was elevated, in 57 only ALT was elevated. In ALT and AST elevated group, in 137 of 272 samples anti-HCV and HCV RNA were positive, in 46 only anti-HCV was positive, in 10 only HCV RNA was positive and in 79 both of them were negative.
Conclusion: Anti-HCV negative but viraemic patients can occur. Therefore, PCR is an important test in determining the cases that show no seroconversion and that are uncertain. Quantitative real time PCR is a very useful tool in monitorizing the answer of patients undergoing therapy, as in our study.

R2201

Acute *Brucella* hepatitis: two case reports

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Introduction: Brucellosis is an infection disease, which is appeared acute or chronic progress and is fall into many organs. Acute *Brucella* hepatitis is a rare manifestation of the disease. It progresses with increasing of liver function tests usually. Because of this reason two cases, which were progressed with acute *Brucella* hepatitis in our clinic was submitted.
Case 1: A 42-year-old male patient which was come with fever, sweating, back pain and appetite complains, was hospitalized. His fever was 38.2°C, pulse 88/minute and the other physical examination was normal. His laboratory findings follow; WBC: 4200/mm^3 (lymphocyte 48%) CRP: 25.5 mg/L (N < 5 mg/L), total bilirubin 0.87 mg/dl, ALT: 484 IU/ml, AST: 401 IU/ml, ALP: 298 IU/ml and PTT were normal. His hepatitis markers (HBsAg, Anti-HBc-IgM, Anti-HCV and Anti-HAV IgM) were negative. His *Brucella* agglutination was 1/320 and Rose-Bengals test was positive. *Brucella* spp. was grown from his blood cultures than doxocycline 200 mg/day and rifampicin 600 mg/day were given to the patient. In 5th days of the therapy his fever decreased and in 19th days the liver function tests decreased to normal level.
Case 2: A 38-year-old female patient goes to doctor with arthralgy and morning rigidity. The doctor diagnosed reactive arthritis and gives her NSAID. The patient came to us with fever 39.2°C, pulse 92/minute a week later, and she was hospitalized in our clinic. Her other physical examination was normal. Her laboratory findings were: WBC: 6200/mm^3 (lymphocyte 58%), CRP: 42.5 mg/L (N < 5 mg/L), total bilirubin: 0.72 mg/dl, ALT: 374 IU/ml, AST: 312 IU/ml, ALP: 238 IU/ml. Prothrombin time was normal. Her hepatitis markers (HBsAg, Anti-HBc IgM, Anti-HCV and Anti-HAV IgM) were negative. Her *Brucella* agglutination was 1/640 and Rose-Bengals test was positive. *Brucella* spp. was grown from her blood cultures then streptomycin 1 gr/day and doxocycline 200 mg/day were given to the patient. In 7 days of the therapy her fever decreased. Her liver function tests were decreased 3 weeks later.
Conclusion: *Brucella* infection is endemic in our region in particular. Acute *Brucella* hepatitis, a rare manifestation of brucellosis, was studied carefully with these cases.

R2202

The effect of pegylated interferon on quality of life in the treatment of chronic active hepatitis C

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Objective: Hepatitis C virus (HCV) infection is a world widespread disease and a very serious health problem. Pegylated (PEG) interferon (IFN) is developed because of disadvantages of IFN alfa, which has been used in the treatment of HCV infection since 1986. Health related quality of life (HRQOL) in patients treated with PEG IFN alfa 2a was superior to patients treated with IFN alfa. This study was designed to evaluate the effect of PEG IFN used in the treatment of chronic hepatitis C on HRQOL.
Methods: 40 patients who have been treated in Eskisehir Osmangazi University Hospital Department of Infectious Diseases and Eskisehir Yunusemre State Hospital Department of Gastroenterology at the period of time between 01.03.2003 and 01.04.2005 attended the study. 22 patients in the first group were treated with PEG IFN alfa 2a (180 mg/week) and ribavirin (1000–1200 mg/day), 18 patients in the other group were treated with PEG IFN alfa 2b (1.5 mg/kg) and ribavirin (1000–1200 mg/kg) for 48 weeks. Patients answered the short form-36 (SF-36) to evaluate the quality of life at the beginning of treatment, 12th, 24th, 48th weeks and 24th week after the treatment.
Results: There was no statistically significant difference between two groups on frequency of adverse reactions and laboratory findings. There was also no statistically significant difference between two groups on reduction of drug doses, making pause during treatment and discontinuing the treatment. Sustained virological response was 80% in naïve patients the first group and it was 83.3% in naïve patients the second group (P = 1). They were 60% and 25% respectively at relapsed patients (P = 0.293). SF-36 was evaluated and physical function, role physical, role emotional, vitality, mental health, social function, bodily pain, general health scores were obtained at the beginning of treatment 12th, 24th, 48th week and 24th week after treatment. There was no statistically significant difference on median of each scores obtained from both groups during the period of treatment. In both groups decrease of the SF-36 vitality score from the beginning to the end of treatment was statistically significant. Decrease of other scores we had during treatment was not statistically significant.
Conclusion: HRQOL should be considered in diseases need long-term treatment and have similar cure rates such as chronic viral hepatitis.

R2203

Evolution of antigenaemia and viraemia in mono-infected and HIV-co-infected patients not responsive to chronic hepatitis C treatment

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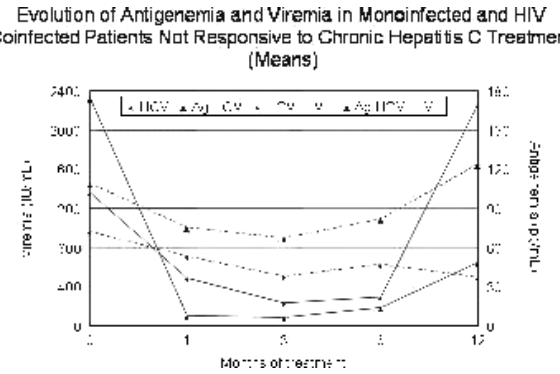
Introduction and purpose: Hepatic disease from hepatitis C virus (HCV) is a serious problem, especially in patients coinfected with human immunodeficiency virus (HIV). In addition to a more rapid progression to cirrhosis, such coinfected patients respond more poorly to treatment than monoinfected patients. The aim of our study is to investigate viral kinetics through the concentrations of core antigen and HCV RNA in monoinfected and HIV positive coinfected patients not responding to treatment with pegylated interferon plus ribavirin.
Materials and methods: Twenty-seven patients with chronic hepatitis C were studied (15 HCV monoinfected patients and 12 HCV-HIV coinfected), controlling their evolution for 12 months following onset of treatment with pegylated interferon and ribavirin. HCV RNA concentration was determined by PCR following reverse transcription of RNA to complementary DNA (Cobas Amplicor, Roche Diagnostics); antigen core concentration was determined by ELISA (Ortho Trak C, Ortho Clinical Diagnostics).
Results: Until the third month of treatment, monoinfected patients showed a stronger decrease in the concentrations of antigen core than in HCV RNA. These levels remained steady until the ninth month; from then until the end of the study period the patients presented a significant increase in HCV RNA concentrations. However, from the six month on, the antigenemia increase was stronger in HIV coinfected patients, HCV RNA levels remaining more or less constant.



Conclusions: Our study shows that the viral kinetics of patients not responding to treatment is different in HCV monoinfected and HCV-HIV coinfected patients. A stronger decrease in antigen core concentrations is produced during the first three months of treatment in monoinfected patients, while a greater increase in antigen core concentrations from the sixth month of treatment on is produced in coinfected patients.

R2204

Hepatitis B virus/hepatitis D virus co-infection in Greece

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Objectives: Hepatitis D virus (HDV) is a single RNA viroid that requires a helper function provided by Hepatitis B virus (HBV) and causes a severe and rapidly progressive form of liver disease. HDV infection is still a major public health problem. HBV endemicity has declined in Greece due to HBV vaccination. Our country has received a great number of socioeconomic immigrants from countries with high endemicity of HBV infection and this might influence the prevalence of HDV infection. Our intention is to investigate the prevalence of HDV infection in Western Attica among Greeks and socioeconomic immigrants with Chronic Hepatitis B(CHB).
Materials/methods: Sera obtained from 484 patients (114 immigrants, 373 Greeks) with CHB as defined by HBsAg (+) and/or HBV DNA (+) and/or HBcIgM Ab(-) were tested for HDV IgG and/or IgM and/or HDVAg. 29 individuals' sera with HBsAg (-)/HbcAb (+) were analyzed for HDVAb. Serological markers for HBV infection were performed by MEIA AXSYM ABBOT and for HDV infection by ELISA. HBV DNA was measured by PCR assay Amplicor Monitor Roche.
Results: 1) Immigrants: 21/114(18%) were HDV Ab and/or Ag(+). 2)Greeks: 82/373 (22%) were HDV Ab and/orAg(+). 3) 28/373 patients with HBV DNA low levels (1000 copies/ml), abnormal ALT and HBcIgM Ab(-) were: 9/28 (32%) HDV Ab(+). 4) 29/373 patients with high HBV levels (>100 000 copies/ml), abnormal ALT and HBcIgMAb (-) were: 1/29 (3%) HDV Ab(+). 5) 4/29 (14%) individuals with HBsAg (-)/ HbcAb (+) were HDV Ab (+).
Conclusions: 1) Although the prevalence of HBV infection in immigrants (17% from our previous study) is much greater than in Greek population (2%), the prevalence of HDV infection in CHB immigrant patients (18%) is less than in CHB Greek patients (22%). 2) CHB patients with low viral load, abnormal ALT and HBcIgMAb (-) exhibit a higher percentage of HDV infection (32%) than CHB patients with high viral load, abnormal ALT and HBcIgMAb (-) (3%). This indicates that in case of coinfection the HDV virus suppresses the HBV virus replication as referred in the bibliography. 3) HBsAg (-)/ HbcAb (+) individuals exhibit an unexpected high prevalence of HDV antibodies (14%). According to the bibliography 10% of individuals with HbcAb (+) as the only marker of HBV infection have detectable HBV DNA levels in their sera. These individuals may be carriers of HDV infection. Further studies have to be done in this setting.

R2205

Quality of life and adherence to treatment in HIV/HCV co-infected patients and co-treated with antiretroviral plus chronic hepatitis C therapy

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Objectives: Assess adherence to treatment and quality of life in HIV/HCV co-infected patients, co-treated for both pathologies.
Methods: An observational prospective study was carried out by the Outpatient Pharmacy Service. Adult patients were eligible considering three conditions: HIV/HCV co-infectioned presence, following antirretroviral therapy for at least 6 months, and starting treatment with peg-interferon + ribavirina combination for chronic hepatitis C. Quality of life as well as adherence to antiretroviral and HCV treatment were evaluated at the beginning of the treatment and 3 months later. Adherence was assessed with the validated questionnaire SMAQ (Simplified Medication Adherence Questionnaire) and the pharmacy records (adherence >90%). Quality of life was assessed with the EuroQOL-5D test (score from 5 (no affected by treatment) to 15 (significantly affected) and visual analogical scale about ''how is your health status today'' from 0 to 100).
Results: During the period of study, 27 patients were enrolled, 3 of them left the study before the 1st month. The median age was 38.73 (28–49), 62.5% were men and the HIV risk factors were: injection drug user 66.7%, sexual contact 16.7% and other 12.5%. 90.9% of the patients had undetectable HIV-RNA viral load and a CD4 basal average recount of 631.28 (237–1360). 12.5% of the patients discontinue treatment due to adverse effects and 16% needed a dosage decrease. Patients considered adherent to treatment according to SMAQ were the 50% at the basal visit and 73% at the 3rd month ($p < 0.05$) and according to the pharmacy records 70.8% to 100% ($p < 0.05$). Quality of life, by EuroQOL-5D health dimensions score, was 5.84 (SD: 1.35) at visit 0, and 6.38 (SD: 1.89) at 3rd month ($p < 0.05$); and in the visual analogical scale 69.9 vs 60.54 (N.S.), respectively. 91.0% of patients had adverse effects related with the HCV treatment and 17.1% because of the antiretroviral drugs.
Conclusions: Adherence to treatment is not decreased by introducing the HCV treatment in HIV pre-treated patients. Despite of the difficult treatment with many adverse effects, the patients compliance is correct. It can be explained because the monitoring of this patients is increased and have educational interventions by pharmacist and physicians in each visit during the HVC treatment. Quality of life is significantly lower after 3 months of treatment compared with the basal measure, although it is not related with non-adherence.

Virology (non-HIV/non-hepatitis)

R2206

Human papillomavirus genotype determination using Inno-LiPA HPV genotyping assay. Is the easy detection of mixed populations of HPV useful?

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HPV, particularly some genotypes (HPV 16, HPV 18...) are recognized as a main cause of cervical cancer, HPV also play a major role in the development of different types of cervical lesions. The aim of the study was to determined the effectiveness of the Inno-LiPA® (Innogenetics) HPV genotyping assay and to looked at the distribution of genotypes or combination of genotypes in our population. This technique of amplification followed by an hybridization on strips allowed simultaneously the detection of 24 genotypes of HPV. 218 endocervical scrapes were tested and compared to results of gynaecological evaluation and cytological examination. All women have atypical or positive index cytology (ASCUS or CIN1 to CIN 3). HPV-DNA was detected in 52% of the samples. According to our results, some genotypes are more frequently represented as HPV 53, HPV 31, HPV 66, HPV 16 all at high risk of cervical carcinoma. 53% of the positive specimens showed only a single genotype on LiPA strip. To consider only ''single types'' HPV 16, HPV 31 and HPV 53 were the most frequent ones. Among the 54 combinations of genotypes a great diversity was observed without any predominance (42 different combinations). To enter all the details, we noticed that inside mixed HPV genotypes we can extract 6 various associations more prevalent than others (53 + 66, 31 + 53, 31 + 66, 45 + 53, 6 + 31, 31 + 68/73). They represent more than 75% of HPV genotypes among the group of patients infected with mixed HPV types and 36% of all HPV positive patients. Informations provided by this test about single or mixed genotypes contained in cervico-vaginal samples is a useful tool combined with cytology and clinical examination results. Inno-LiPA HPV genotyping assay allow to record precise genotypic and epidemiological data of interest for the follow up of the patients. Larger studies are necessary to evaluate the predictive values of the accumulation of multiple HPV genotypes observed for many patients.

R2207

Primary cytomegalovirus infection in patients with Guillain-Barré syndrome

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Objectives: Guillain-Barré syndrome (GBS) is associated with cytomegalovirus (CMV) infection in 10–15% of cases as identified by the presence of CMV-specific IgM-antibodies in serum. Presence of IgM-antibodies may indicate primary CMV infection as well as reactivation from latency. The aim of the present study was to identify primary CMV infection in patients with GBS.
Methods: We studied 46 CMV-seropositive patients with GBS between 1998 and 2003. Sera obtained from these patients were tested by an avidity assay and CMV-specific polymerase chain reaction (PCR). Virological findings were compared with clinical characteristics.
Results: Primary CMV infection that occurred <6 months before onset of GBS was identified in 10/46 (22%) patients by detection of low-avidity IgG-antibodies (n = 9) or IgM-antibodies in the absence of IgG-antibodies (n = 1). High-avidity IgG-antibodies were found in 2/6 (33%) IgM-antibody-positive patients. Recent CMV activity was identified in 17/46 (37%) patients by detection of CMV-DNA in serum. Thirteen of the PCR-positive patients had also high-avidity IgG-antibodies suggestive for reactivation of latent infection. The likelihood of the presence of CMV-DNA in serum decreased significantly with increasing antibody avidity (P = 0.041). Patients with primary infection were younger (P = 0.033) and had more frequently urinary retention requiring catheterization (P = 0.007) than other CMV-seropositive patients.
Conclusions: CMV-associated GBS is common in CMV-seropositive patients and a considerable proportion may be attributed to primary infection. Reactivation of latent virus or reinfection by a different CMV strain, however, also have to be considered as relevant events associated with this neurological illness.

R2208

Symptomatic, primary cytomegalovirus infection in otherwise healthy adults. Increased incidence or increased resort to improved laboratory facilities?

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Background: The increased availability of serological-biomolecular assays for the diagnosis of acute Cytomegalovirus (CMV) infection, allows us to include these assays in the workout of fever of unknown origin (FUO) in immunocompetent adults.
Methods: A retrospective study of patients (p) diagnosed with primary CMV infection during assessment of a FUO, was performed at our centre of Bologna (Italy).
Results: One hundred and 11 p aged 13–42 years (females in 67 cases: 61.4%), were assessed for a FUO since the year 2001. The diagnostic workup also included CMV serology with IgG-IgM search,while pp65 antigenemia and CMV viremia were carried out in selected p. Of 111 p, 16 (14.4%) had a positive CMV IgM assay, confirmed in 6 p by biomolecular testing and antigen search. An altered leucocyte count and differential were always present, while T-lymphocyte subsets showed a transient reversal of CD4+/CD8+ T-lymphocyte ratio, with a reduced percentage and absolute number of CD4+ cells (250–580/μL), and an expanded CD8+ phenotype. Concurrent signs-symptoms included fever in all p, associated with a mononucleosis-like syndrome in 13 p. A moderate (2–4-fold) rise of serum transaminases was found in 12 episodes, in association with an ultrasonography-confirmed hepatosplenomegaly In 3 p, the predominant signs-symptoms were fever, asthenia, fatigue, and anorexia. A normalization of liver enzymes and leucocyte differential preceded the disappearance of lymphadenopathy and hepatosplenomegaly, while positive IgM serology lasted until 36 months in one p (mean time to disappearance: 10–26 months).
Conclusions: Primary CMVirosis is a self-limiting disorder, whose apparent increased frequency is probably attributable to a more easy access to specific and sensitive laboratory testing. Although a treatment is not indicated in otherwise healthy p, clinicians should maintain an elevated suspicion for a primary CMVirosis whem assessing p with FUO, since CMV may cause a prolonged febrile syndrome, and undiagnosed p are at risk to be exposed to further, second level diagnostic workup, due to an apparently unexplained, prolonged disease, usually associated with constitutional symptoms.

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R2209

First detection of human astroviruses in raw sewage samples in Baranya County, Hungary

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Background: Routine procedures for monitoring viruses in water samples have not been drawn up for the water-microbiology screening panel. Enteric viruses, including astroviruses, are able to persist under environmental conditions and may cause public health problems by contaminating natural and drinking water resources.
Objectives: The aim of the study was to clarify whether human astrovirus-associated gastroenteritis cases observed among hospitalized children give a good indication on the real impact of this virus on the population, or whether only a small proportion of the true number of HAstV infections is being observed by physicians.
Methods: To obtain data on whether HAstVs are shed in the environment, 35 raw sewage samples from 22 sewage plants in different regions of Baranya County, Hungary were tested for astrovirus using the polyethylene-glycol method for concentration and the guanidinium thiocyanate-silica procedure for extraction of viral RNA. Reverse transcription-polymerase chain reaction with HAstV-specific primer pairs Mon2/PRBEG and Mon2/JWT4 was used for amplification and the specificity of amplicons was confirmed by sequence analysis.
Results: Among the 35 raw sewage samples, 15 (43%) contained HAstV and by sequence analysis, 10 genotype HAstV-1 and 1 genotype HAstV-2 were identified. Four samples were untypable.
Conclusion: This investigation applied for the first time a molecular virological method to detect human astroviruses in sewage in Hungary, as was this viral screening test in conjunction with a routine bacterial and chemical water assay.

R2210

Seroprevalence of human parvovirus B19 infection

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Objectives: Parvovirus B19, a small, nonenveloped ssDNA virus, causes infections ranging from asymptomatic to potentially fatal. Erythema infectiosum in children, aplastic crisis in patients with chronic haemolytic disorders and hydrops fetalis are clinical manifestations caused by parvovirus B19 viraemia. The purpose of this study was to determine the seroprevalence of human parvovirus B19 acute infection in a tertiary care hospital in Greece.
Methods: Sera from 467 patients of different age and sex groups were examined for the presence of IgM antibodies against human parvovirus B19 for routine virological diagnostics during the period from May 2001 to August 2005. For the determination of specific IgM titre in sera, a commercial enzyme-linked immunosorbent assay (ELISA) using high specific recombinant parvovirus B19 antigen was used (Novatec, Germany).
Results: Out of the 467 patient samples, 329 came from children and 138 from adults (mainly pregnant women). IgM antibodies to parvovirus B19 were present in 35 patients (18 women and 17 men). Positivity rates were 9.7% (32/329) for children and 2.1% (3/138) for adults. No IgM positive sera were observed in patients with haemoglobinopathies. The seasonal distribution of acute infections in rank order was: winter 40%, spring 31.4% and summer 28.5%. No IgM positive samples were found during autumn period. Annual positivity rates were: 9%, 10.7% and 7% in 2001, 2004 and 2005, respectively, while in the years 2002 and 2003 ranged below 4.5%.
Conclusion: In our study, children were most commonly affected by parvovirus B19 and infections were most prevalent during winter season. Clinicians should be alert to suspect parvovirus B19 in some clinical manifestations and laboratories should be able to detect it.

R2211

High-risk human papilloma virus in women with abnormal pap-smears of the uterine cervix in the northwest of Spain

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Objectives: High-risk human papillomavirus (HR-HPV) is considered the most important aetiological factor of uterine cervical cancer. The aim of this study was to know the relationship between high-risk human papillomavirus (HR-HPV) detection and cytological findings among women with primary abnormal pap-smears of the uterine cervix in the North West of Spain.
Methods: 313 women aged 17–66 years with previous cytological abnormalities were included in the study. All patients underwent cytological study and cervical scrapings were collected for HR-HPV detection by PCR using Amplicor HPV Test® (Roche, Diagnostics).
Results: Cytological findings observed in the 313 patients studied were squamous metaplasia and inflammation in 108/313 (34.5%), atypical squamous cells in 61/313 (19.5%), atypical glandular cells in 4/313 (1.3%), low-grade squamous intraepithelial lesions (LG-SIL) in 81/313 (25.8%) and high-grade squamous intraepithelial lesions (HG-SIL) in 20/313 (0.6%). The remainder 39 patients presented a normal cytology. The prevalence of HR-HPV increased from 23% (14/61) in atypical squamous cells to 68% (55/81) in LG-SIL and 75%(15/20) in HG-SIL, respectively. HR-HPV were detected in 28/108 (26%) and 9/30 (23%), of women with metaplasia and without lesions, respectively. For women with age under 30, the overall HR-HPV prevalence were 55.5%, which was significantly higher than those of women aged 30–39 (34.8%, $p = 0.008$) or women age older than 40 (32.5%, $p = 0.002$).
Conclusion: HR-HPV detection was more frequent in patients with LG-SIL, specially in the etary group under 30 years old. HG-SIL correlates with HR-HPV detection in 75% of cases, the most cases were found in women older than 30 years old. The prevalence of HR-HPV in atypical squamous cells, metaplasia and normal cytology described in our study, suggests that a positive HR-HPV screening in conjunction with a previous abnormal cytology can be employed to select women, to whom follow up is recommended.

R2212

Hospital-based study of pneumonia and influenza hospitalisations among adults, Seoul, Korea, 1995–2004: implications for prevention through immunisation

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Background: Epidemics of influenza may cause secondary bacterial pneumonia and exacerbation of chronic underlying diseases in high-risk groups. In 1998, South Korea initiated a national program of mass vaccination targeting the elderly = 65 years of age. At present, there are limited data on the

burden of influenza in adults of South Korea. We analyzed hospital discharge records to describe pneumonia and influenza hospitalizations and excess hospitalizations among those with chronic underlying cardiac and pulmonary disease.
Methods: Among patients 15 years of age and older, we selected hospital discharge records from 1995 through 2004 using a standardized list of pneumonia and influenza (P/I) diagnostic codes from the International Classification for Diseases, 10th Edition. To determine excess hospitalizations associated with influenza, we analsyed hospitalizations for pneumonia, asthma, chronic obstructive pulmonary disease (COPD), and congestive heart failure (CHF) during the influenza A and B epidemics of 2002–03 and 2003–04.
Results: During the 10-year study period, we identified a total of 3553 hospitalizations associated with P/I. Forty-five percent of these hospitalizations occurred among patients aged = 65 - years. A seasonal peak of pneumonia and influenza hospitalizations was identified from November through January. Among patients with P/I, a total of 557 (16%) deaths were identified during the 10-year period. Case-fatality associated with P/I was significantly higher among patients = 65 years of age compared with younger adults ($P < 0.05$). During the 2002–03 and the 2003–04 influenza epidemics, a total of 82 and 88 excess hospitalizations were identified, respectively. During these two influenza seasons, most excess hospitalizations were associated with pneumonia (n = 70), CHF (n = 68) and COPD (n = 24).
Conclusions: A substantial proportion of P/I hospitalizations occur among patients = 65 years of age nearly 1 out of every 6 patients with P/I died. Preliminary data suggest that the influenza season appears to correlate with a substantial number of excess hospitalizations due to pneumonia, CHF and COPD.

R2213

Clinical manifestations, haematologic findings and clinical course in patients with infectious mononucleosis

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Objectives: To study clinical manifestations, haematological findings and clinical course in patients with infectious mononucleosis (IM) caused by Epstein–Barr virus (EBV).
Methods: In 2004 in our clinic 72 cases of IM were diagnosed and treated. The diagnosis was based on clinical criteria (fever, pharyngitis, lymphadenopathy), haematologic manifestations (lymphocytosis >60%) and presence of immunoglobulin M antibodies to viral capsid antigen (VCA) of EBV, detected by enzyme-linked immunosorbent assay (ELISA) technic.
Results: The patients' age ranged between 1.2–29 years. The main clinical features were: lymphadenopathy 88%, pharyngitis 78%, fever 78%, splenomegaly 54%, hepatomegaly 43%, rash 16%, palatal enanthem 12%. Haematologic abnormalities were: leucocytosis (the highest value 27.000/mm^3) 73%, absolute lymphocytosis 72%, atypical lymphocytes 45%, neutropenia (2000–3000/mm^3) 62%, thrombocytopenia (<140.000/mm^3) 44%. Elevated levels of transaminases enzymes (the highest value 621 U/L) were recorded in 68% of patients. *Streptococcus pyogenes* was isolated from throat culture in 38% of patients. The mild and moderate forms of IM counted 95%. Management was consists in supportive treatment and nonsteroidal anti-inflammatory agents; corticosteroids were used in 4 patients (2 cases with severe airway obstruction, 1 case with prolonged high fever, 1 case with severe thrombocytopenia).
Conclusions: The most frequent complication was hepatitis. Streptococcal pharyngitis was associated with IM in 38% of cases. Corticosteroid therapy was used in 5% of cases.

R2214

Correlation of quantitative measurement of BK polyomavirus (BKV) DNA with the clinical course of BKV infection in renal transplant patients

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Objectives: To investigate the use of a quantitative PCR assay for monitoring of BK viral load and to investigate the correlation of BK viral load to the clinical data.
Methods: Four patients with known BK replication were included in the study. One suffered from BK nephropathy (renal dysfunction and viral particles in graft biopsy) and the other three did not show any such renal dysfunction. BK replication was defined as the presence of decoy cells in urine specimen in two or more consecutive specimens in an interval of less than one month. In these patients the immunosuppression therapy was decreased slowly. The presence of the BK virus in urine and blood was monitored using two different tests, a qualitative in-house nested PCR and a quantitative real-time PCR (affigene®BKV trender; Sangtec Molecular Diagnostics, Sweden).Clinical evaluation e.g. serum creatinine levels, and immunosuppression therapy was correlated to analytical data.
Results: As expected, viral loads were higher in urine than in blood as it was expected. After reduction of immunosuppressive drug dose a reduction in virus load in both in urine and blood, together with the disappearance of decoy cells was observed.Patient 1. Treated for BKV nephropathy. The blood samples showed a median viral load of 3.0E + 06 copies/ml (range, 4.28 E + 04 to 9.90E + 06), and the urine samples showed a median viral load of 1.54E + 10 copies/ml (range 0.0E + 00 to 4.65E + 10). The serum creatinine levels were above normal values. Patient 2: The blood samples showed a median viral load in blood of 1.12E + 06 copies/ml (range, 1.67E + 03 to 5.80E + 06). The urine samples showed a median viral load of 1.45E + 12 copies/ml (range 1.50E + 07 to 6.62E + 12). Patient 3: The blood samples in this patient showed a median viral load in blood of 1.975E + 04 copies/ml (range, 0.00 E + 00 to 2.93E + 04). The urine samples showed a median viral load of 8.25E + 11 copies/ml (range 0.00 E + 07 to 6.62E + 12). Patient 4: The blood samples in this patient showed a median viral load in blood of 1.67E + 07 copies/ml (range, 1.60 E + 05 to 3.94E + 07). The urine samples showed a median viral load of 3.47E + 12 copies/ml (range 8.90 E + 09 to 1.64E + 13).
Conclusions: Serial measurement of viral loads by quantitative PCR BKV could be a useful tool in monitoring the course of BKV infection, and so we can prevent early the course of the illness, making adjustments in the immunosupression drugs. Quantitation of BKV DNA should always be interpreted together with clinical findings.

R2215

Cytomegalovirus associated disease in immunocompetent patients

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Cytomegalovirus (CMV) is common and reaches most of population. Clinical CMV disease in normal host varies from an inapparent infection to the mononuclesosis-like syndrome. Severe illness can occur after reactivation of the latent virus in immunosuppressed host. On the contrary CMV severe disease is a relatively exceptional event in the immunocompetent patient.
Case reports: 10 cases of CMV-related disease in previously healthy immunocompetent subjects were admitted to our

Department between 2001 and 2005. 7 were men and 3 women, median age was 42 years (range 21–68). Only 1 pt had comorbidities: NIDDM, MGUS and hepatic steatosis. The most frequent symptoms of presentation were: fever, chills, headhache, fatigue and spleen enlargement. Each pt had acute, anicteric hepatitis. Transaminases levels went above 5/6 times their normal ranges. We performed an ecotomography to 8 pts: in 5 of them a liver enlargment was shown. Levels of LDH and ALP also were high, till twice their normal range. Each pt had increased lymphocyte/monocyte count (>50%). Atypical lymphocytosis was a feature on blood examination in 5/10 pts. T-lymphocytes subset showed a transient reversal of CD4+/CD8+ ratio with reduction of CD4+ percentage and increased number and percentage of CD8+ lymphocytes. Five pts had a more severe form of disease with organ involvement: 1 pericarditis, 1 polisierositis, 1 meningitis, 3 interstitial pneumonia. Diagnosis was performed with pp65 direct antigenemia test in 8 pts: 6 resulted positive. ELISA serology was positive for CMV IgM antibodies in 10/10 pts. The measurement of IgG avidity improved the diagnosis in 2/10 pts. Antiviral therapy was given just in 2 pts, because of their severe clinical conditions. A woman with polisierositis and interstitial pneumonia and another one with meningitis were treated with Ganciclovir (5 mg/kg twice daily) for 14 days. All the patients completely recovered and no sequelae were observed after discharge.
Conclusions: We must remain aware of the protean manifestations of CMV infection in immunocompetent persons. The rapid diagnosis of CMV infection could be important to avoid further, second-level diagnosis workouts. However, early investigation of rapid treatment may be important. Use of gancyclovir treatment should be considered in the rare patient with severe organ-based pathological conditions.

R2216

Herpes zoster and patient age

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Objective: To analyse the correlation between age and clinical form, anatomical site and pain in zoster.
Material and method: We retrospectively analysed the case records of our patients with Herpes Zoster admitted in Hospital of Infectious Diseases between 1 Jan 2000–31 Dec 2004. We performed four groups of different ages excluding all those with acquired immunosuppression. Statistical was performed in EPIINFO 6, Excel statistical analysis and SPSS10.
Results: 289 patients with median age 58.2 (max 86 years and min 2 years of age), 94% adults, 56% women. The divide on age groups was: younger than 18 years 6% of cases, 19–40 years 9% of cases, 41–65 years 37% of cases and older than 65 years 48% of cases. After clinical form we recorded: 147 cases with typical form, 71 case with extension, 19 with dissemination and 50 patients with septic complication. Age did not influence the appearance of clinical form neither the typical nor the disseminated one. Anatomical site for exanthema was: cervical in 50 cases, trigeminal in 39 cases, Ramsay Hunt syndrome in 8 cases, thoracic in 123 cases, lombar-sacrat in 50 cases and dissemination in 19 cases. Except Ramsay-Hunt syndrome which rare appear over 65 ($p < 0.05$) and trigeminal zoster which is more frequent over 65 ($p = 0.08$), age did not influence the anatomical site of exanthema. Post herpetic neuralgia complicated 137 cases frequently over 65 years ($p = 0.00042$).
Conclusions: 1) zoster is rare under 40 years; 2) the clinical form of disease is not influenced by age; 3) patient age not influence the anatomical site excepting Ramsay Hunt syndrome and trigeminal form; 4) post herpetic neuralgia complicate the course of disease over 65 years.

Mycobacterial infections (including diagnosis)

R2217

Performance of Amplicor/Cobas PCR for *Mycobacterium tuberculosis* detection in clinical specimens

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Objectives: We evaluate the commercially available nucleic acid amplification technique Amplicor/Cobas PCR, Roche, USA, which has been suggested as accurate enough to be routinely used for the rapid diagnosis of tuberculosis.
Material and methods: Using the Lowenstein-Jensen (LJ) culture as the 'golden standard' and culture positive results as a reference, we evaluated the sensitivities of microscopy and Amplicor/Cobas PCR in 115 culture positive sputum samples. Samples were derived from TB patients attended in our Hospital.
Results: Overall sensitivities were 46% (53/115 samples) for microscopy and 84.3% (97/115 samples) for Amplicor/Cobas PCR. In particular, Amplicor/Cobas PCR sensitivity was increased in smear positive samples (96.2%), while limited to 74% in smear negative samples.Conclusions: Our results indicate that Amplicor/Cobas PCR is a promising method in TB diagnosis, taking into consideration that it is well standardized and provides a fast and accurate result, especially in smear positive patients. However, cost-effectiveness studies are required to support introduction of this method routinely, at least in high-burden environments.

R2218

Antimicrobial susceptibility rates of *Mycobacterium tuberculosis* against first-line drugs

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Objectives: *Mycobacterium tuberculosis* infects one third of the world's population. Culture is the ''gold standard'' for detecting mycobacteria in clinical specimens. Testing of *M. tuberculosis* isolates for drug susceptibility is important to guide therapy. The aim of this study is to report the antibacterial susceptibility rates of *M. tuberculosis* strains isolated in our laboratory.
Methods: Laboratory records between January 2001 and January 2005 were evaluated retrospectively. Susceptibility rates against first line drugs (Isoniazid (INH), rifampin (RMP), ethambutol (EMB), streptomycin (STM)) were recorded. Lowenstein-Jensen agar and BACTEC 460 TB system were used for culture. Acid-fast stain was performed for each sample.
Results: A total of 5602 materials (2104 respiratory specimen, 1275 urine, 788 body fluids, 445 tissue specimen, 990 others) were admitted to the laboratory for acid-fast stain and mycobacterium culture. *M. tuberculosis* was detected by culture in 145 (2.5%) of 5602 samples. Acid-fast stain was positive in 46 (31.7%) of 145 samples. Growth was detected only in BACTEC 460 TB but not in Lowenstein Jensen agar for 16 (11%) of the 145 samples. Seven of these 16 samples were pus and the others were, bronchoalveolar lavage fluid and tissue specimen. The

results of acid-fast stain were also negative for these 16 samples. One hundred and fifteen (79.3%) of 145 *M. tuberculosis* strains were sensitive to all four antibiotics (INH, RMP, EMB, STM) tested. Sixteen (11%) were resistant to only INH, 3 (2%) were resistant to only RMP, 3 (2%) were resistant to both INH and RMP, 3 (2%) were resistant to INH and EMB, 2 (1.3%) were resistant to INH, RMP and STM and 3 (2%) were resistant to all 4 antibiotics tested.

Conclusion: 1. Eleven percent of the positive culture result were obtained only by BACTEC 460 TB system, this demonstrates that BACTEC 460 TB is superior to conventional methods (Lowenstein Jensen agar and acid fast stain) in the detection of *M. tuberculosis*.2. As approximately 20% of *M. tuberculosis* strains are resistant to at least one first line antibiotic, treatment of tuberculosis should be guided by antimicrobial susceptibility results in our country.

R2219

Salivary gland mass as the only manifestation of tuberculosis

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Objectives: Salivary gland involvement is included among the les commonly encountered forms of cervicofacial tuberculosis. However, since the incidence of extrapulmonary tuberculosis at all sites has been increasing steadily since 1985, the diagnosis of *Mycobacterium tuberculosis* must enter into the differential diagnosis. In this study, the frequency of tuberculosis and its distribution according to sex and type of gland has been determined.

Methods: This is a retrospective case series study and the data were collected through observation of the files of the patients that were operated during 1996–2003 in Imam And Amir Aalam hospital due to salivary gland mass. There are no exclusion criteria.

Results: Overall, 164 patients had salivary gland mass resection between 1996–2003.Among them, 5 cases of salivary gland mycobacterium infection were reported. Four cases (80%) were in submandibular gland and the last one (20%) was in parotid gland. Mean age was 37/4 (SD: 9/09), the minimum of age was 25 and the maximum was 49. All the patients were female and midwife. Malignancy was seen in 36 patients (23/4%). Mean age in malignancy was 48 and that of nonmalignant was 38/3. This difference was statistically significant (p = 0/01). Mixed tumour was the most prevalent diagnosis among the population.

Conclusion: At present study, the frequency of mycobacterium salivary gland infection was 3/2%. In a study done in 1993, this was 2/3which is not statistically significant. In other studies, almost all the cases were in parotid gland; whereas in our study 4 out of 5 were in submandibular gland. These results need to be proved by other prospective studies.

R2220

Susceptibility of *Mycobacterium tuberculosis* on anti-TB drugs in Kosovo

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Objective: Objective of the study is presentation of the level of multi-drug resistance of *Mycobacterium tuberculosis* in bacteriological confirmed cases in Kosova.

Methods: Samples have been examined at the Reference Laboratory for Tuberculosis at the Department of Microbiology; National Institute for Public Health of Kosova.During the study period, that covers mid of 2003–2004, 6267 samples have been processed, mainly referred from suspected TB hospitalized cases at the Clinic for Lung Diseases, University Clinical Center of Kosova, in addition to the ones coming from other clinics and primary health care services. Processing of samples has been based in the standards for processing TB suspected samples and proportion method for susceptibility testing on anti TB drugs has been used. Lowenstein Jensen media, with following concentration of anti TB drugs have been utilized: isoniazid (INH) 0.2 μg and 1 μg, rifampicin (RMP) 20 μg, ethambutol (EMB) 2 μg, streptomycin (SM) 4 μg and Ethionamide (ETH) 30 μg. Incubation, interpretation and reporting of the results have been done according to the international standards.

Results: During the study period, from 6267 samples received at TB reference laboratory of the Department of Microbiology, National Institute for Public Health of Kosova, referred from all levels of the Kosovo health care services, 720 of them have resulted positive based in cultivation. On 264 samples in whom susceptibility testing on anti TB drugs has been determined, following rates of resistance has been gained: INH, (both concentrations), 7.1% during 2003 and 9.7% in the year 2004; EMB rate of resistance 21.4% in 2003 and 29.7% in 2004; SM, 21.4% in 2003 and 4.8% in 2004; RMP 17.8% in 2003, comparing with 5.45% in the year 2004 and *M. tuberculosis* rate of resistance on ETH was 25% in 2003, followed by 40.6% in the year 2004. Five cases have been reported as multi-drug resistant during the year 2004 due to their expressed resistance at all five tested drugs, presenting multi drug resistance of *M. tuberculosis* at 2.2%.

Conclusions: Scale of multi drug resistance of *Mycobacterium tuberculosis* is still at the low level, emphasizing the need for continual monitoring of the susceptibility of *M. tuberculosis* on anti TB drugs with more advanced methods and implementation of TB prevention strategy in Kosovo.

R2221

Predictive values of diagnostic tests in patients with tuberculous pleural effusion in Iran

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Objectives: Tuberculous pleural effusion occurs in 30% of patients with tuberculosis (TB). Rapid diagnosis of a tuberculous pleural effusion would greatly facilitate the management of many patients. The purpose of this study was to determine sensitivity, specificity, and predictive values of clinical, laboratory, radiographic findings in patients with tuberculous pleural effusion.

Methods: The cross sectional study was performed between august 2002 and March 2004 at a referral teaching hospital. Major clinical, laboratory, and radiographic findings were evaluated in 88 cases of pleural effusion, 33 with confirmed TB pleural effusion (TBPE) and 55 with a diagnosis other than TB (NTBPE).

Results: The sensitivity of culture of pleural effusion and tissue were 3% and 9.1% respectively. The mean of adenosine deaminase (ADA) values in TBPE was 36.7 U/L (±18.72), and the mean in the NTBPE was 28.2 U/L (±17.0). Both the sensitivity and specificity of ADA estimation in diagnosing tuberculosis were 55%. The sensitivity of PCR was 3% with specificity of 12.7% (positive predictive value, 50%; negative predictive value, 70%). Younger age ($p < 0.024$), positive history of exposure to TB patient ($p < 0.02$), and the combination of fever, weight loss and sweating ($p < 0.01$), were associated with tuberculous pleural effusion. There were also significant

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association between Positive sputum smear ($p < 0.001$), positive sputum culture ($p < 0.006$), positive pleural biopsy ($p < 0.001$), pleural LDH>200 ($p < 0.005$), pleural lymphocytes>50% ($p < 0.015$) and TBPE.
Conclusions: In our region with a high incidence of tuberculosis, the most frequent cause of exudative pleural effusion is tuberculosis. We suggest that the diagnostic planning of pleural effusion should be determined in each region with a view to the adoption of regionally optimized diagnostic and therapeutic facilities.

R2222

Antituberculotic chemotherapy selects methicillin-resistant *Staphylococcus aureus* in HIV-positive Cambodian children

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Introduction: The aim of this study is to assess the role of tuberculosis (TB) and antituberculotic chemotherapy in HIV positive Cambodian children on HAART (21 months) on immune reconstruction, frequency and etiology of opportunistic infections.
Methods: 28 HIV positive children on HAART (Lamivudine + Stavudine + Nevirapine or Efavirenz), 13 of them with TB on antituberculotic chemotherapy (RIF + INH + PZA) in the project House of Family (HOF), Bl. Maximilian Coble's Clinic, were analysed for CD4 cells, occurrence of clinical symptoms of opportunistic infections, aetiology and antimicrobial resistance of cultures from infection site.
Results: Children with TB (n = 13) had mean increase of CD4 cells somewhat slower than those children without TB (n = 15). After 21 months of HAART the mean CD4 cell count increased from 135 to 802 in TB positive children and from 207 to 1027 in TB negative children. Upper respiratory tract infections (URTI) were commonest opportunistic infections in both groups, children with TB or without TB. In children with TB was on second place pneumonia and in TB negative children bacterial skin and soft tissue infections (SSTI). Pneumonia and upper respiratory tract infections were more often in TB positive children. Those children receiving antituberculotic chemotherapy had higher proportion of multiresistant grampositive bacteria (MRSA, PEN-R pneumococci) and other resistant respiratory isolates (*M. catarhalis*, *H. influenzae*).
Conclusion: Pneumonia and URTI are more frequent infections in HIV positive children with TB comparing children without TB. Moreover, HIV positive children with TB are more likely to be colonized with MRSA which suggests the role of rifampin and/or INH + PZA in selection process of multiresistant bacteria due to prolonged (6–9 months) exposition.

R2223

Moxifloxacin as supportive therapy in pulmonary tuberculosis

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Background: There is increasing evidence on the role of moxifloxacin as therapeutic choice in pulmonary tuberculosis.
Aim: To determine the reasons of administration and the adverse events of moxifloxacin in patients with confirmed pulmonary tuberculosis.
Methods: A retrospective study included all patients between 2000 and 2003 with diagnosis of pulmonary tuberculosis that received at least one dosage of moxifloxacin during hospital stay. Bacteriological findings at admission, reasons of administration of moxifloxacin and adverse events possibly related to moxifloxacin were recorded.
Results: A total of 47 patients (mean age 51.7 ± 17.2 years; 31 (39.7%) males) with pulmonary tuberculosis that received moxifloxacin were identified. Twenty-two patients had positive AFB in sputum at admission. The reasons for administration of moxifloxacin were: resistance to the at least one of the first-line anti-TB drugs, n = 14 (29.7%); adverse events to at least one of the first-line anti-TB drugs, resulting in discontinuation and subsequent replacement with moxifloxacin, n = 13 (27.6%); concomitant respiratory infections, n = 9 (19.1%); empirical treatment before TB diagnosis, n = 9 (19.1%). Mean duration of therapy was 5.8 days (1–30 days). A total of 45 (95.7%) patients presented at least one adverse event that could be possibly related to moxifloxacin; the most frequently observed were: increased hepatic enzymes (n = 25), diarrhea (n = 6) and nausea and vomiting (n = 6). Six patients discontinued treatment with moxifloxacin because of adverse events. At hospital discharge, after a mean of 2.9 months, 18 patients (38.2%) produced a valid sputum sample and 16 had a negative bacteriological finding.
Conclusion: Almost half of patients with pulmonary tuberculosis received moxifloxacin to replace a fist-line anti-TB drug. Moxifloxacin was well tolerated and substantially contributed to the bacteriological cure.

R2224

Quick *Mycobacterium* isolation in cases of fever of unknown origin due to extrapulmonary mycobacteriosis

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Objectives: In order to draw clinician's attention to extrapulmonary mycobacteriosis (MB) as cause of Fever of Unknown Origin (FUO) and to stress the laboratory's ability for quick *Mycobacterium* isolation and determination of antibiotic susceptibilities within a few days, we report on three cases of patients with MB, diagnosed over the past two years in our laboratory.
Methods: Two male (68 and 69 years old) and one female (71 years old) patient were hospitalized with FUO. Two of them were patients with already diagnosed haematological disorders, namely myelodysplastic syndrome and hairy cells leukaemia. There were no indications of pulmonary involvement. Within the course of investigation, bone marrow biopsies were performed and blood samples were taken. Bone marrow aspirations and blood samples were directly inoculated into mycobacterial-specific MB/BacT/Alert Blood Culture Vials (Biomerieux) and placed in the MB/Bact/Alert automatic colorimetric detection system (Organon Teknika). After (mean) 7.2 days of inoculation positive signals were given. Acid-fast staining along with DNA probes (AccuProbe System-GenProbe) and reverse hybridization (GenoType System-Hain) were used for further identification. From the bone marrow samples of the two patients *Mycobacterium tuberculosis* was isolated, whereas from the blood sample of the third patient *Mycobacterium avium* was detected. Susceptibilities were determined with the proportional method. For each patient, proper combined antituberculous chemotherapy was given and full recovery was achieved.
Conclusions: Although the prevalence of extrapulmonary mycobacterial infections is constantly increasing over the last few years, clinicians rarely include MB in their differential diagnosis of FUO, especially when there are no clinical indications of pulmonary involvement. As MB is a curable disease, all efforts towards isolation of mycobacterium are warranted. Since their

introduction a few years ago, the use of mycobacterial-specific liquid cultures has offered quick and easy mycobacterium isolation. Timely diagnosis of extrapulmonary MB is of great relevance, as this could prevent a series of unnecessary invasive practices or even surgeries. The presence of mycobacterium should never be ruled out if no pulmonary symptoms or signs are present and MB should always be included in the differential diagnosis of FUO, especially in patients with underlined haematological disorders.

R2225

Genetic diversity of *Mycobacterium avium* isolates among Slovenian patients

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Objectives: The objectives of this study were to characterize the genetic diversity of human *Mycobacterium avium* isolates collected at the National Reference Laboratory for Mycobacteria (UC Golnik) between 2002 and 2004.
Methods: A total of 60 isolates from 27 patients were collected over a three-year period and identified with the classical biochemical tests and a commercially available AccuProbe® *Mycobacterium avium* Culture Identification Test (Gen-Probe Inc., San Diego, CA, USA). All the isolates were tested with duplex PCR discriminating between *M. avium* and *M. intracellulare* in order to discover any double infections. IS1245-IS1311 spacer PCR typing and IS1245 RFLP were performed to study the genetic diversity of the isolates.
Results: Duplex PCR revealed double infection (*M. avium*/*M. intracellulare*) in four isolates from one patient. IS1245-IS1311 spacer PCR typing showed heterogenic patterns usually comprising up to 6 bands. Albeit simple and rapid, the method lacked sufficient discriminatory power. Fifty-five out of 60 isolates were successfully IS1245 RFLP typed. The patterns showed a high degree of heterogeneity, with the number of the bands mostly ranging from 20 to 30. When using the shorter of the two different IS1245 probes, the patterns generally consisted of fewer bands and were easier to read. In 12 patients, sequential isolates (n = 45) collected in different time periods, were compared. Infections were clinically important and treated in 7 patients. Identical RFLP patterns were seen in the majority of the sequential isolates from certain patients.
Conclusions: In comparison with IS6110 RFLP patterns of *M. tuberculosis*, IS1245 RFLP patterns of human *M. avium* strains are quite diverse and substantially harder to interpret, partly due to numerous bands and partly due to frequent occurrence of faint bands. The latter can be eliminated with careful primer design for the IS1245 probe to avoid cross-hybridization with IS1311. Identical patterns of the sequential isolates collected from certain patients over a longer period of time indicate clinical importance of the infection. *M. avium* infection will remain a challenging clinical problem due to the ubiquitous nature of the bacillus, increasing number of immunocompromized patients and demanding treatment trials.

R2226

Characterisation of *Mycobacterium tuberculosis* populations during chronic infection: an Italian longitudinal study

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Drug-resistance of *Mycobacterium tuberculosis* (MT), due to the selection of carrying mutations clones, is the cause of treatment failures in tuberculosis (TB) infections. The single selected mutations, conferring drug-resistance, are the only molecular changes characterizing the evolution of MT subpopulations in the host, during chronic infections. Up to now, no detailed information on the evolution of the bacterial population in the host is available.
Objectives: To investigate the evolution of the infecting MT population over time, to get a molecular characterisation of drug resistant MT strains circulating in Italy, to clarify the epidemiological relationship of the strains and sub-clones identified in the study population and to elucidate the role of resistant subpopulations in the clinical outcome.
Methods: Longitudinal samples and strains are investigated for their resistance genotype by commercial polymerase chain reaction (PCR) and in house Real Time PCR. MT isolates are further characterized by determination of first and second line drug susceptibility (DST) and by mycobacterial interspersed repetitive units (MIRU) typing.
Results: During first ten months of study 110 longitudinal sputum samples and strains are obtained from 54 patients attending to the Sondalo TB hospital. DST data have been obtained for all strains and molecular work and data analysis are ongoing. Preliminary results show a very good concordance between genotypic and phenotypic drug resistance detection. Six out of 53 baseline sputum samples by real time PCR show a rifampin mixed population (wild type and rpoB mutant). MIRU typing analysis is ongoing, in order to clarify if these cases are co-infections or true heteroresistance.
Conclusion: These preliminary results only allow us to confirm that frequency of resistance and number of serial samples and isolates of MT will permit a suitable statistical analysis of the results.This work is supported by grants from Italian Ministry of Health "AIDS project – Co-infections, opportunistic infections and tumours AIDS-related" grant no 50F.26.

Infection in the immunocompromised host & transplant recipients

R2227

Microbiology of diabetic foot: our experience

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Objectives: To investigate the aetiology of infection in patients with diabetes and foot disease and evaluate the suitability of techniques to obtain a proper sample to identify the pathogens responsible for the infection.

Methods: A total of 13 patients with infected diabetic foot ulcers were studied. Samples were collected after sterile saline washing of the wound and included one swab of skin surrounding the lesion, three swabs of the ulcer base, three tissue curettages obtained by a sterile scalpel taken after flushing the ulcer with povidone iodide solution and saline. Samples for anaerobics were placed in prereduced tyoglicollate broth. Cultures were performed by standard procedures after microscopic examination. Data from all patients were also recorded.

Results: Microorganisms were isolated in 12 out of 13 patients. in 11 patients, a polimicrobial flora was found. In 5 of these, the same microorganisms were isolated from all of the three samples collected. In 3 patients with mixed flora, the same bacteria were isolated from swabs and curettage of the ulcer base but not from the swab collected from the skin surrounding the lesion. In one patient *C. albicans* and in two patients *S. aureus* were isolated only from the bioptic specimens, respectively. Anaerobes (*Peptostreptococcus* spp. and *Bacteroides* spp.) were recovered from two patients. Interestingly, one of these patients had also fever and the same anaerobe was isolated from the blood culture. This patient underwent amputation of multiple fingers. The predominant flora consisted of *S. aureus*, followed by coagulase-negative staphylococci, *P. aeruginosa* and other *Enterobacteriaceae*. *A. baumanii* was also isolated. All *S. aureus* isolated were MRSA and also resistant to macrolides and to fluorochinolones. *P. aeruginosa* was fully susceptible only to meropenem. *Enterococcus* spp. were not GRE.
Conclusions: Our data show that multiple sampling, even if time consuming and expensive, may help to identify microorganisms that may be missed when only one swab of the ulcer is collected. The heterogeneousness of microorganisms involved in infected diabetic foot ulcers and the complex resistance patterns involved suggest that the clinician should obtain detailed information on the infectious agents of the ulcer and the specific drug sensitivity before considering antibiotic treatment of these potentially life-threatening infections.

R2228

Epidemiology and outcome of severe sepsis in patients with diabetes mellitus

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Objectives: Diabetes mellitus is an immuno-compromising disease predisposing to severe infections. The aim of the present prospective study was to determine the epidemiology and outcome of severe sepsis in patients with diabetes mellitus.
Patients and methods: The study included 76 diabetic patients (40 women and 36 men) with severe sepsis. The patients had a mean age of 74 years, (min 40, max 103) and mean ÂÌÉ 27.4 ± 4.1. Thirty-two of the patients (41%) had a first-degree relative with diabetes melitus. Fifteen patients (19.7%) were under insulin treatment. Sepsis was defined according to the following criteria: (a) temperature >38 or <36 (b) heart rate ≥90/min (c) respiratory rate ≥20 breaths/min or $PaCO_2 \leq 32$ mmHg (d) WBC >12 000 or<4000. Severe sepsis was defined as the presence of sepsis plus at least one organ dysfunction. Immunocompromised patients were excluded. The severity of sepsis was classified by the sepsis-related organ failure assesment score (SOFA).
Results: Mean serum glucose values of diabetics at admission were 295 ± 164 mg/ml, mean fasting glucose values in the first 24 hours in hospital were 214 ± 94 mg/ml. The patients had mean SOFA score 3.6 ± 3.2, mean CRP 15.8 ± 10 and mean Hba1c 8 ± 1.7. Thirty one patients (40.8%) had respiratory tract infections, 22 (28.9%) had urine tract infections, 9 (11.8%) had soft tissue infections, 6 (7.9%) had intra-abdominal infections, 2 (2.6%) had endocarditis, one (1.3%) had infection of the central nevrous system, one (1.3%) had ostomyelitis and 4 (5.3%) had infection of unknown origin. Thirteen patients (17.1%) had bacteraemia. Pathogens isolated were *S. aureus* in 4 patients, *E. coli* in 2 patients, *P. aeruginosa* in 1 patient, *Enterococcus* spp. in 1 patient, *E. cloace* in 1 patient, *S. pneumoniae* in 1 patient, *S. viridans* in 1 patient, *S. epidermidis* in 1 patient and *Streptococcus* spp. for 1 patient. Seventeen out of 76 patients died with an overall mortality of 22.1%. There was no statistical difference in mortality of patients without bacteraemia compared to the patients with bacteraemia.
Conclusion: Diabetes melitus is associated with poor clinical outcome and mortality in sepsis. Tight glycaemic control should be a priority in diabetic patients with sepsis.

R2229

Changing assistance issues at a reference infectious disease day-hospital service, compared with the natural history of HIV infection in the HAART era

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Objective: To evaluate frequency and features of admissions performed at a Day-Hospital service in Northern Italy, a retrospective evaluation of all admissions of the last decade (1994–2004) was performed.
Methods and results: Before HAART introduction (years 1994–1996), the proportionally low mean number of admissions (110/year) was linked to the elevated prevalence of HIV disease, which accounted for 89.4% of Day-Hospital hospitalizations, their recurrence, and their prolonged duration. Immediately after HAART introduction, the number of Day-Hospital admissions showed a significant increase, from 171 (year 1997), to 318 (2002), 338 (2003), and 347 (2004) ($p < 0.0001$ versus the pre-HAART era), although this phenomenon paralleled a drop of percentage of HIV-related admissions (from 59.1% of 1997, to a minimum of 25.6% of the year 2001; $p < 0.0001$). While HIV-associated hospitalizations decreased, a temporal increase of admissions due to chronic liver disease occurred ($p < 0.0001$). The reduction of admission duration allowed an increase of overall number of hospitalizations of each examined year ($p < 0.0001$) and the mean bed occupation rate showed a continued rise (8.2 in the year 2000, to 10.9 in the year 2004 ($p < 0.0001$).
Discussion: The modifications occurred at our Day-Hospital service during the last years are largely attributable to the significant changes occurred in the spectrum of infectious disorders which came to our attention: from a low number of prolonged hospitalizations typical of patients with advanced HIV disease, the HAART era led to a progressive broadening of the spectrum of disease and a notable reduction of admission time. Notwithstanding this situation, no significant modification was observed as to mean weight of diagnosis-related group (DRG) features: from a mean 1.03 rate per patient of the year 2000, to a mean 1.05 figure in 2004. The evolution of assistance features in a Day-Hospital setting, seems strictly linked to the modification of prevailing disorders. A permanent monitoring of the features of health care provision at an Infectious Disease Day-Hospital service may allow to consider significant temporal modifications, and contribute to ensure adequate assistant planning, including the eventual revision of structural, professional, technical and funding resources.

R2230

Use of cefazolin for bacteraemia by methicilin-sensitive *Staphylococcus aureus* in chronic haemodialysis patients

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Introduction: Bacteraemia by *Staphylococcus aureus* is a common and serious complication in long-term haemodialysis patients.

Vancomycin is the usual treatment but several cases are produced by methicilin-sensitive strains.
Objective: To evaluate the efficacy and security of cefazolin use in chronic haemodialysis patients with bacteraemia by meticilin-sensitive *S. aureus*.
Patients and methods: Retrospective study of patients in chronic haemodialysis (CH) with bacteraemia by MS *S. aureus* treated with cefazolin during the period between January-2002 and December-2004. Risk factors related to mortality and relapse were analysed.
Results: 33 patients in CH (21 males, 64%) with a median age of 61 years old (18–82) and with MS *S. aureus* bacteraemia, were included. They were treated with cefazolin (20 mg/kg administered IV post-dialysis during a median of 21 days (4–26 days). Only one patient (3%) had to stop treatment due to secondary effects. Bacteraemia was community acquired in 25 cases (76%) and catheter-related in 30 episodes (91%). Charlson index was greater than 4 points in 85% of cases, and the most prevalent pathologies were diabetes and vascular disease. Median Pitt index was 1 pt (0–8) at the onset of bacteraemia and 4 patients (12%) died (two of them were relapses) in the initial follow-up (90 days). Relapse was observed in 4 patients (12%). Factors related to mortality in univariant analysis were: ischemic cardiopathy (37.5% vs. 4%; $p = 0.03$) and the severity of illness graduated by Pitt index (4.2 pts vs 1 pts; $p < 0.01$). Factors related to relapse were: duration of treatment less than 21 days (60% vs. 3%; $p = 0.007$), Pitt index > 4 pts (66% vs. 6%; $p = 0.03$), and Charlson index (10 + 2 vs 6.4 + 3; $p = 0.01$). Duration of treatment was the only factor related with relapse in the multivariant analysis and so was Pitt index respect to mortality.
Conclusion: Cefazolin is an efficacy and safe alternative for bacteraemia by MS *S. aureus* in CH patients. Duration of treatment is related with a high risk of relapse. Pitt́s index is useful for predicting mortality in this group of patients.

R2231

Herpes group virus reactivation in oncologic patients undergoing chemotherapy

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Viral infection is an important cause of morbidity and mortality in oncologic patients, especially those undergoing chemotherapy. Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein–Barr virus (EBV) and Cytomegalovirus (CMV), all members of the herpes virus group, are the most important pathogens, being able to remain latent after the initial infection and be reactivated under immunossupression.
Objectives: To assess the serologic incidence of herpes group viruses reactivation in seropositive oncologic patients undergoing chemotherapy without previous prophylactic antiviral treatment.
Methods: Overal 580 pts (255 M, 325 F), of mean age 49 years, undergoing chemotherapy for solid tumour (n = 335) or haematologic malignancy (n = 245), were studied serologically before, during and one month after chemotherapy course. All patients were IgG seropositive for one or more of the herpes group viruses. HSV: 493/580 (85%), VZV: 487/580 (84%), EBV: 476/580 (82%), CMV: 506/580 (87.2%). ELISA was the method used for the antibodies detection.
Results: IgM serum specific antibodies against HSV, VZV, EBV and CMV were detected in 5.3% (31/580), 5.7% (33/580), 6.9% (40/580) and 8.3% (48/580) of the patients correspondingly. The incidence of the IgM seropositivity was not significantly correlated with the patients' age or sex and was almost identical among the patients with solid tumours and those with haematologic malignancies.
Conclusion: The incidence of serologic HSV, VZV, EBV and CMV reactivation in oncologic patients undergoing chemotherapy is not so high as to justify the administration of prophylactic antiviral treatment in all cases.

R2232

Assessment of *Staphylococcus aureus* colonisation post lung transplantation

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Colonisation with potentially pathogenic bacteria following lung transplantation may reflect some underlying disease process. We reviewed our transplant database for both Cystic Fibrosis (CF) and non-CF recipients for the incidence of *S. aureus* post-transplant. 213 lung transplants were carried out at this centre in 6 years to 2003. 85(40%) were CF patients and 128 (60%) were non-CF. Routine Bronchoalveolar Lavage (BAL) is performed at 1 week, then 1, 3, 6 and 12 months post-transplant, with other routine respiratory specimens being submitted for culture as necessary. Of 85 CF patients transplanted only 8 (8.2%) had *S. aureus* isolated at time of transplant, 1 patient remained colonised at week 1, 2 patients at 1 month, there was no increase by 3 months, 4 patients at 6 months and the same number at 1 year post-transplant. There were 6 patients who had *S. aureus* isolated from other respiratory specimens and the remaining 1 patient never grew *S. aureus* again. Of the 78 (91.8%) patients who did not grow *S. aureus* at time of transplant, only 23 (27%) had positive cultures with *S. aureus*. Of these 23 patients 3 (13%) grew *S. aureus* at 1 week, 5 (22%) by 1 month and 8 (34%), 15 (65%), 17 (74%) by 3.6 and 12 months respectively. *S. aureus* was isolated from other respiratory samples of the remaining 6 patients post transplant. 128 non-CF patients were transplanted in the same period. 36 (28.1%) were colonised with *S. aureus* post-transplant. Only 4 (3.1%) however had *S. aureus* at time of transplant, and of these 4 patients only 1 patient grew the organism again. In the 32 patients who grew *S. aureus* post transplant only, 5 (15.6%) were isolated at 1 week, 14 (43.7%), 15 (46.8%), 17 (53.1%) at 1, 3 and 6 months with no increase by 1 year. The remaining 15 patients had *S. aureus* isolated from routine respiratory specimens only.
Conclusion: *S. aureus* colonisation occurs at a similar rate in CF and non-CF transplant recipients. Further study is required to determine whether or not there is any underlying lung pathology.

R2233

Cryptosporidium parvum infection in a child after allogeneic bone marrow transplant

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Aims: To describe a case of infection by *Cryptosporidium parvum* in a boy receiving an allogeneic bone marrow transplant (BMT) for acute myeloid leukaemia (AML).
Case report: P.S. is an 11-year-old boy received a partially matched allogeneic BMT from his father after radio and chemotherapy and GvHD (graft versus host disease) prophylaxis including antithymocyte globulin, cyclosporine (CSA) and methotrexate. At day+ 19 (after engraftment of white cells) the patient developed severe haematic diarrhoea in absence of bacterial, fungal and viral positive blood and stool cultures. A rectal biopsy demonstrated the presence of red, basophilic microorganism identified as *Cryptosporidium parvum*. The pres-

ence of the protozoa was confirmed at day+ 24 by direct research of it in patient's stool. Despite the discontinuation of immunosuppresant therapies and the introduction of azithromycin alone or associated with paromomycin and metronidazole a colonscopic examination revealed the persistence of *Cryptosporidium* associated to acute bowel GvHD. Therefore methylprednisolone (MPD) was started, but severe diarrhoea persisted, and sclerosant cholangitis followed by acute pancreatitis developed. At day+ 48 *Cryptosporidium* was demonstrated also in gastric fluid. Nitazoxamide, a drug with action against *Cryptosporidium*, was administered (200 mg every 12 hours) with improvement of the pancreatic damage, but persistence of watery diarrhoea. This drug was administered alone or associated to paromomycin, metronidazole and azithromycin for 4 times. Until engraftment the patient presented a low number of T and B lymphocytes with an increase of natural killer. At day+ 94, CSA and steroids were discontinued and other immunosuppressant therapies were administered for treatment of intestinal acute GvHD. An improvement of intestinal disease was observed and 3 negative testing for *Cryptosporidium* were demonstrated from day+ 115. At present the patient is negative for *Cryptosporidium*, GvHD is controlled by specific therapy, his immunological reconstitution reveals an increased of T cells and morphological bone marrow feature confirmed the complete remission of acute leukaemia.
Comments: In our patient immunocompromission maintained the persistent infection by *Cryptosporidium*; in spite of an effective anti-protozoal treatment and of discontinuation of immunosuppressive drugs clearance of the infection was obtained only when immune function reached an ''acceptable'' level.

R2234

Study of bacterial colonisation in in-dwelling bladder catheters

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Objectives: The aim of this study was to identify the microorganisms isolated from the lumen of catheters and to evaluate the antimicrobial resistance of these strains according to the length of catheterization, the antimicrobial therapy and the underlying disease.
Methods: Three different pieces of 204 indwelling catheters were taken from the post surgical patients of urologic ward. The sections were rinsed once in 10 ml of buffer then placed in 10 ml of Tryptic Soy broth and after vortex mixing for 2 min, were plated out on blood agar, Mc Conkey agar No 2 and S.D.A. The resulting isolates were identified using Api system and the antimicrobical susceptibility test was determined with disk diffusion method. A urine specimen was obtained the first 24 h of hospitalization and after the removal of bladder catheter.
Results: 204 post surgical patients with various urologic malignancies were enrolled in the study. The mean duration of catheterization was 8 days (3–18 days). All the patients received postoperative antimicrobial prophylaxis for 5 days. Bacterial biofilm was recovered on 164 of the catheters. Gram-positive cocci were the common biofilm organism (*S. epidermides*: 58/191 and *Enterococus faecalis* 56/191) followed by *E. coli* (30/191) and *Candida* sp. (17/191). No one of the organisms present on the biofilm were recovered from the urine. In the 40 cases in which no biofilm was recovered, the patients were receiving antibiotics at the time of catheter removal. 84% of the catheters were colonized by a single species while 16% were colonized with mixed bacterial communities. 82% of isolated strains of *S. epidermides* were resistant to oxacillin while 50% of *E. coli* produced E.S.B.L.
Conclusion: Urinary bladder catheterization in our urologic patients was frequently followed by bacterial and/or fungal colonization without bacteriuria. The presence of Gram-positive cocci with high prevalence may inhibits the development of extensive biofilm by the Gram negative urinary tract pathogens.

R2235

Staphylococcus lugdunensis early prosthetic valve endocarditis in a HIV-positive patient

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Background: *S. lugdunensis* is considered a perineal skin commensal and it causes a wide range of infections from superficial skin to fulminant clinical disease. Infective Endocarditis (IE) caused by *S. lugdunensis* has been associated with left-sided native valve disease and life-threatening complications resembling disease caused by *S. aureus*. Prosthetic valve endocarditis (PVE) due to this pathogen is rare.
Case report: A 33-year-old HIV positive man was admitted to the CCU with acute chest pain and dyspnoea. He had undergone aortic valve (AoV) replacement because of congenital bicuspid AoV 40 days prior to his presentation. Upon the presentation he was febrile to 38°C and in heart failure. He was diagnosed with acute myocardial infarction on the basis of increased myocardial enzymes and a new left bundle branch block. Transthoracic echocardiography demonstrated dehiscence and rocking movement of the metallic AoV, a small vegetation of the ring, severe paravalvular leakage and regurgitation. 3 sets of blood cultures were obtained and treatment with vancomycin, gentamicin and rifampicin was started as treatment for PVE. Two sets of blood cultures yielded coagulase negative *Staphylococcus* (CNS) identified as *S. lugdunensis*. The pathogen was reported as sensitive to methicillin and vancomycin was changed to dicloxacillin. Three days later he developed acute tubular necrosis and he was transferred to the Intensive Care Unit where he exhaled.
Discussion: 40–50% of prosthetic valve endocarditis cases are due to CNS, particularly *S. epidermidis* and a subacute presentation is typical. *S. lugdunensis* was first described as a separate species in 1988. It is indigenous to human beings; it predominantly causes skin and soft tissue infections and is only occasionally responsible for IE. An association of IE with inguinal skin breaks has been reported. The majority of the patients are older than 50 years and they acquire the infection in the community. Native mitral and aortic valves are involved and the disease is associated with high mortality rates. PVE due to this pathogen mainly concerns bioprosthetic valves. IE is not considered a complication of AIDS. In the HIV positive population it is mainly associated with intravenous drug abuse. PVE in this population has been associated with the same risk factor. To our knowledge this is the first case of early PVE in an HIV positive individual caused by *S. lugdunensis*.

R2236

Aetiologic structure of catheter-associated bloodstream infections in a cancer hospital

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Objective: To analyse aetiologic structure of bloodstream pathogens associated with central venous catheter infections. In 116 pts with fever due to probable catheter-associated

bloodstream infections (CAI) were analysed. Blood from catheter, from peripheral vein and the tip of removed catheter were tested. ''BactAlert'' aerobic and anaerobic vials for blood culture were used. Catheter tip was cultured by standard procedure. Identifications and susceptibility testing was performed with ''ATB-Expression'' and ''Vitek 2''.
Results: CAI was diagnosed in 42/116 (36.2%) pts with suspected CAI. In 39/42 (92.9%) pts catheter blood was positive including: 9/42 (21.4%) pts with pathogens growth from all three specimens, 13/42 (30.9%) pts with positive culture of catheter blood and catheter tip, 6/42 (14.3%) pts with positive catheter blood and venous blood and 11/42 (26.2%) pts with growth only from catheter's blood. In 1 pts growth was revealed from catheter tip and venous blood. In 2/42 (4.8%) pts only catheter tip was colonized. All pts had fever >38ordm;C, 4/42 (9.5%) pts had local infections in catheter site. In 38/42 (90.5%) one microorganisms was isolated from each specimen, in 3/42 (7.1%) – two microorganisms and in 1/42 (2.4%) – three microorganisms. Thus 47 pathogens were isolated. Gram-positive microorganisms were isolated in 38/47 (80.9%). There were: *Staphylococcus* spp. – in 33/47 (70.2%) of cases. Coagulasonegative staphylococci prevailed. 26/47 (55.3%) of strains were MR-CNS and 3/47 (6.4%) – MS-CNS. 4/47 (8.5%) of strains were *Staphylococcus aureus* (2 – MRSA and 2 – MSSA). *Enterococcus* spp. were isolated in 5/47 (10.6%) of cases. All these pathogens were susceptible both to Vancomycin, Teicoplanin and Linezolid. Other pathogens were represented with gram-negative organisms: 5/47 (10.6%) – *Stenotrophomonas maltophilia*, 2/47 (4.3%) – *Acinetobacter baumanii*, 1/47 (2.1%) – *Alcaligenes xylosoxidans*, 1/47 (2.1%) – *Enterococcus aerogenes*. Yeasts were isolated in 2/47 (4.3%) of cases: *Candida albicans* – 1 and *Candida parapsilosis* – 1.
Conclusion: *Staphylococcus* spp., predominantly CNS were the most common cause of CAI. But in some pts non-fermenting gram-negative pathogens and yeasts can cause CAI as well.

Community-acquired infections including CAP, sepsis, STD, ….

R2237

Fatal *Aerococcus urinae* endocarditis: a case report

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Objective: *A. urinae* is a microaerophilic, Gram-positive coccus growing in pairs and clusters that is negative for catalase and PYR and produces a-haemolysis. As a fastidious organism it needs blood supplemented media and sometimes incubation in a CO_2 enriched atmosphere. It strongly resembles viridans streptococci or lactobacilli. Identification is hampered because *A. urinae* is not included in the code-books of commonly used identification systems. *A. urine* is a rare cause of UTI (0.3–0.8% of all cases), mainly in elderly men with underlying urinary tract pathologies, but underdiagnosis is probably common. Moreover, *A. urinae* has been described as a very rare cause of endocarditis. To the best of our knowledge, only 12 cases of *A. urinae* endocarditis, 9/12 in men, have been published so far with a striking mortality of 66.6%. We describe another case of fatal *A. urinae* endocarditis in a 62-year-old man with urinary tract pathology.
Case report: The patient was admitted with pulmonary oedema due to suspected aortic insufficiency. TEE revealed a destructing aortic valve endocarditis leading to aortic insufficiency grade IV and tricuspid valve involvement due to abscess formation. He was empirically treated with beta-lactams and gentamicin. Multiple blood cultures but not urine culture (UC) grew *A. urinae*. The strain was susceptible to penicillin and identified by 16S rDNA sequencing. The patient was considered inoperable and died 4 days after admission of cardiogenic shock and sepsis. 6 months ago the patient was evaluated because of lower abdominal and inguinal pain. An urine dipstick test showed leukocyturia and bacteriuria, a UC grew Gram-positive bacteria reported as lactobacilli. Urological examination showed a benign prostate hyperplasia. A second dipstick test still revealed leukocyturia/bacteriuria but UC was sterile. One might speculate that a) ''lactobacilli'' in the first UC were in fact *A. urinae*, b) isolation of the pathogen in subsequent UC was hampered by the culture conditions, i.e. a lacking CO_2 atmosphere and c) early antimicrobial therapy might have prevented the fatal course.
Conclusion: Isolation of a-haemolytic cocci from urine and blood cultures, especially in elderly male patients, that cannot be identified biochemically should make one think of *A. urinae*. 16S rDNA sequencing identifies the pathogen definitely. Clinicians should be informed about the impact of *A. urinae*, especially about the poor prognosis of endocarditis.

R2238

Aetiology and antimicrobial resistance of community-acquired urinary tract infections in childhood in Turkey

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Objectives: To determine the aetiology and antimicrobial resistance of community-acquired urinary tract infections (UTIs) in childhood in Turkey.
Methods: This study was performed at the Baskent University Alanya Hospital, which has 100 beds capacity. Patients between 3 month and 15 years of age with community-acquired UTI were included in the study from October 2003 to December 2004. We interviewed face to face the patients and filled a form. Midstream urine samples obtained from patients were inoculated onto 5% blood agar and Eosin-Methylene Blue agar with 0.01 mL calibrated loops by semi-quantitative technique. Patients who had dysuria or pollakuria or fever and both ≥105 bacteria and pyuria were included in the study. Recurrence UTI was the exclusion criterion. The isolated bacteria were identified by conventional methods. Antimicrobial susceptibility was performed by a disc diffusion method, according to the NCCLS criteria. Quality was assured by testing *Escherichia coli* ATCC 25922.
Results: A total of 51 patients were admitted in our study. Forty-nine percent of patients were 6 year old or younger and 96% were female. The most common pathogen was *E. coli* (90%). Antibiotic resistance rates for *E. coli* were as follows; Amoxicillin 71.7%, trimethoprim/sulfamethoxazole 54.3%, amoxicilline/clavulanate 43.5%, cefazolin 21.7%, nitrofurontoin 6.5%, ceftriaxone 4.3%, gentamicin %4.3, amikacin 2.2%, ciprofloxacin 2.2%.
Conclusion: *E. coli*, the most common pathogen, had a high rate of resistance to most of the drugs commonly recommended for UTI treatment. We recommend gentamicin and amikacin as first line drugs while waiting results of sensitivity testing.

R2239

Isolation of vaginal pathogens in different groups of women

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Objectives: Vulvovaginitis is probably the most common infection treated by gynecologists all over the world. The environment of the vagina is dynamic since constant changes on the microbial flora are determined by the metabolic activity of the host, the pre-existent bacteria and exogenous factors such as hygiene habits, age or sexual activity. The aim of the study was to assess the prevalence of pathogens in the vaginal secretions of women attending our hospital.
Methods: We studied 1233 symptomatic women, which were divided into 3 groups. Group A comprised 813 non-pregnant women of reproductive age, group B 177 pregnant women and group C 243 postmenopausal women. In order to identify aerobic microorganisms we inoculated vaginal secretions on blood agar, MacConkey agar, Chapmann and Sabouraud, and then incubated the plates at 37°C for 24 hours, whereas anaerobic cultures were carried out on Wilkins-Chalgren agar at 37°C for 48 hours. The isolated strains were identified using the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, USA). *Ureaplasma urealyticum* (Uu) was identified by urease production, whereas *Mycoplasma hominis* (Mh) was detected using DNA agar and incubation for 48 hours in anaerobic conditions.
Results: The most commonly isolated microorganisms were: a) in group A, *Gardnerella vaginalis* (Gv) (30.1%), anaerobic bacteria (24.4%), *Candida* spp. (20.8%) and Gram-negative rods (14.3%) b) in group B, *Candida* spp. (30.1%), Gv (28.2%), anaerobic bacteria (21.1%) and Gram-negative rods (10.5%) c) in group C, Gv (31.4%), anaerobic bacteria (26.9%), Gram-negative rods (17.0%) and *Candida* spp. (12.7%). Moreover, Uu was isolated in 41.0%, 33.3% and 23.1%, whereas Mh was detected in 3.4%, 2.6% and 1.9%, respectively, for groups A, B and C.
Conclusion: The most commonly isolated pathogens were similar between our study groups with small differences in the isolation rates. As expected, candidiasis was increased in pregnancy. In the non-pregnant group as well as in the postmenopausal group more than half of the microorganisms isolated were those responsible for the specific clinical entity of bacterial vaginosis (Gv and anaerobes). High isolation rates were observed for *Ureaplasma urealyticum* in all groups.

R2240

Micro-organisms isolated from leucorrhoea in a military hospital, Algiers

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Objectives: The purpose of this study is to determine the most commonly isolated micro-organisms from leucorrhoea observed in Sexually transmitted infection (STI) clinic in central military hospital of Algiers.
Methods: We performed a retrospective study from January 1998 to December 2004 of 6490 vaginals discharges in microbiology laboratory of central military hospital. This study concern the women between 20 and 60 years old with genital infection symptoms. Every woman was tested for the presence of *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Candida albicans, Gardnerella vaginalis* and other no specific bacteria. Vaginal and cervical samples processed by conventional methods: -microscopic exams, culture in appropriate medium and identification by conventional test or manual biochemical systems(API20 NE,API20 E,API NH). We also used commercials kit for detection of *Mycoplasma* (Mycoplasma Duo) and Direct Fluorescent Assay for research *Chlamydia trachomatis*.
Results: -Global results:from 6490 samples 3166 (48%)were positive.-the frequent micro-organisms isolate were: *Candida albicans* 1568(49%), *Mycoplasma* 371(11%), *Enterobacteria* 313 (10%), *Streptococcus agalatiae* 297 (9%), *Chlamydia trachomatis* 200 (6%), *Trichomonas vaginalis* 143 (4.5%), *Gardnerella vaginalis* 19 (0.6%) and *Neisseria gonorrhoeae* 3 (0.09%).
Conclusion: This study show a low frequency of specific agent of the STI but a high prevalence of endogenic infection as the vaginal candidiasis.

R2241

Extemporaneous biopsy in invasive streptococcal infection: a guide for surgeons?

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Group A streptococci (GAS) affect either the skin (cellulitis) or the deeper soft tissue (necrotizing fasciitis or myonecrosis). In these last two cases, the infection is extremely rapidly progressing infection with systemic choc. Because infection initially spreads along fascia or through muscle with no or minimal skin involvement, early in its course the extent of disease may be not recognized. Early aggressive excision of infected, necrotic tissue is essential for survival, in association with adequate treatment.
Patients and methods: We evaluate the role of extemporaneous biopsy performed in cases of invasive severe GAS infection suspected.
Results: 5 patients (4 men, 1 woman) were admitted in operating room with the suspicion of necrotizing fasciitis because of hypotension and multi-organ dysfunction without cause. The underlying conditions were diabetes mellitus (n = 2). The local signs were erythema (n = 4), oedema (5), bullae (n = 3), anaesthesia (n = 2) of the thigh (n = 4) or of the chest (n = 1), diffuse rash on the trunk and thighs (n = 1). Anaesthesia followed a period of exquise pain treated with non-steroidal anti-inflammatory drugs. The C-reactive protein, leukocyte count were increased (n = 5). In operating room, at the incision into the infected area, the surgeon found small resistance in the fascia plane (n = 2), brownish fluid (n = 3) but no pus. The extemporaneous biopsy performed in operating room, only 5–12 hours after the admission of the patients in hospital showed polynuclear infiltration of the deep dermis (n = 5), the fascia (n = 3), liponecrosis (n = 3), multiple thrombi (n = 3) and myonecrosis (n = 1). The diagnosis of necrotizing fasciitis (n = 2) and myonecrosis (n = 1) was etablished on clinical suspicion and histological basis and was retrospectively confirmed. Immediately, aggressive debridment (n = 2) and amputation (n = 1) of all the entire thigh were decided while they received intravenous immunoglobulins, clindamycin and penicillins. During the first week, wound revision was performed every day, in operating room.The necrotizing cellulitis (n = 1), toxic shock syndrome with rash (n = 1) was diagnosed for the others. They received penicillins-clindamycin (n = 2) and immunoglobulins (n = 1). GAS grew on tissue culture (n = 5), on blood culture (n = 4). The rate of survival was 100%.
Conclusion: We believe that an extemporaneous biopsy can guide the surgeon in the decision of aggressive debridment or amputation, when severe invasive GAS soft tissue infections is suspected.

R2242

Bacteraemia in a general hospital, Bilbao, Spain 1994–2005

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Objective: The aim of this study is to assess the features of bacteraemia in the hospital of Basurto in the last 11 years (Jan 1994–Sept 2005).
Methods: Blood cultures are performed by means of BACTEC 9240. The Infection Control team studies every patient with positive blood cultures. Variables under surveillance are patient's age and sex, underlying illness, predisposing conditions, source of bacteraemia, nosocomial/community acquired, microorganism and antibiotic susceptibility, antimicrobial treatment, complications and outcome. A computer based surveillance system is used.
Results: 7051 episodes of bacteraemia were studied during this period. Incidence from 1994 to 2005 between 17.6 and 22.05 cases/1000 discharges. The incidence remained stable at among 19 cases/1000 discharges with two peaks of incidence in 1995 and 2004. Men (57.51%) aged over 70 y (48.14%) accounted the majority of cases. The most frequent underlying illness was neoplasia present in 1644 patients (23.32%). The urinary (26.86%), gastrointestinal (17.23%) and respiratory tract (14.55%) were the most common sources of bacteraemia. Aetiology: Gram– 54.73% (range: 49.7–58.72), Gram+ 42.61% (range: 39.18–42.98%) and yeast 2.58% (range: 1.39–3.93%). *E. coli* (30.54%), *S. aureus* 10.71%, *S. pneumoniae* 9.56%, *S. epidermidis* 5.95%, *P. aeruginosa* 3.46% and *Salmonella* spp. (3.31%) are the most frequent agents of bacteraemia. Susceptibility to antibiotics: Methicillin resistant *S. aureus* 12.48% (Range 0% 1994 to 21.33% 2004); *E. coli* resistant to Ciprofloxacin 12.82% (Range 9.7% 1994 to 15.20% 2004), and to Cefotaxime 0.86% (Range 0% 1994 to 3.8% 2004). In 81.4% episodes appropriated antibiotic treatment was started the same day that blood cultures were obtained. Crude mortality until the end of the episode 16.89% (range: 13.8–21.36%). Nosocomial bacteraemia accounted 29.88% (range: 26–38.7%) of cases. Incidence of nosocomial bacteremia ranged between 3.82-7.41/1000 discharges. Nosocomial acquired bacteraemia aetiology: Gram+ 53.22%, Gram- 40.51% and Yeast 6.14%. Coagulase Negative Staphylococci 23.09%, *E. coli* 17.73%, *S. aureus* 16.56%, *P. aeruginosa* 5.66%. Crude mortality until the end of the episode 25.11% (range: 17.0–30.34%).
Conclusions: Incidence of bacteraemia remained stable in last 11 years with two peaks of incidence in 1995 and 2004. A trend toward a decrease in fungi was observed in community and nosocomial acquired cases. 51.5% of cases of Methicillin Resistant *S. aureus* bacteraemia were community acquired.

R2243

CAP in patients with diabetes

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Objectives: Infections cause considerable morbidity and mortality in patients with diabetes. Due to impaired immunity infections have more severe and prolonged course. In patients with diabetes many aspects of humoural and cellular immunity is impaired. In our study we wanted to analyse the impact of impaired immunity to clinical course, offending microorganisms, treatment and outcome of CAP in patients with diabetes.
Methods: Study is retrospective. It includes all patients with diabetes hospitalized due to CAP at our hospital in 2004. All data are from patients files.
Results: 94 episodes of CAP in 90 patients with diabetes were treated, 56% were men, aged 39 to 96 years, average age was 74.8 years. 30% of patients had insulin dependent diabetes, in 60% late complications of diabetes were present. 85% of patients had also other chronic diseases. Multilobar infiltrations on chest x-ray were present in 32% of patients, 12% had parapneumonic effusion. Haemoculture was performed in 54% of patients, positive in 18%, *S. pneumoniae* being found in 5 patients, and other pathogens rarely (*Bacteroides* sp., *S. aureus*, *P. mirabilis*). Examination of sputum was done in 61%, was positive in 23%, *S. pneumoniae* was most frequent isolate (7 patients), followed by *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, MRSA and *C. albicans* (1 patient). Microbiologic examination of tracheal aspirate was performed in 20% of patients with positive isolation in 74%. Here *S. aureus* was most frequently isolated (5 patients), followed by *Enterobacteria* (4 patients), *P. aeruginosa* (2 patients) and *S. pneumoniae* (1 patient). 14% of patients had infection of urinary tract simultaneously, caused most frequently by *E. coli*. 2% of patients developed empyema. 60% of patients were treated with amoxicillin clavulanate, 21% with respiratory quinolones. Average length of hospital stay was 13.8 days. Mortality rate was 17%.
Conclusion: CAP in patients with diabetes tends to have a severe and prolonged course. Multilobar involvement on chest X-ray is frequent. Hospital stay is longer and mortality rate higher than in whole population. *S. pneumoniae* and *S. aureus* are most frequent offending microorganisms. Amoxicillin, clavulanate and respiratory quinolones seem to be best therapeutic options.

R2244

FASTCAP Study (FAdoi Study on CAP). Retrospective and perspective study on community-acquired pneumonia in internal medicine

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Objectives and methods: A one-year (Jan–Dec 2002) retrospective study was performed on the management of patients with CAP hospitalized in Internal Medicine, and compared with a one year perspective phase (June 2003–May 2004) on the same kind of patients, following the implementation of FADOI (Internal Medicine Association) Italian guidelines on RTIs.
Results: We report the retrospective results and a trend on perspective. 31 Internal Medicine Departments were involved. 1443 cases, (613 F, mean age 79.3 years, 961/1443 PSI score Class IV and 482/1443 Class V, pathogen isolation: 11.4%) were included in retrospective analysis. Prior antibiotic treatment was administered to 18.4% of the patients. First line antibiotic treatment (50.7% monotherapy, 49.3% combination, mean duration = 9.2 days) obtained a clinical success in 1029 patients (71.3%). Second line treatment was administered to 222 patients, treated more frequently with combination therapy (57.7%). The mean duration of second line therapy was 8.8 days, and a clinical success was obtained in 70.7% of cases. The mean duration of hospitalization was 12.2 days, and the overall mortality was 231/1443 patients (16%). The analysis of perspective phase (about 1400 patients) is ongoing and the results will be available on early 2006. However, some positive trends can be noted: preliminary data showed a slight improvement in aetiological diagnosis (14.7% vs 11.4%), in clinical success of first line therapy (72.8% vs 71.3%) and in overall mortality (14.9% vs 16%).
Conclusion: The data of this study reflect the real management of CAP in Internal Medicine Wards in Italy.

R2245

Fulminant septic shock in a splenectomised patient caused by an *Haemophilus influenzae* type b strain harbouring six copies of the capsulation b locus

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Objectives: *Haemophilus influenzae* type b (Hib) strains isolated from invasive disease generally possess a duplication of the capsulation (cap) b locus. Amplification up to five copies has been reported and has been proposed to be a mechanism to evade host defence. Splenectomized individuals are known to be particularly prone to infections caused by encapsulated bacteria. We report a case of Hib septicaemia, associated with disseminated intravascular coagulation and sudden death in a previously splenectomized adult patient. To investigate whether unusual virulent traits of the microorganism might have contributed to the severe clinical presentation observed, genotypic characterisation of the Hib isolate was performed. In particular, the number of copies of the capb locus was determined.
Methods: Pre-mortem blood culture was positive for Hib. The capsular type b of the case isolate was confirmed by PCR capsular genotyping. Genetic relatedness between the case isolate and the prototype Hib strain 40F, belonging to the major Hib clone endemically present in Italy, was investigated by pulsed-field gel electrophoresis (PFGE). The number of copies of the cap b locus was determined by Southern blot analysis, based on the size of the restriction fragments after digestion of the chromosome with KpnI and SmaI restriction enzymes.
Results: By PFGE, the case isolate showed a restriction pattern identical to strain 40F, indicating that it belonged to the major invasive Hib clone endemically present in Italy. When the number of copies of capb locus was investigated, the case isolate appeared to contain a mixed population of 6, 5, 4, 3 and 2–copy arrangements of the locus. The result was confirmed by analysis of individual colonies showing distinct subpopulations containing exclusively a 6, 5, 4, 3, or 2-copy arrangement.
Conclusion: The detection of six copies of the capb locus in a Hib isolate is noteworthy since it is the highest number of copies yet reported. Although splenectomized patients are at risk for serious systemic infections caused by encapsulated bacteria, severe Hib cases are more common in asplenic or splenectomized children than in adults. Our results suggest that the unusual amplification of the capb locus might have contributed to the particularly severe clinical presentation.

R2246

Prevalence of *Chlamydia trachomatis* infection in Iranian women suffering from cervicitis: a cross-sectional study

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Objectives: The aim of this study was to determine the prevalence of *Chlamydia trachomatis* among a randomized statistical group of women suffering from cervicitis in Iran.
Methods: During a 12-month-period, Jan 2003 to Jan 2004, 142 endocervical samples were taken from women suffering from cervicitis attending to Mirzakoochakkhan Women Hospital in Tehran, Iran. Direct fluorescent antibody (DFA) and polymerase chain reaction (PCR) techniques were used to detect *C. trachomatis* in endocervical samples.
Results: Twenty-two (15.5%) [95%CI, 9.54–21.4] out of 142 samples were diagnosed as *Chlamydia* positive according to PCR results, while DFA diagnosed 20 (14.1%) [95%CI, 8.37–19.8] of cases as *Chlamydia* positive. No statistically significant difference was found among two diagnosis methods applied in this study, (i.e. PCR and DFA). Prevalence was the highest (25%) among women aged 25 to 29 years and 35 to 39 years. The χ^2 test showed there was a significant relationship between positive test result and bearing a history of STI (P = 0)
Conclusion: The results of this study showed high prevalence of *C. trachomatis* infection among women suffering from cervicitis and suggest that patients diagnosed with genital *Chlamydia* infection should be referred to the genitourinary medicine clinic for further STI screening and partner notification.

R2247

Acanthamoeba cornal ulcer as a complication of orthokeratology

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Objectives: Orthokeratology is a recently favoured non-surgical procedure to reduce myopia, which involves nightly use of rigid gas permeable lenses to flatten the cornea to reduce refractile errors. Despite the recent surge in popularity of this procedure, there are serious risks associated with it that may be overlooked. We describe a case of Acanthamoeba keratitis in an adult as a complication of orthokeratology.
Methods: Contact lenses were cultured on non-nutrient 1.5% agar plates with a lawn of non-mucoid *E. coli*. After incubation at 37 °C scrapings from surface of the agar were observed for presence of characteristic trophozoites and cysts. Clinical details were obtained by review of the case with the patient's attending physician.
Results: The patient was a 29 years old otherwise healthy female who presented to her physician with severe eye pain, conjunctival suffusion, photophobia and decreased visual acuity over the past 12 days. On examination she showed unilateral corneal ulcer. The patient was a rigid contact lens wearer for a year prior to this event. She wore contact lenses overnight only, for correction of vision, and wore no lenses during the day. There was no history of eye trauma. No corneal scrapings were available for culture but the hard contact lens grew *Acanthamoeba* species after 24 hours of incubation on the plates as well as in the original container.
Conclusion: Orthokeratology is a recent, increasing trend among the optometrists, encouraged for use particularly in children, who may have problems with handling contact lenses during daily activities. Nevertheless, Acanthamoeba keratitis is an alarming emerging complication of this procedure, particularly because of lack of effective treatment. Ulcerative keratitis occurs at 20.9/10 000 extended wear lenses per year. However, the risk of developing the disease increases 10–15 times in extended daily wear with overnight use. This is a serious potential problem in all orthokeratology recipients. In view of increasing popularity of this procedure questions should be raised about how these lenses and methods are being regulated, marketed and monitored.

Lyme, borelliosis, toxoplasmosis

R2248

Borrelia burgdorferi genospecies in the environment of forestry

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Objective: The objective of the study was detection of three pathogenic *Borrelia burgdorferi* sensu lato (s.l.) genospecies (*Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia afzelii* and *Borrelia garinii*) in Ixodes ricinus ticks and forestry workers from three provinces of the Lublin region (eastern Poland).
Methods: Eighty-seven sera of forestry workers from the Lublin region, seropositive with *B. burgdorferi* s. l. antigen in ELISA test (Bellco Biomedica®, Austria) were examined for the presence of species-specific antibodies against *B. burgdorferi* s. s., *B. afzelii* and *B. garinii* by the use of ELISA test (Test-Line®, Czech Republic). All the seropositives forestry workers were subjected to clinical examinations. Species-specific primers designed for differentiation of *B. burgdorferi* into three above mentioned genospecies were used in nested-PCR reaction in 54 out of 664 examined *I. ricinus* ticks in which *B. burgdorferi* s.l. DNA was found. Ticks were collected from the same three provinces from which forestry workers were examined.
Results: The study showed high percentage of infections with an unidentified genotype *B. burgdorferi* (55.2%). It was observed that among identified genospecies of *B. burgdorferi* s.l. in forestry workers, mixed infections with three genotypes are dominant, i.e. *B. burgdorferi* s.s., *B. afzelii* and *B. garinii*. Considering infections, the percentage of individual or mixed infections was as follows: *B. burgdorferi* s.s. (35.6%), *B. afzelii* (31.0%) and *B. garinii* (33.4%). Out of 46 seropositive workers in 3 clinical borrelioses were diagnosed. The main clinical manifestations included: borreliosis erythema, periodical strong arthralgic pain, dysfunction of extremities, headaches and dizziness. A correlation was observed between clinical symptoms and genotype infection with *B. burgdorferi*. In 54 out of 664 *I. ricinus* ticks in which *B. burgdorferi* s.l. DNA was detected, *B. burgdorferi* genospecies were identified in 51 individuals (94.5%). The majority of examined ticks (96.1%) showed infections with one genotype. Considering identified infections (single and coinfections) the percentage was as follows: *B. burgdorferi* s.s. (51.8%), *B. afzelii* (33.3%) and *B. garinii* (5.5%).
Conclusion: Among identified infections, *B. burgdorferi* s.s. seems to be dominant in forestry workers as well as in *I. ricinus* ticks in three provinces of the Lublin region.

R2249

Socio-economic conditions and other factors influencing *Lyme borreliosis* incidence in the Czech Republic

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Objectives: Analysis of factors influencing the *Lyme borreliosis* (LB) occurrence.
Methods: Analysis of surveillance data collected by field epidemiologists, data from the meteorological service and from the demographic and economic institutions.
Results: Reliable data of LB infections in the Czech Republic are available since year 1988. The hypothesis is validated by comparison of the trend of Tick borne encephalitis (TBE) incidence and LB incidence in years 1988–2004 (correlation r = 0.87). Incidence of both infections reached the peak in 1995. The seasonal distribution of TBE and LB is almost similar (1993–2004, r = 0.9) with the maximum cases occurring in July. During years 1993–2004 sex specific incidence was higher in females than in males(ratio 1.5:1). Age specific incidence of LB has two peaks, in the age group of 5–9-year-old children (30.9/100 000 per year) and in the age group of 55–64 years olds (48.2/100 000). The percentage of forest men and other persons working in the forests or fields among the LB cases in years 1997–2004 was 1.8%. Ticks bite infects the patients during their recreational activities mainly. Unemployment remained in the years 1991–1995 approx. at the same level (between 2 and 3%). During the next years the percent of unemployed persons increased quickly up to 9.3% in the year 1999 (7.8% in year 2001, 8.3% in year 2004). This trend differs significantly from the trend of LB incidence that peaked in year 1995 (r = 0.39). The percentage of unemployed persons among the LB cases was 3.8% in contrary to the Czech Republic figures which were in the same period 5–9%. Gross domestic product in US $ per capita increased from 2600$ in year 1991 to 6700$ in year 2003. Since that year varies between 4800$–5600$. This trend therefore differs from the trend of TBE incidence as well (r = 0.12). Retired persons comprised 25% of LB cases in years 1997–2004.
Conclusions: The behavioural and socioeconomic aspects of TBE cases remained stable despite the political changes, which occurred in Czech Republic since the beginning of nineties and therefore are not responsible for the increase of LB incidence in recent years. The increase of LB incidence was influenced by natural, ecological and climate conditions.
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Antimicrobial clinical trials

R2250

Efficacy of cefepime plus clarithromycin versus cefepime alone in patients with ventilator-associated pneumonia after cardiac surgery: prospective clinical trial

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Ventilator-associated pneumonia (VAP) is the most frequently occurring infectious complication after major cardiac surgical operation. The VAP rate in cardiovascular surgery averages 1–2%. Usually there is early-onset VAP, which is caused by community-acquired pathogens including atypical microorganisms.
Objectives: To estimate potential role of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in early-onset VAP development. To compare efficacy of cephalosporin and macrolide combination versus cephalosporin alone for early VAP treatment in patients undergone cardiosurgical operation.
Methods: A total 50 patients undergone major cardiovascular surgery were administrated cefuroxime or ceftriaxone for antimicrobial prophylaxis. Inclusion criteria – new and persistent infiltrate on chest radiography occurred during first 7 days

of mechanical ventilation (at least 48 hours after mechanical ventilation start) associated with two of the following: body temperature >38°C or <36°C, leukocytosis (>10 × 10^9/L) and/or > 10% of immature forms of leucocytes or leucopenia (<5 × 10^9/L), purulent tracheal secretions. Early VAP patients were divided into 2 groups: 1 group (n = 30) received cefepime plus clarithromycin (IV), 2 group received cefepime alone. We investigated a procalcitonin (PCT) level a 72 hours before antimicrobial therapy beginning. Serological ELISA tests were conducted in 22 VAP patients for finding of antibodies to *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.
Results: We observed diagnostic level of IgM to *Chlamydia pneumoniae* (9%) and to *Mycoplasma pneumoniae* (9%). Clinical efficacy (cure and improve rates) of cefepime/clarithromycin combination was 53%, compare for cefepime alone – 30% (NS). Also we observed reduction of procalcitonin level from 1.65 (25% – 0.75, 75% – 3.32) ng/ml to 0.7 ng/ml (25% – 0.53, 75% – 2.74) with cefepime/clarithromycin therapy that confirmed the evidence of antimicrobial therapy adequacy. Patients who received only cefepime had increase of procalcitonin level from 0.61 (25% – 0.41, 75% – 2.52) ng/ml to 1.31 (25% – 0.38, 75% – 3.41) ng/ml. Total postoperative mortality rate were similar in both groups (30%). Major cause of death was not infection but thromboembolic and other complications.
Conclusion: Combination of cefepime with clarithromycin is more preferable for treatment of early VAP in cardiac patients compare with cefepime alone.

Paediatric infections

R2251

An urban case of lymphocytic choriomeningitis virus encephalitis and hydrocephaly due to a new variant strain traced back to infected wild *Mus musculus* colony

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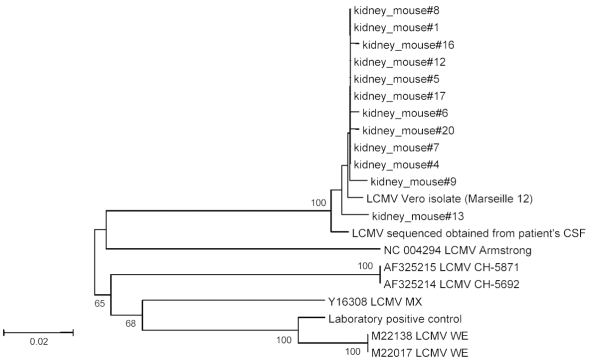
A five-year-old boy was admitted at the emergency ward in August 2004 for a typical summer meningitis with fever, neck stiffness, vomiting and prostration. CSF showed elevated protein level (1.3 g/L) and discrete hypoglycorachia (2.3 mmol/L). PCR and RT-PCR tests searching Herpesviridae and enteroviruses, respectively were negative. Treatment consisted of acyclovir and cefotaxime for 8 days. The outcome was favourable. Forty-five days later, the boy was admitted again with clinical manifestations compatible with encephalitis. Cerebral tomography and MRI showed a major hydrocephaly associated with pachymeningitis which revealed to be non spontaneously resolutive and necessitated to install a permanent ventriculoperitoneal derivation. This convalescent-phase serum contained IgG (1/512) but no IgM directed against LCM virus; the acute-phase serum tested retrospectively showed IgG (1/128) and IgM (1/64). RT-PCR with primers specific for LCM virus was found positive; the PCR product was sequenced and found to be >13% divergent from all LCM virus sequences available in the GenBank database. Interview of the mother revealed that their home was infested with "mice". Attempts to trace this case back to rodents were achieved by trapping rodents outside and inside the apartment lot. A total of 20 mice (presumably *Mus musculus*, definitive identification is pending) were captured inside a apartment lot in the vicinity of the boy's flat. Capture was performed with glue-traps, and mice were still alive when brought to the laboratory. They were euthanized and organs were collected. One kidney of each rodent was used for nucleic acid purification and was tested with the same primers. A total of 13/20 kidneys were positive, and crude extract were used for Vero cells inoculation. Full genome sequencing has been performed and comparative analysis with other LCM virus strains reported in the literature indicated that this strain is a distant variant of LCMV; a detailed phylogenetic and comparative sequence analysis will be presented. Additionally, evaluation of new classic and/or real-time RT PCR diagnostic system will be presented. This case underlines the importance to develop sensitive and specific diagnostic tools for LCM virus, which remains at least in France but probably worldwide a neglected human pathogen.



R2252

Viral, mycoplasmal and chlamydial upper respiratory tract infections among paediatric outpatients

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Objectives: The aim of this study was to investigate the viruses and atypical bacteria in upper respiratory tract infections of children.
Methods: Two throat swab specimens were collected from each children with acute respiratory infections visiting public hospital outpatient department. One of the swabs was used for bacteriological cultures and one of them for PCR analysis. In this study real-time PCR method was used to investigate the viruses and atypical bacteria in respiratory tract infections.
Results: Bacteriological culture negative specimens were chosen for this study. A total number of 100 specimens were evaluated for the detecion of RSV A, RSV B, CMV, Adenovirus, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* and 31% were positive at least 1 virus or atypic bacteria. Among them respiratory synsitial virus A were detected from 8 specimens, while RSV B were detected from 3 specimens. CMV were detected from 17 specimens, and Adenovirus from 1 specimen. *Chlamydia pneumoniae* were detected from 2 specimens and *Mycoplasma pneumoniae* was not found from these specimens. From five specimens RSV A and CMV were detected as mixed viruses. From one specimen RSV A, RSV B, CMV *Chylamidia pneumoniae* were detected as mixed infection.
Conclusion: The results of this study demonstrate that CMV and RSV A are the two most common viruses isolated from paediatric outpatients with upper respiratory tract infection.

Real-time PCR method can be applied in clinical practice for arriving at a correct diagnosis and administration of effective treatment.

R2253

Rotavirus: a new future for an old known virus

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Objective: To show that the infection caused by rotavirus is one of the main aetiologies causing mild to severe diarrhoea in the total amount of children hospitalized due to gastrointestinal symptoms.
Material and methods: This study is an observational and retrospective study performed at the Hospital Interzonal General de Agudos, Eva Perón de San Martín, Buenos Aires, Argentina, between January and September 2005. 173 patients were assessed due to Gastrointestinal symptoms with an age range from 1 months old to 4 years old.
Results: Out of 173 patients hospitalized due to gastrointestinal symptoms, 49 patients (28.32%) were identified with Rotavirus in the stool. The average age of the 49 patients was 9 months. The average days of hospitalization was 6.54 days, being April the months with the highest incidence. 31.07% of the 49 patients showed metabolic acidosis and more than one-third had mild to severe dehydration. 5.88% were admitted to Paediatric Intensive Care Unit with the diagnosis of hypovolemic shock.
Conclusions: Rotavirus is the most common cause in the world of vomits and diarrhea with great morbimortality especially in less developed countries. This disease produces a high rate of admittance as well as a high cost of hospitalization, with loss of working days for the children's parents, being the appearance of the vaccine a major finding to solve this issue of Public Health. We believe that the use of this vaccine is the chosen method to significantly diminish the global incidence of this disease.

R2254

Intestinal *S. aureus* colonisation in Italian and Swedish infants is effected differently by antibiotic treatment, siblings and pets

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Objectives: The skin bacterium *Staphylococcus aureus* has become a frequent colonizer of the intestinal tract of Swedish infants. Whether such colonization is common in other countries as well is not known. Here we compared intestinal colonization by *S. aureus* in Swedish and Italian infants and studied the effect of some life-style factors on such colonization.
Methods: One hundred infants each from Sweden and Italy were followed with rectal swabs at 3 days of age and quantitative stool cultures at 1, 2, 4, and 8 weeks and 6 and 12 months of age. Individual *S. aureus* strains colonizing an infant were identified by RAPD and their toxin production, faecal population counts and time of persistence in the microflora was determined and related to different life-style factors.
Results: Sixty-six percent of the Italian infants had *S. aureus* in their stools at some time-point during the first year of life compared with 83% in Sweden ($p = 0.0003$). Most colonized infants in both cohorts had a strain that persisted in the microflora for more than a month and half of the strains produced one or more superantigenic toxins (SEA-D or TSST-1). Having elder siblings was positively associated with *S. aureus* colonization in the Italian cohort ($p = 0.02$), while the presence of pets in the family correlated negatively with *S. aureus* colonization in the Swedish cohort ($p = 0.03$). Antibiotic treatment was significantly more common in the Italian cohort ($p = 0.0001$) and was negatively associated with *S. aureus* colonization while the vice versa were seen in the Swedish cohort. Sectio-delivery and exclusive breastfeeding showed a marginal and non-significant positive association with *S. aureus* colonization. When examining only vaginally delivered infants breast-fed up to four months of age and not treated with antibiotics, Italian infants were still less likely to be colonized by *S. aureus* than Swedish infants, but the significance was gone.
Conclusion: Variations in antibiotic treatment affected the *S. aureus* colonization, but could not explain the whole difference. The underlying causes of this country-specific colonization pattern remain to be determined. Possibly, Italian infants harbour a more complex microflora, conferring greater resistance to implantation of *S. aureus*.

R2255

Wound infection caused by *V. parahaemolyticus* and *V. alginolyticus*

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The genus Vibrio includes more than 35 species, mostly marine in origin, many of which may cause disease in human. The best known halophilic species belonging to this genus are *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The former is a common human pathogen causing gastroenteritis and, less often, tissue infections. The latter has been associated with otitis and also with wound infection after exposure to sea water. We present a case of a mixed wound infection in a child, from which both *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were recovered.
Case report: A 10-year-old boy was admitted to our hospital on 5th August 2005 because of a wound of the external malleolus of the right foot, lameness, pain and fever. The patient reported that he was injured when he hit his foot against a sharp stone at the seashore the previous afternoon. The wound was treated on cite with povidone iodine, but it kept bleeding and started producing pus. On admission to the hospital, his temperature was 38.5°C. The wound was remarkably deep and wide and covered with pus. Sample was taken for full microbiological investigation, surgical debridement subsequently followed and antibiotic therapy with cefuroxime per os was started. From the specimen's culture, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Clostridium perfrigens* and *Bacillus brevis* were recovered. All *Vibrio's* strains' antibiotic susceptibilities were tested using the automatic VITEK 2 system (Biomérieux, Marcy L'Étoile, and France). *V. parahaemolyticus* showed intermediate susceptibility to ticarcillin and *V. alginolyticus* was resistant to it. They were both susceptible to ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, imipenem, meropenem, gentamicin, amikacin, and ciprofloxacin.The antibiotic regimen failed to reduce the fever and stop the pus production after 8 days of administration, so cefuroxime was replaced with amoxicillin/ clavulanic acid per os for 12 days, which finally succeeded to treat the infection. Nevertheless, the patient's foot recovered full function only after three months.
Conclusions: Our patient suffered from a mixed infection caused by four different microorganisms, two of which were *Vibrio* species. Although appropriate management resulted to the patient's clinical improvement and prevented possible complications, such as necrotizing fasciitis, full recovery was

slow, as *Vibrio* infections are severe and often followed by prolonged morbidity.

R2256

Bacteraemia caused by *Clostridium baratii* and Kawasaki syndrome. First case report

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Background: The clinical spectrum of clostridial bacteraemia ranges from an asymptomatic patient having an incidental positive blood culture to a full-blown, life-threatening infection. Kawasaki syndrome is an acute, self-limited systemic vasculitis of unknown aetiology that occurs in children. Its importance is due to the coronary artery aneurysms that develop in 20–25% of cases if the treatment is not given early in the course of the disease.
Case presentation: A 3-year-old male presented for evaluation of fever, abdominal pain, vomiting and polymorphous erythematosus rash on the extremities of 3 days duration. Three weeks before he presented with irregular fever and micropapulous exanthema over the truncus for 3 days followed after two days by perianal hyperaemia, scrotal oedema and balanopreputial hyperaemia and swelling. On admission, he was febrile (38.5°C). Physical examination showed: periungual peeling on the palms and fingers; severe hyperaemia of perianal region with peeling areas; erythematosus pharynx, strawberry tongue, dry and fissured lips, angular cheilitis; unilateral enlarged jugulodigastric nodes. Laboratory examinations revealed a white blood cell count of 5000/μL with 54% neutrophils and 38% lymphocytes. Platelet count 222.000/μL, C-reactive protein concentration of 50 mg/l and an erythrocyte sedimentation rate of 27 mm/h, serum IgA 297 mg/dl. Within the normal range anti-streptolysin O, IgM, IgG, blood urea, glycaemia, creatinine, plasma bilirubin, transaminases, gamma-glutamyltransferase, CD4+T-cells, CD8+ T-cells, CD19, NK cells, CD4/CD8 rate. No reactive for EBV, CMV, HSV-1, HSV-2, VZV, Adenovirus, Parvovirus B19, Coxsackie, Echovirus and *Chlamydia pneumoniae*. Results of urine analysis showed sterile pyuria. Two blood cultures were processed by the hospital microbiology laboratory using a standard blood culturing system (BACTEC 9120; Becton Dickinson). The anaerobic bottles gave a positive result at day 3 after inoculation. The biochemical profiles produced by the RapID ANA II System (Remel, Inc., Lenexa, KS) showed that the organism was *Clostridium baratii* with a probability of 99%.
Conclusions: Our report highlights the importance of *C. baratii* as a potential human pathogen and documents the association with symptoms never before reported in clostridial infections.

R2257

Hydrocephalus: a rare complication of mumps meningoencephalitis

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Meningoencephalitis is the most frequent and generally benign complication of mumps; however hydrocephalus may develop as a result of mumps meningoencephalitis. In this report two patients with hydrocephalus related to mumps meningoencephalitis are presented.
Patient 1: an 8-year-old boy, who had a 5-day history of fever, malaise, headache and vomiting was admitted to our hospital. Physical examination revealed bilateral parotis swelling, neck stiffness, and positive Kernig and Brudzinski signs. He had also horizontal nistagmus and bilateral papilledema. Cranial computed tomography showed dilatation of lateral ventricles. Cerebrospinal fluid examination revealed pleocytosis (250 leucocytes/μL with 90% lymphocytes and 10% neutrophils). CSF protein concentration was 152 mg/dL, and glucose concentration was 30 mg/dL (simultaneous serum glucose: 91 mg/dL). Serum mumps IgM and IgG were positive. Symptomatic treatment was initiated. Vertigo, ataxia and dysmetria developed; however patient's complaints and neurological findings resolved completely. Hydrocephalus persisted but did not progress.
Patient 2: a 6-year-old boy with fever and vomiting was admitted to our hospital. She had had bone marrow transplantation because of ganglioneuroblastoma 9 months previously. She developed parotis swelling two weeks before and the diagnosis of mumps was established by his paediatrician. Physical examination revealed neck stiffness, and positive Brudzinski sign. Cranial computed tomography showed dilatation of all ventricles. Cerebrospinal fluid examination revealed pleocytosis (160 leucocytes/μL with 85% lymphocytes and 15% neutrophils). CSF protein concentration was 206 mg/dL, and glucose concentration was 17 mg/dL (simultaneous serum glucose was 97 mg/dL). Serum mumps IgM and IgG were positive. The patient was followed with supportive treatment. All complaints and neurological findings of the patient completely resolved. Hydrocephalus persisted but did not progress. Hydrocephalus should be taken into consideration in patients with mumps meningoencephalitis.

R2258

The incidence, serotyping and antibiotic resistance of non-typhoidal *Salmonella* and *Shigella* species in childhood acute gastroenteritis in a district of the capital city of Turkey

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Objective: To determine the incidence, clinical features, serotypes and antibiotic resistance of non-typhoidal *Salmonella* (NTS) and *Shigella* strains among children in our district.
Methods: Children aged 0–16 years, who presented to the Ankara University Medical School, Department of Paediatrics with acute diarrhoea were included in the study. Patients were clinically evaluated. The stool samples were examined directly and then inoculated into McConkey and Salmonella Shigella agar. The NTS and *Shigella* colonies were identified by conventional biochemical methods. The *Salmonella* colonies were treated with *Salmonella* polivalent O anti-sera and serotyped with the Kauffmann–White scheme. *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* antisera were used for *Shigella* serogrouping. *S. flexneri* strains were agglutinated serially and subserotypes were identified. Antimicrobial susceptibility of the isolates was determined with disk diffusion method as advocated by the National Committee for Clinical Laboratory Standards.
Results: In a two-year period, stool cultures were obtained from 1335 children. Forty-four (3.3%) NTS and 132 (9.8%) *Shigella* strains were isolated. There were no significant difference in clinical features between children less than 1 year of age and those who were older. The frequencies of the isolated NTS serotypes were as follows: Sixty-eight percent *S. enteritidis*, 17% *S. typhimurium*, 7.3% *S. irumu* and 7.3% *S. paratyphi* B. The frequencies of the isolated *Shigella* species were as follows: 92% *S. sonnei* and 7.6% *S. flexneri*. Other *Shigella* species were not detected. Among 10 *S. flexneri* isolates, three were identified as *S. flexneri* type 4. Twenty-three percent ampicillin and 9% trimethoprim-sulfamethoxazole resistance were found among NTS

species. No ciprofloxacin and ceftriaxone resistance were detected. Eighteen percent ampicillin, 70% trimethoprim-sulfamethoxazole, 8% chloramphenicol and 83% tetracycline resistance were found among *Shigella* species. All *Shigella* strains were found to be susceptible to ciprofloxacin and cefixime. *S. flexneri* was more resistant to ampicillin, chloramphenicol and cephalothin than *S. sonnei*.

Conclusions: *Shigella* and NTS species are still major bacterial pathogens in acute gastroenteritis of children. The changing pattern of the antibiotic resistance and serotypes among districts should be studied and treatment recommendations should be determined accordingly.

R2259

A pragmatic clinical score to reduce unnecessary antibiotic use in children with pharyngitis in developing countries

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Background: Clinical diagnosis of Group A Streptococcus (GAS) pharyngitis has a low positive predictive value. Diagnosis has to be confirmed by bacteriology. Some clinical scores help to identify children at risk of GAS pharyngitis necessitating a microbiological diagnosis. In developing countries, the access to microbiological resources is limited and all children with pharyngitis are empirically treated with antibiotics to prevent subsequent rheumatic fever. The efficacy of this prevention remains controversial.

Objectives: To develop a clinical score allowing, by identifying ''non GAS'' pharyngitis, the reduction of empirical antibiotherapy for children with pharyngitis in developing countries.

Methods: We prospectively included children with pharyngitis in 3 public hospitals of Brasília (Brasil) during 9 months in 2004. We filled a clinical form and performed throat swabs. Conjunctivitis, coryza, cough, diarrhoea and viral exanthema were considered as ''viral signs'', while fever higher than 38.5°C, tender cervical node, headache, petechia on the palate, abdominal pain and sudden onset (<12 hours) were noted as ''bacterial signs''. Children under current antimicrobial treatment were excluded. Bilateral chi-square and multivariate analysis were performed to determine the score categories. The outcome measures were: sensitivity, specificity, likelihood ratios and post-test probabilities of ''non GAS'' infection with the score approach as compared with throat culture.

Results: 163 (74%) of the 220 children had ''non GAS'' pharyngitis (negative culture). We established 3 categories (age, viral and bacterial signs) with 3 possible answers each. Based on the score results, the children were sorted in 2 groups. Antibiotics treatment was indicated for children in group 1 while those in group 2 were treated without antibiotics. Use of this score would prevent 41% (67/163) of unnecessary antimicrobial prescriptions. The specificity of the score for ''non GAS'' pharyngitis in group 2 was 84%. Therefore, 16% of children with pharyngitis and positive GAS culture would not have been treated with antibiotics; this is less than the expected carriage rate.

Conclusion: Such a clinical score could be helpful to reduce unnecessary antibiotic prescriptions for pharyngitis in children from developing countries. In our children population, it would have reduced the antibiotic prescription by 41% without resorting to microbiology. However, it should be validated for routine clinical use.

R2260

Penicillin + gentamicin versus ampicillin + gentamicin in the empiric treatment of early neonatal sepsis

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Increasing antibiotic pressure with rising resistance and narrowing of antibiotic choices have been the leading trends in antibacterial treatment over the last decades. Narrow spectrum penicillins have been shown to have the least effect on normal bowel colonisation in neonates but their comparative efficacy for empiric treatment of early neonatal sepsis is poorly documented.

Objective: The aim of our study was to compare the clinical efficacy of two antibiotic regimens – Pen + Genta and Amp + Genta – in the empiric treatment of early neonatal sepsis.

Materials: Case histories of all neonates, treated in the NICU of Tartu University Hospitals from the 1st of January 2003 to 31st of December 2004 (n = 256) were analysed retrospectively. From January to October 2003 the preferred primary antibiotic regimen was Amp + Genta, since November 2003 Pen + Genta was used. Primary end-point was change of antibiotic treatment from the study regimen within 96 hours.

Results: Overall 218 infants needed early empiric antibiotic treatment, 108 received Amp + Genta and 95 Pen + Genta. The two groups were comparable in demographic parameters; there were 24 (23.5%) and 28 (30.1%) VLBW infants in the Amp + Genta and Pen + Genta groups, respectively. Change in antibacterial treatment within 96 h was considered necessary in 13 (12%) and 9 (9.5%) patients in Amp + Genta and Pen + Genta group, respectively. The number of sepsis episodes and their aetiology is presented in the table.

	Amp+Genta (n=108)	Pen+Genta (n=95)
Pt. with early sepsis (n)	18	16
Pt. /episodes with late sepsis: (n)	11/14	9/10
Aetiology of early/ late sepsis (n):		
GBS	1/0	2/0
CONS	0/8	1/4
Listeria monocytogenes	1/0	0/0
Gram-negative enterobacteria	3/4	1/1
Enterococci	0/2	0/0
Haemophilus influenzae	1/0	0/0
Candida spp	0/1	0/0
unknown	12/2	12/5
NICU stay (d, mean +/–SD)	13 +/– 22	9 +/–15
Died (n)	7	8

Conclusion: In the empiric treatment of early neonatal sepsis the combination of Pen + Genta is as effective as Amp + Genta.

R2261

A series of unfortunate events after paronychia due to *Staphylococcus aureus*: pericarditis, pneumonia, lung abscess, empyema and osteomyelitis

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Staphylococcus aureus is an important agent of soft tissue infections. If staphylococcal infections are inefficiently treated,

more severe infections such as septicemia, endocarditis, pneumonia, arthritis, and osteomyelitis might occur. In this report, a child with paronychia is presented. A 4-year-old boy presented with dyspnoea, and abdominal pain was admitted our clinic. His complaints had started with painful swelling in the left second finger. He was initially treated with topical mupirocin. His paronychia did not resolve and one-week later oral amoxicillin-clavulanate had also been initiated. Because of the progressive infection the patient was hospitalized, surgical drainage was performed and parenteral antibiotic therapy was initiated; however, the patient's condition had deteriorated, and the patient had been referred to our hospital. He had fever (38.4°C), tachypnea (52/minute), tachycardia (143/minute). His arterial blood pressure was 99/73 mmHg. Cracking rales were determined on the right hemithorax. His heart sounds profoundly low. The liver and the spleen were palpable for 7 cm and 2 cm, respectively, from the costal margins. Paronychia was present on the left second finger. White blood cell count was 25 200/μL, erythrocyte sedimentation rate was 74 mm/hour, and C-reactive protein was 8.7 mg/dL. Chest X-ray revealed cardiomegaly, and massive pericardial effusion was demonstrated by echocardiography. Approximately 500 mL of purulent fluid was drained by pericardiocentesis, and pericardial tube drainage was applied. Vancomycin and ceftriaxone were initiated. Chest computed tomography revealed pneumonia, lung abscess, and empyema on the left lung. On the follow up, osteomyelitis of the left tibia also developed. However, these localized infections did not require surgical intervention. Pericardial fluid culture and blood cultures were positive for methicillin-sensitive *S. aureus*, and antibiotic therapy was changed to ampicillin-sulbactam. On follow up, the patient's clinical condition improved. Pericardial drainage was discontinued. Antibiotic therapy was switched to oral therapy and the patient was discharged on day 38 of hospitalization. Oral antibiotic therapy was discontinued two weeks later. The patient did not develop any sequelae. Localized infections due to *S. aureus* may cause life-threatening complications especially if they are not treated sufficiently. Systemic antibiotic therapy with early surgical drainage is the mainstay of therapy.

Immunology, host defenses, immunotherapy

R2262

Safety and efficacy of recombinant interferon-gamma-1b immune adjuvant in 20 patients receiving high-dose donor granulocyte transfusions. An observational study during 2000–2004

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Background: Response to antimicrobial therapy alone is often sub-optimal in severely immunosuppressed cancer patients with difficult-to-treat opportunistic infections (OIs).
Methods: Patients receiving G-CSF donor primed (~5.5×10^{10} per transfusion) granulocyte transfusions (GTX) plus recombinant interferon-gamma-1b (rIFN-g1b) were evaluated.
Results: The mean age was 43 ± 17 y, 10 (50%) were men, 16 (80%) had acute leukaemia, 3 (15%) MDS and APACHE –II score was 19 ± 17 (range, 17–22). Most patients (n = 18, 90%) had relapse or refractory cancer. In 6 (30%) allogeneic haematopoietic stem cell transplantation (HSCT) recipients, GTX plus rIFN-g1b was given 59 ± 100 (range, 12–372) days after transplantation. Seventeen patients (85%0 had severe neutropenia during GTX plus cytokine therapy. In 19 patients (95%) with invasive fungal infection (IFI), 5 (25%) had possible, 3 (15%) probable and 8 of 11 (55%) with definitive IFI had disseminated systemic mycosis. One patient (5%) had refractory Pseudomonas sepsis. Eight patients (40%) were receiving >600 mg prednisone equivalent dose during GTX-cytokine therapy. Cytokine doses that accompanied 10 ± 7 (range, 4–28) GTX included, 9 ± 7 doses of rIFN-g1b (mean cumulative dose [c.d.] 1188 ± 2621 μg). Other concomitant cytokines were G-CSF 12 ± 3 G-CSF doses (c.d. 7128 ± 4721 μg) in 15 patients (75%) and GM-CSF 12 ± 9 doses (c.d. 5624 ± 4410 μg) in 14 patients (70%). In 2 patients with fever and one patient each with skin rash and transient dyspnoea were attributed to GTX. In 8 patients, 3 each with fever, and reversible liver dysfunction, and 1 each with fever, and tachycardia were considered rIFN-g1b associated adverse reactions. Four weeks after therapy, 9 patients (45%) had completed or partial response; another 3 (15%) had stable systemic mycosis.
Conclusions: Adjuvant GXT plus rIFN-g1b therapy was tolerated without serious adverse reactions. Therapeutic efficacy needs further prospective evaluation.

Vaccines

R2263

Comparison of immune response in immunised and non-immunised mice after challenge with tick-borne encephalitis virus

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Objectives: Tick-borne encephalitis (TBE) virus is an important human pathogen that causes dangerous central nervous system disease in large endemic areas. Despite the efficacious vaccines existing, the understanding of immunopathogenesis of TBEV infection is relevant for treatment development.

Methods: As it was described earlier, BALB/c mice is a suitable animal model for TBE. We investigated the immune response in BALB/c mice immunized with TBE vaccine and infected after with TBE virus (strain Absettarov). We also compared it with the immune response in non-immunized mice infected with the same dose of the virus. Specific humoural (IgM and IgG) and cellular (enzyme-linked immunospot assay and cell proliferation test) immunity was studied, as well as serum levels of some cytokines (tumour necrosis factor-alpha - TNF-a, interleukine/IL-1beta, 2, 6, 10, 12 and interferon-gamma – IFN-g).
Results: Control group of animals demonstrated 100% mortality rate. In contrast, immunized mice challenged at day 7 after

the last vaccination showed 100% protection. We found the significant difference in the specific and nonspecific immune responses between immunized and non-immunized mice. No specific IgM were found in immunized group after infection with TBEV in contrast to the control group. Serum IgG antibody titres against TBEV increased on day 5 post infection in immunized animals. A statistically significant increase in proliferation rate of splenocytes and number of IFN-g producing cells in immunized group was observed since day 7 post-infection. The animals from control group did not develop specific cellular immunity against TBEV after the lethal challenge. The most interesting finding was the prominent difference in dynamics of IL-6, IL-2, IL-12 and IFN-g. TBE vaccination induced dominant Th1 response (IFN-g, IL-12) at the early stage of the infection. Whereas serum levels of proinflammatory cytokines (TNF-a, IL-1beta) and IL-6 progressively increased in non-immunized mice until the death.
Conclusion: Immunization of BALB/c mice with TBE vaccine revealed the formation of both cellular and humoural immune responses. Probably the strong cellular response and early shift to Th1 cytokine profile are markers of protective immunity against TBE virus in immunized animals.

R2264

Evaluation of measles-specific humoural and cell-mediated immunity in infants aged 9 months after vaccination with AIK-C and Schwarz measles vaccine strains, in Ilam, Iran

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Objectives: The aim of this study was determination of immunogenicity of AIK-C and Schwarz measles vaccine strains in infants aged 9 months, in Ilam, Iran.
Methods: One hundred infants aged 9 months were enrolled in two groups (fifty infants in each group). In this study two live attenuated measles vaccines including Alk-C and Schwarz were used. Each group of infants was injected by one of these vaccines. After vaccination, the humoural and cellular immune response directed against Alk-C and Schwarz vaccines were evaluated by Viral Neutralization Test (VNT) and Lymphocyte Transformation Test (LT) methods
Results: Injection of Alk-C resulted in 58% cellular immunity and 88% humoural immunity against measles. The rate of inducted cellular and humoural immunity against measles after injection of Schwarz were 44% and 68% respectively. There is a significant difference between Alk-C and Schwarz mediated immunity.
Conclusion: We conclude that Alk-C vaccine is more immunogenic than Schwarz vaccine in infants and can induce effective immunization against measles at nine months of age particularly as a first dose.

R2265

Anti-rabies vaccine and its application for predator rabies prevention in Ukraine

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Objectives: Rabies is the emergent disease, which is registered now in more than 80 countries all over the world. This disease goes on without clinical character and the virus conservation and transfer occur in natural conditions and the infectious agent's reservoir is formed in ecological system. The biological fire-wall in the problem of rabies virus dissemination could be created with the use of living vaccines. The scope of the proposed research is the enhancement of the anti-rabies vaccine efficacy as the result of its use in the wild-life conditions.
Methods: The rabies vaccine strain Vnukovo-32M has passed successfully the tests on the reversions' absence during ten passages. The proposed preparation contains the additional component, which assists the vaccine virus penetration through animal tissues. The vaccine material was placed along with colouring agent into pharmaceutical blister packs, which were freezed afterwards. The vaccination was conducted during the autumn-winter period of 2001/2004 year in 9 districts of Odesa region. Proposed vaccine bites were placed manually on the borders of the forests and fox pathways in a number of 20 bites per square kilometre. The wild animals were shot in a planned manner and the laboratory diagnostics of the rabies was performed through Babes-Negri bodies detection in the brain tissues of the animals.
Results: We have shown that the main exponent of the rabies distribution in the southern region of Ukraine is the level of the fox rabies detection. The results of this factor monitoring taken for the last 6 years show that the foxes are the main reservoir of rabies in wild nature in Ukraine. We have registered that the modernized per-oral anti-rabies vaccine content accompanied by the special way of its application resulted in 16.8% decrease of rabies cases among wild-life foxes in comparison with the previous year.
Conclusions: The experience gained during the anti-rabies vaccine preparation and use has shown that it is necessary to modernize the vaccine preparation technology with the scope of prolongation of the longitude of its action. It is shown also that the proposed form of the vaccine bite and compilation of the bio-dependent calendar of vaccination serve the success.

R2266

Vaccination coverage and attitudes towards influenza of hospital healthcare workers, Piraeus

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Objective: To estimate coverage and attitudes toward influenza vaccination (INV) among healthcare workers (HW) of a tertiary Piraeus Hospital.
Methods:
Time: October, 2005. An anonymous questionnaire completed by HCWs regarding INV coverage, INV intention for the forthcoming season, reasons if not vaccinated, awareness and influenza epidemic/pandemic risk estimate, as well as history of influenza like illness (ILI) reported during the previous influenza season. Data entry in PC, analysis by SPSS.
Results: A return of 208 HCW (M: 86 F: 122, medical: 87, nursing/technicians: 121, m. age: 38.4 ± 8.2 years) questionnaires was entered for analysis. INV last season was reported in 20/208 (9.6%) a rate higher in medical personnel [13.7%, but non significantly so v nursing HCW]. Similar rates observed for previous seasons (10.3%, fall 2003). Awareness of INV indication was 71%, non-INV reasons given were negligence, doubts about necessity, fear of adverse events/injections and difficulty in obtaining. Intention for INV was expressed in 50.2% of HCW for the coming season, a rate lower than those anticipating a possible flu pandemic [56.7%]. Álso of note was the rate of HCW reporting ILI during the previous flu season (33%).
Conclusions: The rate of influenza vaccination coverage was found quite low (~10%), as shown in similar European papers,

and far lower than the US respective figures. Strategies to increase acceptance, compliance are warranted as are prompt central and local administrative interventions to ensure optimal coverage at the soonest possible time.

R2267

Effect of two different strategies to raise influenza vaccine coverage among medicine students

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Objectives: Influenza vaccine coverage is low among health care workers in Spain, including medicine students. The main goal of this study is to assess the effect of two different strategies to rise this coverage among Medical students.
Method: Two influenza vaccine campaigns were compared: season 2003 (October–November) versus season 2004 (same months). During the first season an indirect method of motivation was used: a news sheet about influenza was located on the students panel inside the different classrooms, except first and second year of Medicine. During the 2004 season we planned a direct strategy which consisted in a briefing with student elected delegates or representatives of each year from the forth to the sixth year (the last year) of Medicine. This meeting was performed in September and was conducted by one of us. The content of the meeting was a deep explanation of the reasons to be vaccinated against influenza. We used in-home slides and a set of slides downloaded from the CDC web site. Furthermore, we designed a data collection sheet for each student with a full explanation about the campaign, the aims and reasons of vaccination and the possible adverse reactions. The students had to fill this data collection sheet and signed it. This sheet was given out and explained to all students by their representatives. All the process was performed again the first week of November. Vaccines were purchased by the Gobierno de Canarias (Government of the Canarias Autonomous Community).
Results: 95 students were vaccinated in 2003: 6 in the third year (5.1% of the students in this year), 16 in their fourth year (15.6%); 31 from fifth year (32.6%) and 42 from sixth (40.0%). The results in 2004 were 6.6% (no briefing), 43.3%, 23.2% y 67.8% for 3rd, 4th, 5th and 6th respectively. Overall, the coverage among classes with briefing was 29.4% in 2003 and 42.6% in 2004. In both years, the same class showed the lower coverages: they were studying forth year in 2003 and fifth year in 2004. The class who studied third year in 2003 and forth year in 2004 rised from a coverage of 5% to 43%.
Conclusions: To rise low coverages of influenza vaccination among Medicine students is necessary to involve the students themselves through their delegates and representatives. A full explanation of the reasons, and a reiteration in a short period of time as well, is desirable. We also felt that we established a strong bond with our students for further collaboration.

R2268

Evaluation of protective antibody to measles virus in vaccinated medical students

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Background: Measles remains one of the dangerous infections worldwide that regarding to World Health Organization's report, still takes life of more than one million of under the age of five children. Vaccination has been available since 40 years ago. In Iran, it has started with national program of vaccination since 1973 in 2 occasions at 9 and 15 months of age. Recently several cases of early middle age measles infection have reported from different areas in Iran. To find out the reasons of increased prevalence, we decided to evaluate antibody against measles in early adulthood, which is the infection's most dangerous range of age.
Method: In a randomized cross-sectional study, we selected 241(127 male and 114 female) students from Isfahan University of Medical Science. These people have the age between 18 and 22 and the history of vaccination against measles (9 and 15 months). They did not have any past medical history or clinical evidences of infection with measles. IgG level against measles has been measured by ELISA method in their serum and the results have been analysed by SPSS software, Chi square statistical test and analysis of variance test (ANOVA).
Results: Regarding to brochures available with ELISA kit, results of serum antibodies evaluated as follow: Titre less than 8 IU/μl as negative, between 8 IU/μl and 12 IU/μl as intermediate and more than 12 IU/μl as positive. Among 241 samples, 98 samples (40.7%) were positive and104 (43.1%) and 39 (16.2%) samples were negative and intermediate respectively. (Table 1). There was not any meaningful difference in antibody titre between males and females. (Table 2).
Discussion: This study cleared that just 40.7% of the samples had protective antibody against measles and nearly 60% of them had potency of infection while exposed to wild virus. It is important that measles makes high mortality and morbidity rates and control of this disastrous infection needs lots of investment by the government. With reviewing and performing similar studies, it could be possible that reconsidering the vaccination program is worth wise.

R2269

Opsonophagocytic killing of *S. aureus* by PMNs from haemodialysis patients

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Objective: Haemodialysis patients are at increased risk for *S. aureus* infection, which is usually cleared by a functional opsonophagocytosis mechanism mediated by specific antibodies, complement, and PMNs. These patients would benefit from a vaccine directed against *S. aureus* capsular polysaccharides but may have an impaired neutrophil killing activity related to the underlying disease.
Patients and methods: *S. aureus* Loewenstein, freshly prepared leucocytes from 10 healthy human subjects or 11 haemodialysis patients, anti-staphylococcal human hyperimmune sera (AltaStaph, Nabi Biopharmaceuticals in concentrations of 1:100 to 1:2000), and baby bunny complement were mixed and incubated on a rotor rack at 37°C for 90 minutes. Aliquots were plated before and after incubation and the % killing rate was calculated.
Results: Healthy individuals showed an average killing of 62% using a 1:100 dilution of AltaStaph (range 36.8–76.7%) while leukocytes from haemodialysis patients showed an average killing of 64.3% with a range between 36.9 and 86.8%. Dilution of the sera resulted in reduced killing in all patients. The differences between the two groups were not statistically significant.
Conclusions: Our data indicate that PMNs from haemodialysis patients are capable of phagocytosis and that opsonic killing of *S. aureus* is not impaired in these patients compared to healthy controls. Susceptibility to infections in this population may be related to other factors involved in opsonophagocytosis such as

complement and other conditions (e.g. uncontrolled glucose levels and uraemia). Further studies to explore these components are under way.

R2270

Determination of anti-HBS titre mean induced by hepatitis B vaccine among health care workers in an Iranian hospital

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Objectives: HBV is a major cause of viral hepatitis, cirrhosis and liver cancer worldwide. Health care workers are one of the high-risk groups in affecting and transmission of this virus.
Methods: In this descriptive cross-sectional study, 102 HCWs of firoozgar hospital including faculty members, residents, interns, students, nurses and clerks were selected and enrolled through convenience sampling. All participants had to complete three-dose vaccination schedule. After blood sampling the samples were transferred to laboratory. Check list was consisted of age, sex, occupational group, smoking, alcohol abuse chronic renal and hepatic failure , diabetes and duration after the last dose of vaccine.
Results: Statistical analysis was done with SPSS 11.5 software. $P < 0.05$ was considered statistically significant. Multiple linear regression model was used to predict effecting factors on Anti-HB titre .102 Anti-HBS titres of HCWs were determined and the mean was 70.43(95% CI, 57.02–83.84). Also, Anti-HBS titres of 30 individuals (29.4%)were less than 10 mIu/ml and 72 individuals (70.6%) were equal or more than 10 mIu/ml. There was a significant statistical correlation between Anti-HBS titre and age and duration after the last dose of vaccine ($p < 0.05$).
Conclusion: Following-up for complete three-dose injection, vaccination before or at the onset of working should be attended. HCWs who are one of the high-risk groups in affecting and transmission of HBV, are recommended to determine their Anti-HBS titre after three-dose vaccination.

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