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Foot & mouth disease

K7 The UK 2001 FMD outbreak

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The United Kingdom had been free of FMD since 1981, when there had been a single outbreak in a dairy herd on the Isle of Wight, off the coast of southern UK. On February 19th, this year, pigs waiting slaughter in an abattoir in southern Essex, to the north of London were identified by the on-duty veterinarian with feet lesions consistent with FMD, and this was confirmed positive the following day at the high security Institute for Animal Health laboratory, Pirbright. This laboratory is also the World Reference Laboratory for FMD, and apart from having the largest research group working on FMD, also maintains a library of FMD virus isolates collected from around the world during the last 60 years. The virus strain was quickly identified by nucleotide sequencing to be the PanAsia strain of serotype O FMD virus, which was known to be present throughout most of Asia, and had recently caused new outbreaks in Japan (free since 1908), South Korea (free since 1934) and South Africa (this was the first outbreak of serotype O ever recorded), Mongolia and eastern Russia. The index farm contained over 500 adult, mostly cull sows and boars, and following clinical examination it was apparent that disease had probably been introduced as early as the beginning of February, as most of the pigs had lesions of approximately 10 days old. The farmer fed almost exclusively a diet of waste food (swill), collected from nearby schools, hospitals and restaurants. Regulations relating to swill feeding make it compulsory to boil all waste food before feeding to pigs, but this is difficult to enforce. Control of the outbreaks was taken over from MAFF by the Chief Scientist, supported by four teams of mathematical modellers and other involved groups, who reported directly to the Prime Minister's Office. The slaughter policy was extended to all neighboring farms and those within a 3 km radius of the infected farm; slaughter of the infected farm was to be completed within 24 h of diagnosis, and neighbouring farms within 48 h, and this became the responsibility of the Army. The use of vaccination was considered, but would not have significantly altered the disease distribution or the final outcome, and was likely to confuse subsequent re-establishment of disease free status. The total cost of the outbreak has not been calculated, but including the effect on tourism, will exceed 15 billion pounds sterling.

Streptococcus pyogenes: new features of an old pathogen

S13 Pathogenesis and epidemiology of invasive *Streptococcus pyogenes* isolates

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Aachen, D

During the last two decades, a remarkable change in the epidemiology of *Streptococcus pyogenes* infections with a marked increase in reporting of invasive diseases, such as bacteremia, necrotizing fasciitis, and myositis was observed worldwide. A substantial portion of these deep-seated infections was associated with the streptococcal toxic shock syndrome. It has been hypothesized that the emergence or re-emergence of more virulent strains, particularly those with type emm1, emm3 or emm28 is related to the type distribution of the organism among superficial infections partially depending on an absence of protective immunity against these types in the population. Intense research on pathogenesis has investigated the role of the pyrogenic exotoxins SpeA through SpeJ some of which act as superantigens. While the distribution of emm-types among invasive isolates parallels the distribution of emm-types among isolates from superficial infections to some extent, a strong association between the *Streptococcus pyogenes* emm-type and the presence of certain genes coding for pyrogenic exotoxins has been observed in invasive and in non-invasive isolates. Since neither factor can solely explain all the manifestations of streptococcal toxic shock syndrome so far, the genetic control of exotoxin production or expression of other virulence factors, such as the hyaluronic acid capsule, streptokinase, streptolysin S and the mitogenic factor F, has become a major focus for pathogenesis research of this organism. Additionally, subtyping by sequencing of the *sic*-gene encoding the streptococcal inhibitor of complement of isolates from invasive and noninvasive infections with emm1 has demonstrated a remarkably heterogenous array of subclones. It has been postulated that *sic*-variants arise by natural selection on human mucosal surfaces, and that this process may sustain epidemic waves and contribute to the emergence and reemergence of *Streptococcus pyogenes*. The overall implications of these aspects in the concept of invasive *Streptococcus pyogenes* infections will be reviewed.

Vaccination issues (Joint symposium ESCMID/ISID)

S17 Introduction of MenC vaccine in UK

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Objectives: To measure impact of MenC conjugate vaccines on age-specific incidence of invasive MenC disease, to derive age-specific efficacy estimates and to monitor impact on the phenotypic and genotypic characteristics of the organism.
Methods: Vaccination history was obtained for all group C meningococcal cases confirmed in targeted age groups. Efficacies estimated for up to 21 months of follow-up based on vaccine coverage data for the targeted age groups as at June 2001. Evidence of herd immunity was sought by comparing incidence of group C disease in unvaccinated individuals before and after the MCC campaign. To monitor evidence of capsular switching, all invasive isolates are phenotyped, 50% are porA sequenced and multilocus sequence typing performed on 10%.
Results: Up to 31/9/01, 23 confirmed vaccine failures were identified. Efficacy estimates are 91.5 (64.9–98.0) for the routine 2/3/4 months schedule, and 89.3 (72.7–95.8), 100 (84.9–100), 95.3 (88.3–98.6) and 91.9 (73.3–98.4), respectively, for the 12–23 months, 2–4 years, 5–14 years and 15–17 years catch-up cohorts, respectively. Percentage reduction in attack rates in unimmunized individuals are for 1–4 years 50%, 5–8 years 57%, 9–14 years 34% and 15–17 years 61%. No changes suggestive of capsular switching are evident over the 18-month period.
Conclusions: Based on the antibody persistence data from prelicensure clinical trials, serum bactericidal antibody levels in the youngest age groups are currently declining to near baseline so vaccinees will be reliant on memory for protection. However, efficacy estimates remain high in toddlers. Reductions in group C disease in unvaccinated individuals both in the cohorts targeted for immunization are consistent with herd immunity. There is no evidence of capsular switching to date.

S19 Is the MMR vaccination safe and/or necessary?

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Objectives: To investigate the data on the safety and efficacy of measles–mumps–rubella (MMR) vaccination.
Methods: Literature search of the epidemiology of MMR diseases, and the impact of MMR vaccines.
Results: Wherever the MMR diseases have been looked at, their high prevaccination incidence was common feature. Also the incidence of complications has been rather similar, with some exceptions. Measles vaccination

was introduced in the 1970s, while mumps and rubella components are still searching for their way to general use in many countries. The combined MMR vaccine was introduced in the 1970s, and thorough research has been carried out with this combination. Although the antigens vary in different MMR vaccines, especially in terms of the mumps component, the vaccine efficacy has been good – again with the exception of the mumps component whose efficacy has been the highest with the Jeryl Lynn and the lower with the Rubini strain. The reactogenicity of all MMR vaccines is low, the best data deriving from prospective double-blind studies on vaccine using the Moraten strain for measles, Jeryl Lynn for mumps, an RA 27/3 for rubella. This is also the only vaccine from which long-term data are available on the clinical efficacy and the (low) complication rates. Whatever MMR vaccine in question, at least two doses are necessary for the elimination of all sMMR diseases.
Conclusions: MMR vaccines are, generally speaking, safe and efficaceous, but they are not identical. One should take into account no only the short-term but also the long-term effects before large-scale vaccinations are introduced. Only few vaccine brands can show hard data on all these important issues. Bearing in mind the severity of MMR diseases, there is no doubt MMR vaccines are among the most necessary weapons we have in immunizations at hand.

Management of central nervous system infections in risk populations

S21 Parasitic infections of the central nervous system

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Al-Khod, OM

Parasitic involvement of the central nervous system (CNS) is integral in many protozoal and helminthic infections. Falciparum malaria, African and American trypanosomiasis, and toxoplasmosis involve millions of people, and produce distinctive, diagnostic clinical features. Immune evasion is exhibited by many protozoa, e.g. *Toxoplasma gondii* may remain silent for many decades, only becoming symptomatic when immunosuppression develops. In helminthiasis, CNS invasion is less prominent, but trichinosis, angiostrongyliasis cantonensis, and cysticercosis produce well-recognized syndromes. In the case of the brain, meningoencephalitis or space-occuping lesions result, often with seizures as in cysticercosis and schistosomiasis japonicum. Myelopathy is caused by deposition of parasitic ova (e.g. in schistosomiasis), invasion by adults (e.g. in paragonimiasis), or indirectly as in diphyllobothriasis. Ophthalmic involvement variously causes iritis (e.g. onchocerciasis), choroidoretinitis (e.g. angiostrongyliasis), or optic neuritis (e.g. trypanosomiasis). The AIDS pandendemic and the increasing use of immunosuppressive drugs have resulted in an unprecedented rise in the incidence of CNS parasite infection, notably toxoplasma brain abscess. Cases of meningoencephalitis in microsporidiosis, disseminated stronyloidiasis, and trypanosomiasis cruzi have also increased. Identification of CNS parasite invasion has improved with the development of investigations such as antigen detection in CSF, and magnetic resonance imaging. Although treatment in some conditions, e.g. falciparum malaria and trypanosomiasis gambiensehas, has improved significantly, there has been little or no progress in control and prevention of the major parasitic zoonosis.

S22 CNS infections caused by viruses. Focus on herpesviruses and JC virus

P. Cinque
Milan, I

Viral infections of the central nevous system (CNS) still represent a major threat in immunocompromised people, especially those infected with the human immunodefiiency virus (HIV). These are mainly caused by herpesviruses, including cytomegalovirus (CMV) and, to a lesser extent, herpes simplex viruses type 1 and 2, varicella-zoster virus and human herpesvirus 6, and by the papovavirus JC virus (JCV), the etiologic agent of the progressive multifocal leukoencephalopathy (PML). Over the last decade, the diagnosis of these infections has substantially been improved by the application of nucleic acid amplification techniques in the cerebrospinal fluid (CSF). Viral infections of the CNS can now be specifically identified by detection of the respective genomes in CSF, thus reducing the time for diagnosis and the use of invasive procedures. More recently, quantitative amplification techniques have also been introduced in clinical practice, providing an estimate of microbial replication at the time of diagnosis and during follow-up. The prognosis of these diseases has benefited from advances not only in diagnostics but also in drug development. However, despite the large armamentarium of antiviral drugs, CNS infections caused by herpesviruses often fail to respond to specific therapy. This might be due to the severity of disease, limited access of certain drugs to the CNS or development of antiviral drug resistance. With respect to PML, a few drugs are known that are active against JCV in vitro, but none of these has so far been shown to be effective in vivo. On the other hand, the frequency of these diseases among HIV-infected patients has drastically declined in the western world following the introduction of potent anti-HIV combination therapies, as in the case of CMV encephalitis. Furthermore, anti-HIV treatment may *per se* be effective in treating some of these infections, such as PML, by improvement of the host immune defences.

S24 Tuberculous meningitis: still a challenge to the clinician?

J.-C. Garcia-Monco
Galdacano, E

Tuberculous involvement of the central nervous system is most frequently caused by mycobacterium tuberculosis and still represents a formidable diagnostic challenge. tuberculous meningitis, the most severe form of neurologic tuberculous infection, presents most frequently as a subacute syndrome with fever, headache and abnormal sensorium. In children, hydrocephalus is particularly frequent. The presence of human immunodeficiency virus infection does not seem to modify significantly the clinical picture of tuberculous meningitis. Neuroimaging reveals enhancement of the basal cisterns and a variable degree of hydrocephalus. Cerebrospinal fluid (CSF) typically shows lymphocytic pleocytosis with low glucose levels, although initial polymorphonuclear predominance may occur. Cerebrospinal fluid culture is positive in approximately 60% of patients and takes several weeks for the bacilli to grow. Polymerase chain reaction of the CSF has improved the diagnosis but is not yet widely available. Empirical therapy is often initiated upon suspicion based on a clinically consistent picture and CSF findings. Most of the regimens contain three drugs that should include rifampin and isoniazid, although some experts advise four-drug regimens. To date, multiple-drug resistance has not been a problem in neurological tuberculosis, although the mortality rate of this infection remains at approximately 25% of the patients. Parenchymal CNS involvement can occur in the form of tuberculomas or, more rarely, abscesses. Although surgery was initially advocated as the mainstay of therapy, more recent evidence suggests that they both can be cured with medical treatment alone. Also, damage of the spinal cord, roots and spine can occur in the form of spinal meningitis or radiculomyelitis, spondylitis or even spinal cord infarction. Less frequently, opportunistic nontuberculous mycobacteria, especially *Mycobacterium avium*, can cause meningitis or meningoencephalitis, particularly in HIV-infected patients. Therapy in these cases includes a macrolide, ethambutol, and a third agent (rifabutine, rifampin, ciprofloxacin or clofazimine). Overall prognosis in nontuberculous mycobacterial infection is somber, with a mortality rate close to 70%.

Diagnostic methods: molecular

O25 Multicenter performance evaluation of NucliSens EasyQ HIV-1

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Objectives: A HIV-1 viral load assay was developed based on NASBA amplification and real time detection with molecular beacon probes. Molecular beacons are oligonucleotides with a stem-loop structure and with quenching and fluorescent moieties on their 5′-and 3′-ends. Binding of the specific loop sequence with its complementary target RNA produced by the NASBA reaction results in unfolding of the stem and hence the emission of a fluorescent signal upon excitation at the appropriate wave length. In this study, the performance of this new test was evaluated in routine settings at three sites.
Methods: The real-time NASBA assay (NucliSens EasyQ HIV-1) uses an HIV-1 gag-based primer set in combination with bioMerieux's proprietary silica-based extraction technology to isolate HIV-1 RNA from plasma. As a control for the entire procedure an internal calibrator is added to the sample prior to nucleic acid extraction. Two distinct molecular beacons are added to the amplification mixture: one molecular beacon with a loop complementary to a highly conserved region of the wild-type HIV-1 RNA and another complementary to the calibrator RNA. To obtain broad subtype reactivity, the HIV-1-specific beacon contains inosine nucleotides at positions known for sequence variation hot spots in the viral genome among HIV-1 subtypes.
Results: Linearity was investigated at each site by testing a dilution series with 8–16 replicates at each concentration of a HIV-1 RNA reference material calibrated against the WHO standard. A linear dose–response was observed in a range of 25–1 000 000 copies/mL plasma with SDs of about 0.10 log at concentrations above 1000 copies/mL and higher SDs at lower concentrations. Quantitations at all sites were very comparable, demonstrating a good interlab reproducibility. Of more than 50 non-B clades tested, representatives of group M subtypes A-J and the majority of the group O specimens were detected and correctly quantified in comparison to, e.g. bDNA 3.0 (Bayer) and other commercially available assays. Parallel testing of 101 clinical samples showed good correlation with the FDA-approved NucliSens HIV-1 QT ($R = 0.956$, CI 0.936–0.970). Average time to result for amplification and real-time detection was approximately 90 min for 48 samples including 30 min hands-on.
Conclusions: This multicenter trial demonstrates excellent performance of a high throughput real-time NucliSens assay for HIV-1 viral load measurement in routine settings.

O26 A broadly reactive real-time NASBA assay for rapid detection of Norwalk-like virus RNA in stool samples

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Norwalk-like viruses are a genetically diverse group of viruses now recognized as the most common cause of viral gastroenteritis. RT-PCR has been the conventional method for detecting NLVs in stool samples. We have previously reported the development of a rapid and sensitive nucleic acid sequenced based amplification (NASBA) assay for the electrochemiluminescent (ECL) detection of the 8FIIb Genogroup (GG) I Norwalk virus strain RNA using NucliSens® Basic Kit (bioMérieux, Durham, NC). This assay provided an alternative means for detection of NV RNA in stool samples. However, the two-step assay detected only a small number of GGI strains and detected no GGII strains. Additionally, even though more rapid than RT-PCR analysis, the NLV NucliSens® Basic Kit amplification and ECL detection assay required 3 h to complete.
Objective: We set out to develop a one-step accelerated broadly reactive NASBA assay using the NucliSens® Basic Kit assay and molecular beacons for real-time detection of NLV GGI and GGII RNA in stool samples.
Methods: A 10% stool suspension of known NLV RT-PCR negative and positive stool samples from various GGI and GGII NLV outbreaks was prepared in sterile nuclease-free water. The prepared stool suspension was centrifuged at $5000 \times g$ for 15 min at room temperature. A 100 µL of the cleared stool suspension was added to 900 µl of NucliSens® Basic Kit lysis buffer. Viral RNA was isolated using reagents supplied in the kit. NLV RNA was detected using the NASBA based NucliSens® Basic Kit, broadly reactive amplification primer sets, and molecular beacons.
Results: The one-step broadly reactive real-time NASBA assay detected NLV RNA in both RT-PCR positive and RT-PCR negative samples in 60 min.
Conclusions: The results of our study indicate that the NucliSens® Basic Kit using broadly reactive amplification primers and molecular beacons for real-time detection of viral RNA provides a more rapid method than ECL to detect NLVs in stool.

O27 Clinical evaluation of a NucliSens Basic Kit application for the detection of enterovirus in cerebrospinal fluid (CSF) by nucleic acid sequence-based amplification (NASBA)

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Background: Enteroviruses are a common cause of pediatric infections. The accurate, rapid differentiation of enteroviral illness from other bacterial and viral infections is important for therapeutic and prognostic reasons. Our laboratory previously determined the technical performance of a modified NucliSens Basic Kit NASBA assay (Fox et al.) for the detection of enterovirus. This study evaluated the clinical performance of the assay using CSF samples collected during the 2001 enteroviral season.
Methods: Nucleic acid isolation, amplification and detection were performed using the NucliSens Basic Kit reagents (Organon Teknika/bioMerieux, Boxtel, NL). Nucleic acids from CSF and an enterovirus-specific internal control (IC) were coextracted and coamplified with a single enterovirus 5′-NTR specific primer pair. Electrochemiluminesence (ECL) detection of both enterovirus RNA and IC RNA were performed using target specific capture probes and ruthenium (Ru 2+) labeled detection probes. Samples were reported as negative (ECL signal <350 units), indeterminant (ECL signal 350–649 units) and positive (ECL signal >650 units). Negative results were reported as indeterminant due to amplification inhibition if the IC ECL values were <50 000 units. All the results were correlated with clinical presentation, CSF cell counts, bacterial, fungal and viral cultures, performed when indicated.
Results: In total, 144 CSFs were tested by the NASBA assay of which 74 (51%) were positive (mean ECL signal of 630 000 units, range 1640–10 000 000 units) and 68 (49%) were negative (mean ECL signal of 12 units, range 1–123 units). CSF viral culture was performed on 95 of the samples submitted. Enterovirus RNA was detected in 14 samples that were viral culture negative. There were no CSFs culture positive and NASBA negative. For two samples, NASBA positive and culture negative, repeat analysis of additional CSF and stool were found to be NASBA positive also. The NASBA reaction was inhibited in two visibly bloody samples (1.4%).
Conclusions: The NucliSens enterovirus assay demonstrated superior sensitivity over viral culture, excellent specificity, a clear delineation of positive samples and minimal amplification inhibition. Daily testing and the rapid turn-around-time for results greatly impacted patient care by reducing hospitalization and the inappropriate use of antibiotics for viral infections.

O28 Use of internally controlled real-time NASBA for the detection of HBV DNA

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Objectives: The detection of HBV DNA has been important for the definition of clinically important thresholds, as well as to monitor the effect of antiviral treatment and the detection of resistant related variants. Nucleic acid amplification assays with real time detection enable the detection of HBV DNA in clinical samples over a very broad dynamic range. The use of an

internally controlled real time assay based on NASBA amplification in combination with detection using molecular beacons was evaluated for clinical performance.

Methods: The performance of the NucliSensEasyQ HBV assay (Organon Teknika/bioMérieux) assay was established using well-characterized reference samples ranging between 1 and 8.7 log. WHO IU/mL. Furthermore, reference panels from quality control studies were evaluated as well as samples containing different genotypes of HBV. The clinical use of the assay was established using samples from patients under antiviral treatment. Comparisons with commercially available assays (Roche Amplicor Monitor, and Digene Hybrid Capture II) were performed to determine concordance on the standardization of results.

Results: The real-time based assay, in combination with the NucliSens Extractor (Organon/bioMérieux), could accurately measure HBV DNA between 2 and 8.7 log WHO IU/mL, depending on the amount of input material. Standard deviation was less than 0.25 log below 1000 WHO, and less than 0.15 log above 1000 WHO IU/mL. Both serum as well as plasma could be measured accurately within a 0.25 log variation. The real time assay accurately compared with both the Digene Hybrid Capture II as well as the Roche monitor assay, and depending on the amount of internal standard or calibrator added, could be used for both high as well as low HBV DNA containing samples. This enables the monitoring of patients under antiviral treatment with a single assay.

Conclusion: The real-time based assay enabled the amplification of HBV DNA within 60 min, which combined with automated extraction could detect and quantify 48 samples in a single working day. The assay showed to be clinically useful in monitoring patients under antiviral treatment. Furthermore, the uses of well-known standards enable the comparison with data generated with different assays.

O29 Novel isothermal nucleic acid amplification technologies for the detection and discrimination of infectious diseases and genetic mutations

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Objectives: The development and evaluation of a novel isothermal nucleic acid amplification technology as a simple and robust molecular diagnostic assay suitable for rapid, accurate detection and discrimination of human infections and genetic mutations.

Methods: The amplification systems utilize DNA polymerase and/or RNA polymerase for the isothermal generation of a target-dependent signal. A unique target-dependent structure is formed by the hybridization of DNA probes that are partially complementary to each other and the target sequence, which can be DNA or RNA. The RNA polymerase promoter encoded within this structure is only functional in the presence of target, following which transcription of a unique, short nontarget RNA sequence occurs. The transcribed RNA signal and end detection sequences are universal for all targets. A number of detection methods applicable to both manual and automated versions of the assay have been validated. These include end-point detection using an enzyme linked oligo-sorbent assay (ELOSA) detecting color or luminescence (AP, HRP), fluorescence using molecular beacons for real-time quantitation and TRF and DeFRET. The systems have the advantage that they are isothermal and do not involve copying long tracts of target sequence to which specific probes are required. Moreover, they are quick and performed on standard low-tech laboratory equipment requiring no molecular expertise.

Results: The assays can detect and discriminate between wild type and variant targets in several human, bacterial and viral pathogens. Complex genetic material has been successfully used from pure nucleic acid extracts (*E. coli*, 23S rRNA; RSV, RNA) and simple crude cell lysates (*C. trachomatis* rRNA, 23S rRNA; MRSA gDNA, *mecA/coa*). Our results suggest numerous applications, including the diagnosis of bacterial and viral pathogenic infections, antibiotic resistance genes, and food- and water-borne diseases. One version of the assay has additional benefits. Firstly, it can detect and discriminate single nucleotide polymorphisms (SNPs), or three base deletion mutations such as in cystic fibrosis (delta f508). Secondly, the ability to detect RNA without the requirement for reverse transcriptase allows feasible gene expression analysis.

Conclusions: When fully developed these novel isothermal assays will be sensitive, rapid, simple to perform, amenable to automation for HTS, and a realistic alternative to culture and PCR with numerous applications.

O30 Less than one hour real-time PCR detection of toxigenic enteric pathogens directly from faeces using the Smart Cycler®

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Objectives: Shiga toxin-producing *Escherichia coli* (STEC) and cytotoxin-producing *Clostridium difficile* are important toxigenic fecal pathogens of clinical significance. The methods currently used in the clinical microbiology laboratories for the detection of these two classes of pathogens require at least 48 h. We have developed two rapid real-time fluorescence-based PCR assays for the detection of the toxin genes responsible for the virulent manifestations of these two pathogens.

Methods: We designed two pairs of PCR primers targeting the shiga toxin genes *stx1* and *stx2*. Two molecular beacons bearing different fluorophores were used as internal probes specific for each amplicon. For the *C. difficile* assay, we also designed two pairs of PCR primers and two molecular beacons targeting the cytotoxin genes *tcdA* and *tcdB*. Internal control DNA was incorporated into both assays to monitor potential PCR inhibition.

Results: Using purified genomic DNA, we showed that the analytical sensitivity of both assays was around 10 genome copies for each target. The specificity was demonstrated by the absence of amplification with 1 ng of DNA ($\sim 10^5$ genome copies) from 20 strains not producing shiga toxin for the STEC assay and from 15 non-*C. difficile* bacteria for the *C. difficile* assay. These assays, respectively, detected efficiently all STEC and *C. difficile* strains tested. Liquid or unformed feces samples obtained from patients were processed by a rapid (10 min) DNA extraction protocol and submitted to nucleic acid amplification on the Smart Cycler®. The shiga toxin gene PCR assay was validated by testing 38 feces samples obtained from 27 patients. Twenty-six of these samples were positive with both the PCR assay and the culture-based assay. Of the 12 culture negative samples, there was one PCR positive sample which could be explained by the presence of shiga toxin-producing bacteria other than *E. coli* O157:H7. The *C. difficile* assay was performed on 58 samples. Both *tcdA* and *tcdB* were efficiently amplified from 28 of 29 *C. difficile* cytotoxin-positive feces samples. There was no amplification observed with all 27 cytotoxin-negative feces samples.

Conclusions: We have developed the first less than one hour real-time PCR assays for the sensitive and specific detection of toxigenic *C. difficile* and shiga toxin-producing bacteria directly from stools.

O31 Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) by a novel isothermal amplification assay (CytAMP)

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Objectives: Specific and rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in patient specimens is essential for the effective control of MRSA nosocomial infection. This study evaluated the specificity and sensitivity of a novel isothermal amplification assay (CytAMP) for detection of MRSA in comparison with conventional culture and PCR-based identification methods.

Methods: The CytAMP assay is based on the formation of a three-way junction (3WJ) structure between the target DNA sequence and two target-specific oligonucleotide probes. For detection of MRSA, two sets of probes were designed to simultaneously detect the methicillin resistance (*mecA*) and coagulase (*coa*) genes found in MRSA isolates. Following formation of the 3WJ, an isothermal RNA amplification reaction occurs in a single tube/microtiter well and is detected by an enzyme-linked oligosorbent assay (ELOSA). The assay takes approximately 4 h to complete from initial cell lysis to detection.

Results: In a survey of 436 clinical isolates, comprising 359 staphylococci and 77 other organisms, CytAMP recognized 116 (98.3%) of 118 MRSA isolates identified by the 'gold standard' of PCR, compared with 98 (83%) identified by conventional culture. In addition, CytAMP identified as MRSA five *S. aureus* isolates which were PCR-negative for *mecA*, but which were recognized as MRSA by latex agglutination tests for the PBP2′ product of *mecA*. CytAMP incorrectly identified three (0.7%) of the 436 isolates as MRSA in comparison with 13 (3%) misidentified by conventional culture. In addition to culture confirmation, the CytAMP assay has also been used successfully to identify MRSA from patient screening swabs which have been

incubated overnight in Brain Heart Infusion broth >2 μg/mL oxacillin. In this application, results from patient MRSA screening swabs arriving in the laboratory by late afternoon are available to the infection control team by lunchtime the next day.
Conclusion: The CytAMP assay is a user-friendly and specific molecular method for the direct detection of MRSA in patient specimens and is a promising alternative to PCR and culture for the rapid identification of patients with MRSA colonization or infection.

O32 The role of PCR in the diagnosis of visceral and cutaneous leishmaniasis: a 6-year prospective study in Italy

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Objective: To compare polymerase chain reaction (PCR) employed on peripheral blood, bone-marrow aspirate and skin scraping with traditional methods for the diagnosis of visceral (VL) and cutaneous leishmaniasis (CL).
Methods: A 6-year prospective study was carried out from September 1996 to October 2001. 142 patients living in Milan (82.4%) and Palermo presenting with clinical signs of either VL or CL were included. The PCR assay was compared to microscopy and in vitro cultivation for primary diagnosis of VL and to microscopy only for primary and follow-up diagnosis of CL. The DNA extracted from peripheral blood (PB) and bone-marrow aspirate (BMA) was assayed by means of Leishmania-specific PCR. Primers R223 and R333 which amplify a 359-bp fragment of the 18S rRNA gene were employed. Identification of Leishmania to the species level was obtained by PCR-RFLP analysis of a Leishmania-specific nuclear repetitive genomic sequence. Leishmania stocks isolated in vitro were characterized by means of starch-gel electrophoretic analysis of 15 isoenzymes.
Results: Thirty-four patients (14 with AIDS and 20 immunocompetent) were diagnosed as having VL; four patients, two ICT adults and two with AIDS) had CL. Comparative results are reported in the table.

		Bone-marrow		PCR	Skin biopsy or scraping			
Category	Diagnosis	Micros vs	Culture	PB	BMA	Mic	Culture	PCR
16 ICT children	VL	15/16	11/13	16/16	14/14	1/1	ND	ND
14 AIDS adults	VL	12/13	4/6	14/14	13/13	ND	ND	ND
4 ICT adults	VL	2/2	ND	4/4	2/2	ND	ND	ND
2 ICT adults	CL	ND	ND	0/2	ND	2/2	ND	2/2
2 AIDS adults	1 CL, 1 PKDL	0/2	0/2	0/2	0/2	2/2	ND	2/2

Post-therapeutic follow-up was performed by PCR on peripheral blood for patients with VL (119 samples for ICT patients and 187 for AIDS patients) and allowed detection of all cases of relapse (1 ICT children and 13 AIDS patients). By means of PCR-RFLP analysis we identified *L. infantum* as the responsible species in all but 2 subjects which were infected with *L. donovani*. Molecular identification was confirmed by isoenzyme analysis in all cases.
Conclusions: PCR is at least as sensitive as traditional methods in the primary diagnosis of VL and CL. Post-therapeutic monitoring by PCR in peripheral blood is a reliable method to detect clinical disease relapse and allows precise and noninvasive follow-up of patients.

Nosocomial infections

O33 Risk factors for nosocomial infections: results from the Lombardy prevalence study

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Objective: To describe site-specific risk factors for nosocomial infections (NI) using data from a prevalence survey of NI in Lombardy.
Methods: A descriptive study by one-day point prevalence survey. The survey involved 88 out of the 113 public hospitals of Lombardy, voluntarily participating. All adult patients (>15 years) admitted in the 24 h preceding the survey in any department, excluding psychiatric ones, were investigated. The survey was conducted by local teams trained by Prevention Unit of Regione Lombardia. NI infections were defined according to CDC criteria. Data analysis was performed on separate models for the four main types of NI: primary bloodstream infection (PBI), pneumonia, urinary tract infection (UTI) and surgical wound infection (SWI).
Results: In multivariate analysis, vascular catheter (OR: 6.3), immunocompromission (OR: 3.5), alterations of consciousness (2.6), parenteral feeding (2.6) emerged as independent clinical predictors of nosocomial PBI. Risk factors significantly associated with nosocomial pneumonia were: age (OR: 1.01), male sex (OR: 1.6), previous admission (OR: 1.6), chronic respiratory disease (OR: 2.3), immunocompromission (OR: 1.8), alterations of consciousness (OR: 4.2), mechanical ventilation (OR: 5.1), parenteral feeding (OR: 3.4), surgical procedure (OR: 1.5). For nosocomial UTI, age (OR: 1.01), previous admission (OR: 2.8), diabetes (OR: 2.8), chronic renal failure (OR: 2.8), trauma, alterations of consciousness (OR: 2.8), urinary catheter (OR: 2.8) were found to be significant risk factors in both univariate and multivariate analysis. Only two factors significantly increased, in the multivariate analysis, the risk for SWI: parenteral feeding (OR: 3.2) and CDC classification of surgical procedures. Time-span from the occurrence of infection to surveillance day was not longer in infected patients than in noninfected ($P < 0.005$).
Conclusions: This study provides useful data to identify conditions or patients groups at increased risk of acquiring nosocomial infections, which might be target for NI control measures. Logistic regression allowed to determine that most of the factors influencing the occurrence of NI were those modifiable factors associated with treatment received by the patients during the stay in hospital. Many NI are avoidable if suitable precautions are taken when treating patients who must undergo instrumental diagnosis or treatment.

O34 Risk factors for nosocomial *Stenotrophomonas maltophilia* acquisition: does carbapenem treatment make a difference?

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Objectives: To conduct a prospective study of risk factors for acquisition of the emerging nosocomial pathogen *Stenotrophomonas maltophilia* (*Sm*): in particular, to investigate the relative importance of antecedent therapy with carbapenems, to which *Sm* is inherently resistant.
Methods: Patients admitted to an adult hematology unit over a one year period were enrolled into this prospective controlled study. Specimens were obtained on admission and twice weekly thereafter until discharge. These included feces, sputum, urine, blood, and swabs from vascular catheters. Specimens were plated onto a selective/differential agar and putative isolates identified using the API 20NE system. Concomitantly, epidemiological data, including parameters previously suggested as risk factors for acquisition of *Sm* were collected. These included; indwelling vascular catheters, previous hospitalization and antibiotic therapy, Neutrogena, duration of hospitalization and of antimicrobial therapy, in particular carbapenems. Data from *Sm*-positive patients were compared with *Sm*-negative controls using univariate analysis. Those variables found to be significant ($P < 0.05$) were analyzed using forward stepwise logistic regression analysis. Environmental samples were taken from handwash basins, cleaning items, baths, lavatories, etc., throughout the study. Isolates were typed using ERIC-PCR and CHEF-PFGE and profiles analyzed using Bionumerics software.
Results: *Sm* was isolated from 7/71 patients. 280/693 environmental sites yielded *Sm*. Univariate analysis indicated 10 variables associated with *Sm* acquisition. Significantly more *Sm*-positive patients (57%) had mucositis compared with controls (19%). In addition, 86% of *Sm*-positive patients

received vancomycin and aztreonam therapy compared with controls (45 and 44%, respectively). Logistic regression analysis indicated that the flucloxacillin therapy was significantly associated with *Sm* acquisition. 4/7 *Sm*-positive patients and 20/64 *Sm*-negative patients received meropenem during the hospital admission in which *Sm* was cultured: this was not statistically significant.
Conclusions: There have been conflicting reports from retrospective studies as to whether carbapenem therapy is a risk factor for *Sm* acquisition. Although the number of patients examined was small, our prospective study indicates no statistically significant association between antecedent or contemporaneous carbapenem therapy and *Sm* acquisition.

O35 Trends in micro-organisms isolated in bloodstream infections after the implementation of an infection control program

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Introduction: The continuous surveillance of bloodstream infections is important to detect the etiologic agents in an Institution, in order to establish protocols for empiric therapy and to improve infection control strategy.
Objective: Trend evaluation of clinically significant isolates from blood cultures over a 3-year, 6-month period.
Setting: SAMS Hospital, a Private Community Acute-Care Hospital with 121 beds in Lisbon.
Methods: Retrospective analysis of 3507 blood cultures performed in Bactec 9050 (Becton Dickinson, USA) during the study period (1 November 1997 through 30 April 2001).
Results: Of the 3507 blood cultures performed, 395 (11.2%) were positive corresponding to 185 bloodstream infections (10.9% of the 1691 hypotheses of bloodstream infection) and 186 micro-organisms were detected. The leading pathogens were coagulase-negative staphylococci, *Staphylococcus aureus*, enteric Gram-negative rods, in particular *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Since 1997, a MRSA endemic problem had been observed in SAMS Hospital. An intensive infection control program was initiated in the end of 1998 to prevent and control these nosocomial infections. We observed that the Gram-positive bacteria decreased in relative frequency (77% in 1997 to 50% in 2000), the Gram-negative increased significantly (23% in 1997 to 50% in 2001) and yeasts did not have significant alteration in their trend. MRSA nosocomial bloodstream infections decreased substantially with no single case registered until November 2001. *Escherichia coli* isolates increased in 2000.
Conclusions: The control measures adopted in order to control the MRSA endemic problem, were the major factor for the decrease in Gram-positive. The increase in Gram-negative, specially *Escherichia coli* isolates (genitourinary tract source), was probably due to guideline implementation for blood culture collection in upper urinary tract infections, at hospital entrance.

O36 Clinical relevance of the three major *Klebsiella pneumoniae* phylogenetic subgroups: unequal distribution in clinical sources and different levels of antimicrobial resistance of blood isolates

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Objectives: Based on the nucleotide sequences of genes *gyrA* and *parC*, three *Klebsiella pneumoniae* and two *K. oxytoca* phylogenetic subgroups were previously identified (Brisse and Verhoef, 2001, Int. J. Syst. Evol. Microbiol. 51: 915–921). These subgroups are separated by considerable evolutionary distance (e.g. higher than the distance among *Escherichia coli* subgroups A, B1, B2 and D) and each contains extensive strain diversity. The aim of the present study was to determine the distribution of the *K. pneumoniae* subgroups (KpI, KpII and KpIII) in clinical samples.
Methods: Clinical isolates of *K. pneumoniae* were collected from hospitals of 13 European countries during the years 1997–2001. Only one isolate per patient was included. 141 blood isolates, 35 urinary tract infection (UTI) isolates, 45 respiratory tract infection (RTI) isolates and 19 wound isolates were included. Identification at the *Klebsiella* genus level was performed using the VITEK apparatus (BioMerieux) and conventional biochemical tests. Species identification and phylogenetic subgroup assignment of the isolates was achieved by characterization of the *gyrA* gene. MICs of 16 antimicrobials were determined by the microdilution method (NCCLS guidelines).
Results: Out of 201 randomly selected strains, 180 (90%) belonged to subgroup KpI, 12 (6%) to KpII, and 9 (4%) to KpIII. Based on a negative adonitol fermentation test, 39 additional KpII (5 strains) and KpIII (34 strains) were added to the study. The percentages of subgroups KpI, KpII and KpIII in the total sample of 240 isolates were, respectively, 69, 8.5 and 23% in blood isolates; 94, 6 and 0% in UTI isolates; 78, 4 and 18% in RTI isolates, and 79, 5 and 16% in wound isolates. The absence of KpIII subgroup in UTI was striking. Each subgroup was geographically widely distributed across Europe. Antimicrobial resistance of the three subgroups was compared for 98 blood isolates. The frequency of resistant isolates was generally decreasing (for most agents) from subgroup KpI to KpIII. For example, 34% of 61 strains of subgroup KpI, 23% of 14 KpII strains, and only 6% of 23 strains of KpIII were resistant to ceftazidime, the hallmark of ESBL production.
Conclusion: These results indicate important clinical, epidemiological and/or ecological differences between the three major phylogenetic subgroups of this genetically heterogeneous species known as *K. pneumoniae*.

O37 Surveillance of antibiotic-resistance of staphylococci in a large teaching hospital in Rome

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Objective: To control the resistance of strains of staphylococci consecutively isolated from clinical specimens at the University Hospital "Policlinico Umberto I" of Rome from January 2000 until June 2001.
Methods: Strains were considered only if representative of a single infective episode occurring in various body sites: respiratory tract, urinary tract, blood and central venous catheters, cutaneous wounds, vaginal tract, pus and others. Identification and susceptibility tests were performed by automated method (Vitek2, Bio-Merieux). Oxacillin and vancomycin sensitivity was screened by commercially available agar and resistance confirmed by broth dilution method according to NCCLS guidelines. All the results were repeatedly validated using *S. aureus* 29213ATCC as quality control strain.
Results: A total of 826 strains were considered. *S. aureus* was the most frequently isolated strain (42.98%), whereas *S. epidermidis* (31.84%), and *S. haemolyticus* (10.77%) were the most representative among CoNS. Oxacillin resistance ranged from 55% of *S. aureus* to 71 and 72% of *S. epidermidis* and *S. haemolyticus*, respectively. Most of the staphylococci showed resistance to penicillin (from 84% of *S. haemolyticus* to 100% of *S. warneri*). No resistance or intermediate resistance to vancomycin was observed. Some strains of *S. haemolyticus* (6.7%) showed reduced sensitivity to teicoplanin (MIC90 = 16 mg/L) and up to 19% were completely resistant to the drug (MIC90 > 16 mg/L). All the teicoplanin-resistant *S. haemolyticus* were isolated from blood stream infections mostly occurring in the I.C.U. Further to vancomycin, netilmicin was the most effective drug since resistance was limited to 25% of *S. aureus* and 19% of *S. epidermidis*.
Conclusions: Data of the present study evidenced high frequency of isolation of oxacillin-resistant staphylococci. Furthermore, the appearance of relatively high frequency of isolation of teicoplanin-resistant *S. haemolyticus* was observed. As compared to our previous investigation performed during 1997–1998, the results of this study showed decreased oxacillin-resistance of *S. aureus* during the observation period and, simultaneously, increased oxacillin-resistance of CoNS. Finally, our results showed that all the isolates were sensitive to vancomycin.

O38 Determining the significance of coagulase-negative staphylococci isolated from paired blood cultures of neonates by species identification and strain clonality

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Object: Coagulase-negative staphylococci (CONS) are the most common pathogens isolated from neonates with nosocomial infections. However, discriminating true infection from contamination is usually a challenge to the physician. One criterion is the isolation from sequential blood cultures or from separate sites, which is based on the assumption that the isolates are the same. Using reference methods to obtain species identification and molecular methods to perform strain genotype, we sought to

understand better and possibly to improve the interpretation of CONS blood isolates.
Methods: During July 1999 and November 2000, infants hospitalized in NICUs at Chang Gung Children's Hospital with clinically suspected nosocomial sepsis were included in this study and two blood culture sets were obtained simultaneously from different peripheral sites. Both blood culture sets positive for CONS were further analyzed for species identification with ID 32-Staph gallery and strain clonality with two genotyping methods (infrequent restriction site polymerase chain reaction and pulse field gel electrophoresis).
Results: Two blood culture sets were obtained in 142 episodes. No bacterial organism was isolated from either sample in 73 episodes (51%). One bacteria was isolated from one of the two blood cultures in 14 episodes (10%) and CONS encountered in seven episodes. Two different bacterial organisms isolated from either sample were noted in three episodes (2.1%) and CONS were involved in each episode. Bacterial organisms isolated from both blood cultures were same in 52 episodes (37%), among which CONS were isolated in 26 episodes. Only 13 pairs of CONS strains were available for assessment. A consistent CONS species was noted in all 13 pairs. (*S. epidermidis* 9; *S. hominis* 3; *S. intermedius* 1) Both genotyping results were concordant and a consistent genotype was also noted in all 13 pairs. However, eight genotypes from nine *S. epidemidis* strains and two genotypes from three *S. hominis* strains were identified, respectively.
Conclusion: CONS were not only the most common pathogens of nosocomial bacteremia in NICU but also the most common blood culture contaminants. CONS isolated from two blood cultures obtained simultaneously from separate sites in infants at NICU usually represent true infection.

O39 Impact and changes in Enterococcal bloodstream infections in a teaching hospital: a 5-year study

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Objective: To assess the impact of enterococcal bloodstream infections (BSI) in a teaching hospital, and identify trends in patient morbibity and antibiotic susceptibility over time.
Methods: All patients with enterococcal BSI with bacteriology collected from 1996 to 2000 were studied and compared for morbidity and microbial susceptibility.
Results: A total of 115 cases were studied. The number of cases per year was stable over time. Most cases were *Enterococcus faecalis* (79), the rest were *E. faecium* (31) and *E. avium* (5). Most infections were nosocomial (65.2%), those with *E. faecium* significantly more so than with the other two species (90.3% vs. 56%, $P < 0.0006$). The fraction of cases by vancomycin-resistant enterococci (VRE) dropped (32–20.8%), but not significantly. Patients with *E. faecium* BSI (vs. *E. faecalis*) were significantly more likely to have a VRE (71% vs. 11.4%, $P < 0.001$, OR 19), had longer hospitalizations (51.8 vs. 25.4 days, $P < 0.001$), used the ICU more often (58.1% vs. 34.2%, $P = 0.04$), and had a higher mortality (71% vs. 20%, $P = 0.004$). Patients with VRE BSI (vs. non-VRE) had significantly longer hospitalizations (48.1 vs. 27.7 days, $P = 0.004$), and a higher mortality (65.5% vs. 38.6%, $P = 0.02$). Length of stay in these patients dropped (56–35 days) and mortality increased (62.5 to 80%) over time, but not significantly. *E. faecium*'s resistance to vancomycin, penicillin, gentamicin, and ciprofloxacin increased, but not significantly. *E. faecalis* showed a significant drop in gentamicin resistance (61.1% to 6.3%, $P = 0.0002$) and a trend to decreased vancomycin and increased penicillin resistance.
Conclusion: The rate of VRE and non-VRE BSI at this teaching institution has remained stable in the last 5 years. *E. faecium* BSI were overwhelmingly nosocomial, VRE, and trending to increased antibiotic resistance. *E. faecium* and VRE BSI were associated with a worse prognosis, unchanged over time. *E. faecalis* was found to be increasingly gentamicin sensitive. It is unclear, whether newer antibiotics can change the impact of enterococcal BSI.

O40 Gastroenteritis outbreak in a university hospital due to Norwalk-like virus

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Norwalk-like viruses (NLVs) are the most common cause of outbreaks of nonbacterial gastroenteritis worldwide. These outbreaks are frequently diagnosed in semi-closed communities such as educational institutions and hospitals. Infection caused by NLVs is commonly manifested by a short and self-limiting diarrhea. However, severe disease may occur. We report an outbreak of acute gastroenteritis in a tertiary care center between 28 February and 20 March 2001.
Objectives: To describe the epidemiological features and infection control measures of the outbreak.
Methods: case definition: Rapid onset of vomiting, diarrhea without fever, and spontaneous rapid recovery within 48 h. NLVs were detected by use of reverse transcriptase PCR targeting a 334-bp-long conserved fragment of the RNA polymerase gene.
Results: Sixty-two patients met the case definition. 27 (42%) were patients, 36 (58%) healthcare workers. Microbiological diagnosis was made within 3 days after the onset of the first case. Twelve (38%) out of 32 fecal specimens examined were positive for NLV. Nine of these 12 specimens had a 100% identical nucleotide sequence of about 300 bp. Phylogenetic analysis of this genotype indicated clustering within genogroup II near Bristol Virus (92% nucleotide similarity). The outbreak started in the Department of Dermatology and rapidly spread through the Department of Internal Medicine. Only dedicated healthcare workers were allowed to take care of case patients. Routine disinfection of the innate environment including floors was enforced. None of the applied techniques stopped the outbreak, but spreading to the Bone Marrow Transplant Unit was prevented. The most likely source was an infected patient who introduced SLV into the hospital.
Conclusions: Rapid identification can limit spread of SLV, but the impact of applying published guidelines appears limited. To our knowledge, this is the first reported, microbiologically documented outbreak of acute gastroenteritis in a large hospital associated with NLVs in Switzerland.

Antibiotic resistance of anaerobic bacteria (Organized by the European Working Party on Antimicrobial Resistance in Anaerobic Bacteria (EWPARAB))

S76 Epidemiology of resistance in *Bacteroides fragilis* and related species

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Bacteroides fragilis and related species of the genus *Bacteroides* are the most prevalent pathogens involved in human diseases. *Bacteroides* are the predominant organisms in the fecal flora as well, making up nearly 30% of the indigenous microbial population of the intestinal tract. It is considered an extremely stable community, however, many transient organisms passing through for short periods may give the opportunities for frequent genetic exchange. This has an important implication in the dissemination of antibiotic resistance genes. The phylogenetic position of *Bacteroides* together with the frequent opportunities for direct contact with diverse species is likely related to the wide variety of genetic elements (plasmids, transposons, IS elements) found in *Bacteroides* spp. and may explain the spread of resistance genes among them.
Besides chromosomally mediated β-lactamase production tetracyclin resistance is the most prevalent among *Bacteroides* strains worldwide. Conjugative transposons carry the *tetQ* gene which can be found with broad host range within *Bacteroides* and species of related genus. Clindamycin resistance in the *Bacteroides* is mediated by a macrolide–lincomycin–strepto-gramin (MLS) mechanism. The gene can be found in transposons on plasmids or on chromosal conjugative elements. The frequency of the spread of clindamycin resistance differs in different countries in Europe and worldwide. The occurrence and spread of resistance to imipenem and metronidazole among

Bacteroides strains merit special clinical importance. It has been shown by us and by other investigators that the presence of the *cfiA* gene is much more prevalent than the expression of the imipenem resistance. The spread of the *cfiA* gene among species other than *B. fragilis* is still very rare. A series of *cfiA* positive *B. fragilis* strains (some of them with high MICs for imipenem) were investigated in our laboratory for the presence of IS elements. Known and new IS elements can be involved as active promoters of the gene. Our recent results looking for the genetic background of metronidazole resistance among *Bacteroides* strains obtained from different parts of the world, indicate that well-described carriers, like *pIP417*, account for the majority of *nimA–D* genes, but *nimA*, *C*, *D*, *E* genes could also be chromosomally coded.
The epidemiology of antibiotic resistance of *Bacteroides* strains: this ubiquitous, host-associated organisms shows the need of parallel presence of three critical components required for the spread of antibiotic resistance: the presence of the antibiotic resistance gene, the ability to express the gene and the mechanism for widespread dissemination.

S78 Carbapenem resistance in *Bacteroides fragilis*: much mechanism, little resistance

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The species *Bacteroides fragilis* is divided into two DNA homology groups, I and II, which comprise, respectively, >95 and <5% of the *B. fragilis* strains isolated in medical practice. Group I strains typically contain the chromosome-borne *cepA* gene encoding a class A/group 2e β-lactamase (which does not confer carbapenem resistance), while the group II strains contain the *cfiA* gene encoding a carbapenemase of class B/group 3. Overall carbapenem resistance rates in *B. fragilis* vary, according to most studies, between <1 and 2% and only exceptionally exceed 5%. There are currently no data indicative of a trend toward increase.
Expression of carbapenem resistance (MIC of imipenem = or >16 mg/L) requires activation of the otherwise silent *cfiA* gene. This activation is brought about by mobile promoters carried by insertion sequence (IS) elements which target a ca. 100 bp stretch immediately upstream of the *cfiA* gene. Five such IS elements (and isoforms thereof) are currently known, IS942, IS1186, IS1187, IS1188 and IS4351. They occur preferentially, but not exclusively, in the *cfiA*-positive group. The transcriptional start sites of *cfiA* on the IS elements have been mapped. All were found downstream from a promoter region typical of *B. fragilis* as previously characterized by Bayley *et al.* (FEMS Microbiol. Lett. 2000; 193: 149–154) and different from that recognized by the principal sigma factor of Gram-negative bacteria. The relative strengths of the individual IS-borne promoters vary by a factor of ca. 5 in *B. fragilis* and are fairly well correlated with the carbapenem resistance levels. Preliminary data indicate that they are functional also in *Porphyromonas distasonis* but not in *Escherichia coli* or *Pseudomonas aeruginosa*.

Fluoroquinolones

O88 Effects of fluoroquinolones on the development of fluoroquinolone-resistant *Salmonella*

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Objectives: (1) To study the in vitro development of fluoroquinolone-resistant (FqR) *Salmonella enterica* serotype Typhimurium. (2) To detect the presence of mutations in the topoisomerase gene of FqR mutants.
Methods: Thirty clinical isolates and one standard strain (ATCC13311) of *S.* Typhimurium were subjected to daily passage in Mueller–Hinton broth containing increasing serial two-fold dilutions starting at 0.5× MICs and up to 128 mg/L of pefloxacin, norfloxacin, ofloxacin, ciprofloxacin, levofloxacin, gemifloxacin and moxifloxacin. The overnight cultures were tested for their susceptibility to the NCCLS recommended sensitive breakpoint concentrations of these fluoroquinolones (Fqs) (except gemifloxacin) by the NCCLS agar dilution method. Mutations in the topoisomerase gene *gyrA* were detected by amplifying DNA from the tested strains using primers specific for the quinolone resistance-determining region of the gene followed by single-strand conformational polymorphism analysis and DNA sequencing.
Results: The tested strains were sensitive to all the Fqs tested (MIC range: 0.03–1 mg/L). Ciprofloxacin and levofloxacin were most efficient in selecting resistance (resistance developed at the lowest concentration range of 0.5–4 mg/L), and pefloxacin the least efficient (4–8 mg/L). However, pefloxacin was the first to which isolates became resistant regardless of the Fq used in the selection procedure while levofloxacin was the last to which isolates became resistant when a Fq other than itself was used in the selection procedure. Although gemifloxacin and moxifloxacin were quite efficient in selecting resistance, eradication of strains could be observed at the lowest concentration (0.12–0.25 mg/L) in contrast to 8–16 mg/L for the other Fqs. Two mutations in *gyrA* that were present in clinical isolates were detected in resistant mutants: TCC → TTC resulting in Ser83 → Phe and GAC → GGC resulting in Asp87 → Gly.
Conclusions: (1) Resistance to pefloxacin developed most rapidly regardless of the Fq used for selection. (2) Levofloxacin and ciprofloxacin were most effective in selecting for resistance to Fqs. (3) Gemifloxacin and moxifloxacin were most efficient in eradicating strains. (4) Mutations in *gyrA* identical to those found in clinical isolates were detected in resistant mutants. (5) The selection potentials of Fqs besides their activities are important factors in the choice of therapy.

O89 In vitro killing effect of moxifloxacin on multidrug-resistant *Stenotrophomonas maltophilia*

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Objective: The limited number of antimicrobial agents active on nosocomial *Stenotrophomonas maltophilia* led to the study of the in vitro time-kill effect of the novel methoxyfluoroquinolone moxifloxacin on that species.
Methods: Twenty isolates from different patients with nosocomial infections resistant to ampicilli/sulbactam, cotrimoxazole and ciprofloxacin and susceptible to moxifloxacin (MIC < 1 μg/mL) were applied. They were exposed over time to concentrations equal to 1 and 4× MIC. Moxifloxacin was added with a 6 log-phase inoculum of each isolate in Mueller–Hinton broth and bacterial growth was determined at standard time-intervals during incubation at 37 °C. Killing effect was determined as any equal or more than a 3 log 10 decrease of viable cell counts from the baseline.
Results: Bactericidal activity of 1× MIC of moxifloxacin was found in four (20%), eight (40%) and five (25%) isolates after 4, 6 and 24 h of growth, respectively, and of 4× MIC of moxifloxacin in seven (35%), nine (45%), 14 (70%) and 12 (60%) isolates after 2, 4, 6 and 24 h of growth, respectively. Mean (±SD) decreases of viable cells were 0.85 ± 0.24, 1.89 ± 0.38, 2.39 ± 0.41 and 0.73 ± 0.47 after 2, 4, 6 and 24 h of growth, respectively, in the presence of 1× MIC and 1.35 ± 0.49, 1.58 ± 0.52, 2.84 ± 0.79 and 1.03 ± 0.82 after 2, 4, 6 and 24 h of growth, respectively, in the presence of 4× MIC.
Conclusions: Based on its early time-kill effect on *S. maltophilia*, moxifloxacin might be considered as a promising alternative for the therapeutic management of nosocomial infections by that species.

O90 Intracellular accumulation and bactericidal activity of moxifloxacin and ciprofloxacin in primary respiratory epithelial cells

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Due to their chemical structures, fluoroquinolones may penetrate and accumulate in human cells, thereby increasing their potential to protect man against bacterial pathogens. Therefore, we investigated the intracellular

concentration of moxifloxacin and ciprofloxacin in human primary respiratory epithelial cells (vesicles). Additionally, we determined the antimicrobial activity of moxifloxacin against *Staphylococcus aureus* and *S. pneumoniae* and ciprofloxacin against *Haemophilus influenzae* and *Pseudomonas aeruginosa*. Vesicles were incubated for 30 min with 5 μg/mL moxifloxacin or ciprofloxacin. Then, washed or unwashed vesicles were incubated with 107 cfu/mL of the various pathogens for 5–18 h. Bacterial viability was determined by plate counting and survival of vesicles using light and electron microscopy. Intracellular fluoroquinolone concentrations were determined using HPLC. Intracellularly, 51.1 μg/mL moxifloxacin and 36.9 μg/mL ciprofloxacin were found after a 30-min incubation in washed cells, corresponding to a ~10- and 7-fold drug accumulation, respectively. In unwashed cell cultures, 100% of all pathogens were killed by the fluoroquinolones. In washed cells, the intracellular drug concentrations led to the killing of 85% *S. aureus*, 95% *S. pneumoniae*, 100% *H. influenzae*, and 91% *P. aeruginosa*. Conversely, after bacterial challenge, 48–94% of unwashed vesicles and 42–76% of washed vesicles preincubated with drugs were rescued, compared to the survival of 0–38% of vesicles without drugs. The intracellular accumulation of fluoroquinolones in respiratory epithelial cells and the high activity against sensitive bacterial pathogens provide an excellent rational for their use in human respiratory tract infections.

O91 Anti-anaerobic activity of BMS 284756 (T-3811) a new des-F(6)quinolone antibiotic compared with those of moxifloxacin, levofloxacin, ciprofloxacin, metronidazole and three β-lactams

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Objectives: MICs were determined on BMS 284756 (BMS) moxifloxacin (MOX), levofloxacin (LEV), ciprofloxacin (CIP), coamoxyclav (AMC), piperacillin-tazobactam (PTZ), imipenem (IMI), clindamycin (CLN) and metronidazole (MOL) against 330 anaerobes isolated from human clinical samples.
Methods: Reference agar dilution (standard M11 A5, NCCLS).
Results: BMS, MOX, LEV and CIP, inhibited at 4 mg/L, respectively, 100, 97, 80 and 47% of the *Bacteroides fragilis* group strains whereas resistance rates were, respectively: AMC 5.4%, PTZ and IMI 0.8%, CLN 30%, MOL 0.8%. Considering the three fluoroquinolones,the $MIC_{50/90}$ (mg/L) were:

Microogranisms (*N*)	BMS	MOX	LEV	CIP
B. fragilis group (129)	0.25/1	0.5/2	2/8	8/64
Other Gram-negative bacilli (74)	0.125/0.5	0.125/2	0.5/4	1/8
Clostridia (34)	0.125/1	0.25/2	0.5/16	0.5/32
Non-sporulated Gram+ rods (27)	0.25/1	0.25/1	0.25/2	0.5/4
Peptostreptococcus spp. (66)	0.125/1	0.25/1	1/4	1/4
All Gram+ (127)	0.125/1	0.25/2	0.5/4	1/16
All anaerobes (330)	0.125/1	0.25/2	1/8	2/32

BMS 284756 and MOX unlike other fluoroquinolones demonstrated high activity against the *B. fragilis* group and clostridia but BMS 284756 was the more potent fluoroquinolone. Only one strain of *B. fragilis* was resistant to AMC,PTZ, IMI. Another strain of *B. fragilis* was resistant to metronidazole (MIC = 64 mg/L). BMS 284756 was also more potent than LEV and CIP against gram positive rods and anaerobic cocci. At concentration of 1 mg/L, BMS 284756 inhibited all strains of *Fusobacterium*, *Porphyromonas*, *Propionibacterium* and Gram-positive cocci. At concentration of 2 mg/L, BMS 284756 inhibited all Gram-positive anaerobes (127 strains) and 199 out of 203 Gram-negative anaerobes. Overall 330 anaerobes, BMS 284756 inhibited 95, 99 and 100% of the strains investigated at concentrations of 1, 2, and 4 mg/L, respectively. Comparatively, at the same concentrations, moxifloxacin inhibited 73, 93 and 97.5% of the whole anaerobes. Overall clindamycin resistance rate was 18.5%.

Conclusions: The broad anaerobic spectrum of BMS 284756 demonstrated in vitro is very promising to treat anaerobic or mixed infections; further clinical evaluation is needed.

O92 In vitro assessment of alternatives to penicillin in the treatment of invasive pneumococcal disease

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Objective: The prevalence of penicillin-resistant pneumococci from blood and cerebrospinal fluid in Ireland is high as previously demonstrated by data from the European Antimicrobial Resistance Surveillance System (EARSS). We assessed the in vitro activity of two recently launched antibiotics that might be useful in alternatives treatment.
Methods: Minimum inhibitory concentrations (MIC) to benzylpenicillin, cefotaxime, moxifloxacin and linezolid using E-test strips were carried out on all pneumococcal isolates isolated from Irish laboratories as part of the EARSS study, i.e. blood and cerebrospinal fluid. The participating laboratories in EARSS in Ireland cover about 80–90% of the population.
Results: A total of 317 isolates were received during 1999 and 2000 of which 16% were fully resistant (MIC * 2 mg/mL) or less susceptible to penicillin (MIC * 0.1 mg/mL). The MIC_{90} for cefotaxime, moxifloxacin and linezolid were 0.08, 0.064 and 0.25 mg/mL, respectively. For the 51 isolates that were moderately susceptible or fully resistant to penicillin, the MIC_{90} to the three agents were 0.4, 0.05 and 0.30 mg/mL, respectively.
Conclusion: Whilst the prevalence of pneumococcal isolates resistant to penicillin is quite high in Ireland, all isolates to date are sensitive to cefotaxime, moxifloxacin and linezolid. Therefore, as well as cefotaxime, moxifloxacin and linezolid are potential options for the treatment of invasive infection.

O93 Levofloxacin-resistant *Streptococcus pneumoniae* continue to exhibit cross-resistance to other fluoroquinolones

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Objectives: Antimicrobial resistance among *S. pneumoniae* (SP) is a worldwide concern. As fluoroquinolones are more frequently prescribed for respiratory tract infections, concerns arise regarding the prevalence of fluoroquinolone-resistant mutants and the effects of mutations conferring resistance to one fluoroquinolone on the activities of other fluoroquinolones. We examined 40 levofloxacin (LEV)-resistant (R) SP collected during a 1999–2000 surveillance study for concurrent resistance to other fluoroquinolones, including gatifloxacin (GAT) and moxifloxacin (MXF).
Methods: During 1999–2000, 5015 SP were collected from 13 countries in Europe, Asia, and South America. Isolates were submitted to our central laboratory (Focus Technologies, Herndon, Virginia) and tested by NCCLS broth microdilution against levofloxacin and comparator agents. 40 LEV-R SP were identified and tested on a second broth microdilution panel against LEV, ciprofloxacin (CIP), GAT, MXF, norfloxacin (NOR), and trovafloxacin (TRO). In addition, the quinolone-resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* of these isolates were sequenced.
Results: Overall, 0.8% of the isolates collected were LEV-R. The MIC_{90} of LEV was 1 mg/L in all countries, irrespective of the prevalence of LEV resistance. All the 40 LEV-R SP had GyrA (Ser81– or Glu85–) alterations and 37 had ParC (Ser79Phe, Asp83Asn, or Lys137Asn) alterations. Two isolates had a LEV MIC of 32 mg/L and possessed GyrA (Glu85Lys) and ParC (Ser79Phe/Tyr) alterations. Among the 40 LEV-R SP tested, all were nonsusceptible to GAT (10 intermediate, MIC = 2 mg/L; 30 resistant, MIC ≥ 4 mg/L), 39 were nonsusceptible to MXF (31 intermediate, MIC = 2 mg/L; 8 resistant, MIC ≥ 4 mg/L), 38 had TRO MICs ≥ 2 mg/L, and 39 had CIP MICs ≥ 8 mg/L. No isolates had a NOR MIC < 16 mg/L.
Conclusions: The LEV resistance remained rare in a 1999–2000 13-country surveillance study with a prevalence of <1% overall. Irrespective of the QRDR sequences, when LEV resistance was detected, most isolates were also nonsusceptible to other marketed fluoroquinolones.

O94 Comparative in vitro activity of BMS-284756, a novel Des-fluoro (6) quinolone, and other antichlamydial agents against respiratory, vascular, and cerebral strains of *Chlamydia pneumoniae*

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Objective: The obligate intracellular bacterium *Chlamydia pneumoniae* is a frequent cause of respiratory infections and can be cultured from atherosclerotic vascular lesions. Less than 60 strains of this frequent but fastidious pathogen are available worldwide, and data on their susceptibility profiles are scanty. BMS-284756 is a novel Des-fluoro (6) quinolone proposed to be efficient against a broad range of fastidious bacteria. Thus, we evaluated its in vitro efficiency to inhibit growth of 20 *C. pneumoniae* strains in comparison to established antichlamydial agents using a recently proposed system of standardized MIC determination for Chlamydiae. This system monitors activity against actively replicating chlamydiae and is an in vitro model for drug efficiency in acute chlamydial infection.
Methods: Standardized testing of *C. pneumoniae* MICs was done using HEp-2 cell monolayers, serum-free conditions, and a seed of 500 inclusion forming units per well. Inclusion formation was monitored by immunofluorescence microscopy. MICs were determined for 20 *C. pneumoniae* strains from the US and Europe (nine respiratory isolates, 10 vascular isolates, and one from cerebrospinal fluid) in parallel for BMS-284756, moxifloxacin, levofloxacin, azithromycin, and doxycycline.
Results: MIC90s were: BMS-284756: 0.0025 mg/L; moxifloxacin: 0.05 mg/L; levofloxacin: 0.4 mg/L; azithromycin: 0.08 mg/L; doxycycline: 0.1 mg/L. Thus, on a weight base, BMS-284756 was the most efficient substance to eliminate chlamydial growth in cell cultures. The origin of the isolates had no impact on their susceptibility profiles as respiratory, vascular, and cerebral strains showed nearly identical patterns.
Conclusion: Actively replicating *C. pneumoniae* can be eliminated with considerable efficiency by a variety of drugs. BMS-284756 was the most effective substance overall when compared to moxifloxacin, levofloxacin, azithromycin, and doxycycline. Resistance was not observed. All *C. pneumoniae* strains tested so far had comparable susceptibility patterns. An animal model and clinical studies are needed to establish the in vivo activity of BMS-284756 against the chlamydial infections.

Infections in the immunocompromised host

O95 Viral dynamics in cytomegalovirus infection in solid organ transplantation

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Objective: To study the dynamics of CMV replication in patients undergoing solid organ transplantation.
Methods: CMV viral load was studied in 25 patients (median follow-up 82 days) on anti-CMV therapy (i.v. GCV, 10 mg/kg/day for 21 days) and in eight patients with CMV reactivation not requiring GCV (median follow-up 89 days). Viral load was measured in sequential blood leukocytes by a quantitative polymerase chain reaction (PCR, Cobas Amplicor CMV Monitor, Roche), lowest detection limit 100 genome per 106 cells. Samples were taken from patients on GCV at day +4, +7, +12, +14, +18, +20, +21 and after GCV was dismissed (day 4, 7, 14 and 21). Two hundred and sixty-four samples were analyzed.
Results: In patients on GCV baseline viral load was 4.37 log genome per 106 cells, significantly higher than the one in patients not on GCV (3.1 log, $P < 0.0001$). By day 21, GCV treated patients showed a 1.86 log reduction of pretreatment level corresponding to 83% of baseline load. CMV-DNA cleared by 1.5 log during the first 13 days of therapy and 0.3 log by day 21. Being symptomless, the patients' therapy was discontinued (the mean end-of-treatment viral level: 3.01 logs). At days 4, 7, 14 and 21 after GCV was dismissed, CMV level ranged from 2.51 to 3.15 logs in 21/25 patients. Four patients (16%) experienced a CMV relapse 4–34 days after GCV treatment. Viral load picked to 4.72 logs. A second 21-day GCV course was administered at the end of which the CMV-DNA was below the PCR detection limit. The relapse was not related either to the pre-transplantation CMV serum status (all the four recipients were CMV seropositive before transplantation) or to changes in immunosuppressive drug regimen.
Conclusions: Our findings show that CMV replication and clearance are dynamic processes whose knowledge during the GCV therapy may have important implications for the efficacy of antiviral regimen and controlling CMV. In transplanted patients who are at high risk of CMV infection, viral clearance can be a slow process in which dosage and duration of the antiviral treatment as well as the availability of sensitive methods to detect CMV reactivation are important to prevent CMV disease.

O96 Cytomegalovirus (CMV) infection in kidney recipients: are CMV genotypes and other herpes viruses (HHV-6, 7, 8) associated with clinical manifestations?

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Objectives: CMV is one of the most important major causes of clinical manifestations in kidney transplant patients. Symptoms may vary according to the genotypes of the virus or co-infection with other herpes viruses. This study aimed at investigating: (a) the possible association of CMV *gB* genotypes with clinical manifestations, and (b) the role of other herpes viruses in the outcome of CMV infection.
Methods: In the present study 42 symptomatic and 87 asymptomatic kidney transplant patients were involved. For diagnosing a CMV infection/disease, the antigenemia assay (detecting the pp65 antigen in leukocytes) was used. Genotypes of CMV were identified by the combination of a PCR–RFLP assay. Genomes of HHV-6, 7 and 8 were detected by nested PCR tests.
Results: The four CMV *gB* genotypes were determined in symptomatic ($n = 28$) and asymptomatic patients. There was no association between any particular genotype and the clinical symptoms. DNA of HHV-6 could be detected at a relatively low incidence (7%), and there was no difference between symptomatic and asymptomatic patients. Distribution of HHV-7 DNA showed an interesting picture. While it could be detected in 37 of 87 (43%) asymptomatic patients, it was detectable only in 7 of 42 (17%) symptomatic patients. HHV-8 DNA was found only in 3 of 129 patients tested (2%).
Conclusions: The results suggest that there is no association between genotypes of CMV and disease manifestations. The pathogenic role of HHV-6 and HHV-7 remains unclear. The low prevalence of HHV-7 DNA in symptomatic patients may be explained be the impact of ganciclovir therapy. The importance of HHV-8 requires further studies in larger number of transplant patients.

O97 Cobas Amplicor CMV monitor compared to Antigenemia or rapid quantitative culture in CMV monitoring in solid organ transplant patients

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Objective: To evaluate Cobas Amplicor CMV Monitor (CACM) in comparison with tests used currently used in Swiss centers to monitor CMV in a preemptive approach in solid organ transplant (SOT) patients.
Methods: CMV viral load was monitored weekly after transplantation in 36 SOT recipients. CMV antigenemia (pp65 leukocyte assays, $n = 17$) or infectivity by the shell vial assay ($n = 19$) in PBL was used to monitor and guide preemptive treatment. Using stored plasma samples, we compared the kinetics of CMV viral load appearance in the blood by routine assays (RA) or CACM.
Results: Samples of 36 patients aged 23–72 (median = 51, 26 males, 10 females) with a documented CMV viremia/antigenemia (27 kidneys, 6 hearts, 3 livers) from four centers were analyzed. The patients had from 1 to 13 samples (median = 5) assayed before peak viral load or treatment initiation. The median (range) time from transplantation to detection of CMV in the

blood by RA was 35.5 days (7–74), but 39 days (7–67) to reach the thresholds for preemptive R_x, $P = 0.008$, Wilcoxon's signed-rank test when compared to RA detectable): the time to CACM detectable was 35 days (7–61, $P = 0.36$), CACM >1000 copies/mL: 39 days (9–61, $P = 0.42$), CACM > 5000 copies/mL: 43 days (17–67, $P = 0.03$). Eight patients did not require treatment, two were treated prophylactically, 14 preemptively and 12 therapeutically (after developing CMV disease). Of the latter, three had not been treated preemptively earlier despite having met a threshold for treatment by the RA. Using a threshold for preemptive action of 1000 copies/mL, CACM would have met this trigger point before RA using the usual thresholds in 12 patients, offering the potential to prevent the occurrence of CMV disease in four patients.

Conclusion: The CMV-DNA monitoring in the plasma using Cobas Amplicor offers a standardized, commercially available method with a sensitivity similar to currently used RA. In our experience, 1000 copies/mL plasma appears as a potential threshold for preemptive action corresponding to those defined with in-house assays. This is of interest for the future organization of multicenter studies with preemptive treatment arms.

Acknowledgement: Supported by Roche Diagnostics and Roche Pharma (Switzerland).

O98 Are CMV-seronegative donors a risk factor for CMV seropositive stem cell transplant recipients?

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Objectives: In this retrospective analysis, we evaluated the incidence of CMV reactivation, CMV disease and death from CMV infection in CMV seropositive allogenic stem cell transplant recipients depending on the CMV status of the donor.

Patients and methods: Sixty-two consecutive patients (male : female = 37 : 25) aged from 19 to 64 years (median: 42) were allografted using either bone marrow ($n = 26$) or peripheral blood progenitor cells ($n = 36$) from related ($n = 34$) or unrelated ($n = 28$) donors. The underlying hematological malignancies were: AML: $n = 24$, CML: $n = 15$, ALL: $n = 5$, NHL: $n = 13$, MM: $n = 3$, SAA: $n = 1$ and MDS: $n = 1$. Conventional dose conditioning was performed in 51 patients and reduced conditioning in 11 patients. Antithymocyte globulin (ATG) for GVHD prophylaxis was administered in all recipients of unrelated grafts and in 6/34 patients with related donors. The high-dose acyclovir was given for CMV prophylaxis, and PCR-based preemptive therapy was carried out using either ganciclovir, foscarnet or cidofovir. The following CMV donor/recipient status could be found: positive/positive: $n = 18$, negative/negative: $n = 19$, positive/negative: $n = 8$ and negative/positive: $n = 17$.

Results: At least two positive CMV-PCR results requiring preemptive therapy could be detected in 14 patients, 12 of them developed CMV disease (interstitial pneumonia: $n = 9$, encephalitis: $n = 1$, hepatitis: $n = 1$, gastrointestinal manifestation: $n = 1$). Seven patients died from CMV disease; they were all CMV-seropositive pre-transplant, and six of them had CMV-seronegative donors. Among the CMV-positive patients with negative donors 11/17 (64.7%) showed at least two positive PCR results, whereas in only 35.3% there was no evidence of CMV reactivation. Among the CMV-positive patients with positive donors in 3/18 (16.7%) at least two positive PCR results could be detected and the majority (83.3%) didn't show any sign of CMV reactivation.

Conclusion: Regarding the incidence of CMV reactivation, CMV disease and especially death from CMV disease the subgroup of seropositive patients with seronegative donors had the worst outcome. Therefore, the question arises, whether CMV-positive donors should be preferred for CMV-positive stem cell transplant recipients.

O99 One-year analysis of CMV-PCR surveillance data in heart transplant recipients: a safe strategy to prevent CMV disease?

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Description: Cytomegalovirus (CMV) infection can cause severe disease in transplant patients. Numerous protocols exist to prevent the (re-)occurrence of CMV-disease, but there is no consensus as to which of these strategies is optimal. In January 2000, we introduced quantitative CMV-PCR (QPCR) monitoring of CMV-IgG+ve and CMV- status mismatched patients (CMV-MM) post-transplantation (Tx). The QPCR was performed twice weekly on in-patients and weekly thereafter, or for 3 months post-therapy if the patient received ganciclovir (GCV). All patients received oral aciclovir for 3 months.

Purpose: (1) To establish whether QPCR results can be used as early predictors of CMV disease. (2) To review the utility of a cut-off value above which preemptive GCV therapy should be started. (3) To assess the necessity of QPCR surveillance of CMV-IgG+ve patients.

Methods: We conducted a retrospective survey of QPCR results of patients who underwent cardiac Tx between January 2000 and January 2001. Twenty patients (15 adults and 5 children) who survived 3 months were included.

Results: Four patients were CMV-MM: one patient remained QPCR–ve, while three became QPCR+ve, among which one was asymptomatic (QPCR load 10^5 copies/mL). Two patients became symptomatic (QPCR load 10^5–10^6 and 10^5–10^5 copies/mL) and both were treated with GCV. Of the 16 patients who were CMV-IgG+ve at the time of Tx, seven remained negative and nine became positive by QPCR. Two of these became symptomatic but did not require specific therapy. Among these, there was no obvious correlation between QPCR load and the development of symptoms.

Conclusions: (1) The number of CMV-MM patients was too small to permit any conclusion about the value of QPCR as an early predictor of disease. (2) It may not be worthwhile to monitor the asymptomatic CMV-IgG+ve patients by QPCR, but this area requires further study. QPCR should be included in the diagnostic work-up of symptomatic patients, where appropriate.

O100 Prevention of CMV disease: cost evaluation of two regimes in heart and lung transplantation

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Description: Optimal strategy for prevention of CMV disease following solid organ transplantation is unknown. Several studies have demonstrated the beneficial effects of prophylaxis with i.v. immunoglobulin, ganciclovir and valaciclovir, or preemptive treatment based on viral load measurements. Few studies have assessed the cost-effectiveness of different regimes. In January 2000, we introduced a CMV surveillance protocol based on weekly quantitative PCR viral load measurements in addition to selective ganciclovir prophylaxis to replace i.v. immunoglobulin for all CMV-mismatched patients and oral ganciclovir for CMV-IgG-positive lung transplant recipients. We compared the costs of the new protocol with the previous for patients transplanted between January 2000 and January 2001.

Methods: Patients records were reviewed and cost-calculations were made based on the two protocols.

Results: A total of 78 cardiopulmonary transplants (32 lungs, 43 hearts, 3 heart/lung) were performed at this center between the dates specified. Forty-one recipients fulfilled our surveillance criteria. The comparison of costs between the old and the new protocols are shown below:

Htx recipients: Old protocol US$ 8547; new protocol US$ 11567; difference: +US$ 3020.

Ltx recipients: Old protocol US$ 85930; new protocol US$ 41939; difference: –US$ 43991.

Conclusions: We can conclude from these results that the preemptive therapy strategy in heart transplant recipients is slightly more expensive than prophylaxis. However, in lung transplantation considerable savings can be made.

O101 BKV-associated nephropathy after kidney transplantation: a single-center analysis in a pediatric cohort

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Objectives: BKV reactivation is known as cause of ureteral stenosis in the renal transplant recipients and of hemorrhagic cystitis, particularly in bone marrow transplantation patients. Recurrent BKV reactivation occurs frequently in immunocompetent individuals resulting in asymptomatic viral urinary shedding. Recently, the human polyomavirus BK has been recognized as a possible cause of interstitial nephritis in kidney transplantation recipients, a complication which may lead to graft failure in as many as 45% of affected patients. Moreover, it has been even more recently observed that the human polyomavirus JC also could be involved in post transplant interstitial nephropathy.

We have performed a retrospective analysis on pediatric kidney-allograft recipients with the aim of evaluating the incidence and clinical relevance of BKV and or JCV infection under new immunosuppressive drugs.
Methods: Serum and urine samples from 100 pediatric kidney allograft recipients, referred to G. Gaslini Institute in the last 5 years, were analyzed by PCR assays for BKV-DNA presence. In a group of BKV-DNA positive samples and in a group of BKV-DNA negative samples, a PCR for the JCV DNA was also carried out. Moreover, the BKV viral load was measured by a quantitative PCR.
Results: BKV viruria was observed in 26 out of the 100 patients (26%), while BKV viremia was concomitantly demonstrated in 5 out of 26 patients with viruria (19%). All five children with viremia showed renal damage consistent with interstitial nephropathy and increase of serum creatinine levels. The damage stabilized after immunosuppression reduction in four patients, while the remaining recipient lost the graft owing to a histologically confirmed interstitial BKV nephritis. JCV sequences were shown in 15 out of 26 (58%) urine samples BKV DNA positive and in only 1 out of 20 urine samples BKV DNA negative. JCV sequences were not detected in serum samples.
Conclusions: These data indicate that BKV viremia and high viral urinary shedding are closely related to kidney graft damage. Our clinical data also indicate that the BKV-related kidney-graft damage is becoming a relevant complication after renal transplantation possibly owing to the use of new, more potent, and immunosuppressive regimens.

O102 Bacteremia coincides with impaired gut integrity in HSCT recipients

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Introduction: Mucosal barrier injury (MBI) of the oral cavity and gut is a severe and dose-limiting complication of ablative regimens used to prepare for hematopoietic stem cell transplant (HSCT). Gut MBI results in increased permeability and decreased absorption. Here we describe the onset of bacteremia in relation to changes in gut permeability and absorption as measured by a multisugar test.
Study population: Sixteen adults received a regimen consisting of idarubicin 42 mg/M2 given by continuous infusion for 48 h on HSCT day 13 followed by cyclophosphamide 60 mg/kg i.v. on days 6 and 5 and total body irradiation with 4.5 Gy on days 2 and 1 in preparation for an HLA-matched, MLC-negative T-cell depleted sibling HSCT. All were managed throughout the study period with a triple-lumen central venous catheter (CVC) that was inserted on HSCT day −13
Methods: Beginning on day 12 before HSCT, after an overnight fast, subjects emptied their bladders and then drank an isotonic solution consisting of 5 g lactulose, 1 g L-rhamnose, 0.2 g 3-O-methylglucose, 0.5 g D-xylose in 100 mL. Urine was then collected over 5 h, the total output was recorded and an aliquot was stored at 80 °C. The test was repeated on HSCT days −7, 0, +7, +14, +21. The sugars were detected using high-performance liquid chromatography and the fluorescence label 9-fluorenylmethyl chloroformate hydrazine. Lactulose/rhamnose (L/R) ratios were used as an index of gut integrity. Blood was drawn through each lumen of the CVC for culture twice weekly and also peripherally at the onset of fever. Bacteremia was considered present when any organism was recovered from a single blood culture except coagulase-negative staphylococci and micrococci for which two cultures yielding the same species were required. Antimicrobial prophylaxis consisted of oral ciprofloxacin 500 mg q12h from admission. seven patients received meropenem 1 g q8h from HSCT day +1 onwards, whereas eight patients were given cefepime empirically and only one patient continued taking ciprofloxacin.
Results: Mean L/R ratio was significantly higher on HSCT days 0 (3.00), 7 (6.17) and 14 (4.87) than on days −7 (0.06)and 21 (2.59) ($P < 0.05$) with respect to the baseline HSCT day −12 (0.07), and bacteremia occurred predominantly on these days (0.7,14) involving mainly the coagulase-negative staphylococci (23/27 [85%] episodes).
Conclusion: Gram-positive bacteremia occurs when gut integrity is perturbed in allogenic HSCT-transplant recipients.

Antimicrobial needs and options: the importance of using the best in class for appropriate empiric therapy (Symposium arranged by Bayer)

S106 Variability of antibiotic use and resistance in Europe

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Across Europe, there is a range of both antimicrobial preferences and consequent resistant patterns. The first line choices of physicians in Italy and Spain tend to be macrolide agents, such as erythromycin and clarithromycin, while the UK and Germany prefer β-lactams, such as the amoxicillin and the cephalosporins. There are several susceptibility systems in place which monitor either point prevalence or continuous surveillance. These surveys include the Alexander Project, PROTEKT, EARSS, SENTRY, MRL/Focus and others, which include most European countries, whereas specific country surveys include the SEPRA in Italy. There are clear patterns of resistance to macrolides, with the highest rates being observed in Southern European countries with *Streptococcus pneumoniae* (>25–40%), whereas Northern and Central European countries, such as the UK and Germany, have corresponding resistance rates of <8%. β-Lactam resistance for the *Pneumococcus* is highest in Spain, Italy, France and Greece with rates >30%. *Haemophilus influenzae* also has resistance issues, particularly with β-lactamase-producing strains. Reports suggest that these are 15–40% across Europe. Other pathogens noted to have growing resistance issues include *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa*. The outcome of the combination of this geographic variability and increased travel is the need for an empirical antimicrobial which is active against all the possible resistant strains currently encountered in Europe. The new 8-methoxyquinolone, moxifloxacin covers many community respiratory species, while ciprofloxacin would be the drug of choice for the Gram-negative pathogens.

S107 Preserving the efficacy of an antibiotic class

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The introduction of new members of an antimicrobial class, although exciting, may also carry some risks unless the potential for resistance emergence to the new agent and other class members is fully appreciated. It is hypothesized that use of less pharmacodynamically potent class members may enrich a population for first-step mutants, thus setting the stage for resistance developing to the new agents with possible loss of activity. Fluoroquinolones, an expanding class, exert their action at two sites in the bacterial cell: DNA gyrase and DNA topoisomerase IV. Different agents have varying affinity for the two sites in different species. The structure of the quinolones affects the binding of the drug to these enzymes, with poor-binding encouraging the selection of resistant mutants. The impact of a poor-binding drug used extensively in a population encourages rapid emergence of resistance. To prevent this high-affinity, compounds should be used in preference. Analysis of the C7 and C8 moieties on the quinolone molecule have an impact on binding. The C8 methoxy improves binding to DNA gyrase, while large, bulky C7 molecules block efflux pumps. Both of these structures are found within moxifloxacin; this affords a lower propensity for resistance selection compared with agents such as levofloxacin. The significance of molecular structure and resistance emergence will be discussed.

S108 Pharmacokinetic predictors for clinical outcome

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The basic pharmacodynamics of fluoroquinolones is well understood. The principle pharmacodynamic predictor of efficacy is the AVC/MIC ratio, though C_{max}/MIC also has an important role. The magnitude of the AVC/MIC, which predicts the clinical efficacy of fluoroquinolones has been estimated for free drug fractions and *Streptococcus pneumoniae*. These values are for levofloxacin 24–48; gatifloxacin 35–70; gemifloxacin 75–300 and moxifloxacin 180–375. A number of factors, such as bacterial species, bacterial inoculum, host status and the end point chosen are known to have an impact on the size of the AVC/MIC required to ensure optimal outcomes. One size does not fit all, as this ratio magnitude will not predict outcome in all situations. Pharmacodynamics can also be applied to predict emergence of resistance. It has also been suggested that these ratios may be an indicator of rate of response. The relevance of pharmacodynamics to efficacy and resistance will be highlighted.

S109 Moxifloxacin IV – a novel parenteral therapy for CAP

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Moxifloxacin has been administered orally to over 10 million patients worldwide with respiratory tract infections. Moxifloxacin i.v. has been approved in the US and other countries for the treatment of CAP in the hospital, and is awaiting approval in Europe. Data will be presented from two multicenter studies that assess the safety and efficacy of intravenous moxifloxacin in hospitalized CAP patients. The sequential therapy approach of i.v./p.o. moxifloxacin was evaluated against the ERS standard of coamoxiclavulanate and/or clarithromycin in a European setting. Over 500 evaluable patients showed moxifloxacin i.v. to be statistically superior to the ERS combination in terms of clinical response, 93% versus 85% (95% CI = 2.9, 13.2%). Furthermore, the patients treated with moxifloxacin defervesced significantly sooner. This has marked implications for both patient and health-care setting. The rate of bacterial eradication was also significantly higher with moxifloxacin, 94% versus 82% (95% CI = 1.2, 22.9%). In the second study, conducted in North America, moxifloxacin i.v. was found to be equivalent to trovafloxacin or levofloxacin therapy. Moreover, adverse events were similar in both treatment groups. The main adverse events, like diarrhea, transiently elevated LFTs and nausea, were mild. Together, these studies demonstrate that moxifloxacin i.v. as monotherapy is as effective or event superior to standard combination therapy, is well tolerated, and is convenient to use with a once-daily dosage.

S110 Providing complete coverage for rapid resolution

H. Lode
Berlin, D

The convergence of emerging resistance, newly recognized pathogens, an altering healthcare system, and an evolving patient type yields a more complex situation for today's prescriber. The need to treat more resistant pathogens in a more rapid manner reduces the empiric options available. The recognition of mixed infections has added to this management dilemma. Clearly, no single antimicrobial can cover all these variables and use of one agent, to try to do this is fraught with difficulties. Moxifloxacin and ciprofloxacin are two fluoroquinolones with complimentary activities and so can be used as initial antimicrobial therapy to provide rapid and reliable coverage in both parenteral and oral forms. These drugs have a good to excellent safety record. As the infectious presentations evolve these two agents, in particular, i.v. moxifloxacin will meet many of the clinician's expectations.

Impact on human health of non-human use of antibiotics

S120 Impact on human health of non-human use of antibiotics

T. Shryock
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Antimicrobial agents are approved for use by competent regulatory authorities to prevent, control, and treat disease in food animals, and to increase efficiency of feed utilization. The beneficial effects of these uses in food animals include (1) improved human health (e.g. improved immune response to infection) by assuring the supply of healthy animals into the food chain, so that adequate, affordable nutrition can be maintained; (2) increased feed efficiency and lower manure production that fosters good land stewardship and environmental protection; and (3) improved animal health and welfare. Many reviews, committees and meetings have used a variety of indirect evidence to conclude that antimicrobial use in food animals might negatively impact human health through the selection of bacterial pathogens resistant to antibiotics used in human medicine followed by the subsequent transfer of the pathogens to humans via food. The facts of the matter actually suggest that the use of antimicrobial agents in animal production has an overall negligible negative effect on human health, limited primarily to only a few types of foodborne bacteria (e.g. *Salmonella* and *Campylobacter*). Not all antimicrobial agents used in animals are or will be used extensively in humans (e.g. ionophores, orthosomycins, bacitracin), and of those that are used in animals, most are of classes for which there is already a high prevalence of resistance in some genera of human-isolated bacteria (e.g. tetracycline and macrolide resistance). Moreover, alternate treatment options are available to physicians. The magnitude and extent of exposure of humans to viable foodborne bacteria, some of which may have resistance genes, is minimized by processing interventions and cooking. Finally, the magnitude and medical consequence of resistance gene transfer and selection among bacteria within the human intestinal tract remains unclear. Aproportionate response to address the potential risk to human health should include (1) risk assessment to appropriately guide the most effective interventions throughout the entire food chain; (2) continued application of judicious use guidelines for antimicrobial use by producers, veterinarians and physicians; (3) national antimicrobial resistance monitoring programs; and (4) continued support for science-based regulatory processes for drug evaluations.

Antifungal susceptibility testing

O125 Sensititre YeastOne colorimetric antifungal panel for testing voriconazole against isolates of *Candida* spp.: a comparison with the NCCLS M27-A microdilution reference method

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Objectives: The purpose of this study was to evaluate the commercial Sensititre YeastOne Colorimetric Antifungal Panel for susceptibility testing of yeast to the new triazole voriconazole. The available National Committee for Clinical Laboratory Standards (NCCLS) microdilution method for the antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* (M27-A document) may not be the most efficient and convenient procedure for use in the clinical laboratory. It has been demonstrated that the Sensititre YeastOne panel provides comparable MICs to those obtained by the NCCLS method for testing reference agents against yeast.

Methods: We compared MIC values obtained simultaneously by Sensititre YeastOne Colorimetric Antifungal Panel and NCCLS M27-A broth microdilution methods after 24- and 48-h of incubation for reference agents amphotericin B, fluconazole, flucytosine, and itraconazole and the novel triazole voriconazole. The 100 clinical isolates evaluated included 38

C. albicans, 24 *C. glabrata*, 7 *C. krusei*, 5 *C. lusitaniae*, 10 *C. parapsilosis*, and 16 *C. tropicalis*. Colorimetric MICs of amphotericin B corresponded to the first blue well (no growth) and MICs of the other agents to the first purple or blue well.

Results: Three comparisons of MIC pairs by the two methods were evaluated to obtain percentages of agreement (+2 dilution range): 24- and 48-h colorimetric versus corresponding 24- and 48 h NCCLS MICs and 24-h colorimetric versus 48-h reference MICs. The agreement between the methods for voriconazole MICs was 95.9% when both 24- and 48-h colorimetric MICs were compared to 24- and 48-h NCCLS MICs. The agreement was slightly lower (92.7%) when 24-h colorimetric values were compared to 48-h NCCLS MICs.

Results: For most of the other agents were similar (91.7–98%). Comparison of amphotericin B MICs provide excellent levels of agreement (96.9–100% by all comparisons).

Conclusion: These data suggest the potential value of the YeastOne panel for use in the clinical laboratory to test the susceptibilities of common *Candida* isolates to voriconazole.

O126 In vitro antifungal activity of voriconazole against clinical isolates of *Candida* and *Aspergillus* species

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Objectives: Voriconazole is a new triazole, derived from fluconazole, with a broad spectrum of antifungal activity against *Candida* spp., including fluconazole resistant strains, and fungicidal activity against *Aspergillus* spp. Voriconazole is considered to be an ideal antifungal agent for therapy of persistently neutropenic patients in whom *Candida* and *Aspergillus* spp. have become a major cause of invasive infections. We tested voriconazole against clinical isolates of *Candida* and *Aspergillus* spp. with the purpose of verifying its *inhibitory* and *fungicidal* activity in vitro.

Methods: Voriconazole (concentrations ranging from 0.008 to 8 mg/L) susceptibility was tested with 200 clinical *Candida* isolates (105 *C. albicans*) and two ATCC control strains with the broth microdilution method NCCLS M27A, and 37 clinical *Aspergillus* isolates (22 *A. fumigatus*) with the broth microdilution method NCCLS M38P. Microplates were read spectrophotometrically (540 nm) for the yeast (MIC = reduced turbidity equal or greater than 80%) and visually for *Aspergillus* spp. (MIC_0 = absence of visible growth, and MIC_2 = prominent reduction in growth). MFCs were obtained inoculating (0.01 mL) wells with voriconazole concentrations greater than the MIC values. With the aim of reducing clinical reporting of susceptibility data, 20 *Aspergillus* isolates were tested contemporaneously with inocula made from 48-h and 7-day-old cultures.

Results: The MIC_{90} value for the *Candida* isolates was 0.25 mg/L, while the value for MFC_{90} was greater than 8 mg/L. The most susceptible species was *C. albicans* (MIC_{90} 0.03 mg/L). For the *Aspergillus* isolates the results were: MIC_{2-90} (0.5 mg/L), MIC_{0-90} (2 mg/L), and MFC_{90} (4 mg/L). No differences were demonstrated in the susceptibility results to voriconazole of the 20 *Aspergillus* isolates tested with inocula made from 48-h-old cultures in comparison with inocula made, according to M38P protocol, from 7-day-old cultures.

Conclusions: Voriconazole showed very effective inhibitory activity against both *Candida albicans* and the non *C. albicans* isolates, including *C. krusei*. Significant fungicidal activity was demonstrated against *Aspergillus* isolates. Moreover, the use of inocula made from 48-h-old cultures permitted timely reporting of susceptibility results for *Aspergillus* clinical isolates. Owing to its excellent bioavailability, voriconazole is free of the main drawbacks encountered with fluconazole and itraconazole, viz. a narrow spectrum and poor delivery, respectively.

O127 Quality control limits for disk susceptibility tests of fluconazole and voriconazole

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Objective: Methods for performing yeast disk susceptibility testing of F using Mueller–Hinton agar (MHA) supplemented with glucose (G) and methylene blue (MB) dye have been proposed earlier. This study was undertaken to establish quality control ranges for both F and V disk tests using the standard NCCLS QC yeast strains.

Methods: MHA was supplemented either during production or post production with G to a final concentration of 2% and MB dye to a final concentration of 0.5 mg/L. Ten replicates of each QC strain were tested on three lots of MHA using two lots of 25 µg F or 1 µg V disks at eight international laboratories. Zones of inhibition were read at 24 and 48 h of incubation at 35 °C.

Results: The results are presented in Table 1.

Table 1 Results

	Prepared plates (mm at 24 or 48 h)				Supplemented plates (mm at 24 or 48 h)			
QC strain	F-24	F-48	F-24	V-48	F-24	F48	V-24	V-48
C. albicans	28–39	30–41	32–42	32–43	30–41	30–41	30–41	31–42
C. krusei	NR	NR	16–25	12–21	NR	NR	16–25	12–21
C. parapsilosis	22–23	20–31	28–37	27–38	23–33	26–31	29–38	28–38
C. tropicalis	26–37	20–31	NR	NR	26–37	NR	R	NR

NR = not recommended.

Conclusions: QC ranges for disk diffusion tests of F and V using MHA containing G and MB are proposed.

O128 Antifungal susceptibility testing by the National Committee for Clinical Laboratory Standards of *Candida* species isolates from blood cultures in hospitalized patients

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Objective: The aim of the study was to institute a prospective surveillance program for *Candida* species isolated from blood cultures of patients admitted at the University Hospital of Modena and to determine the in vitro activity of four antifungal agents against the yeast isolates.

Materials and method: Data were collected between January 2000 and October 2001. The susceptibility testing was performed by a broth microdiluition method following the recommendations of the National Committee for Clinical Laboratory Standards (document M27-A) guidelines; the antifungal agents tested were fluconazole, itraconazole, amphotericin B and flucytosine. One reference strain, *C. krusei* ATCC 6258, was included to ensure quality control.

Results: During the study period the distribution of the species was as follows: 17 (57%) *C. albicans*, five (17%) *C. parapsilosis*, three (10%) *C. tropicalis*, two (7%) *C. glabrata*, one (3%) *C. pelliculosa*, one (3%) *C. guillermondi*, and one (3%) *C. krusei*. Resistance to fluconazole (>64 µg/mL) and itraconazole (>1 µg/mL) was observed in 10% (two *C. albicans*, one *C. tropicalis*) and 17% (two *C. tropicalis*, one *C. pelliculosa*, one *C. glabrata*, one *C. krusei*), respectively. The resistance to fluconazole was correlated with antifungal prophylaxis (fluconazole 100 mg per day). Only, 3% (one *C. tropicalis*) of these isolates were found to be resistant to flucytosine (> 32 µg/mL) in a patient with hematological neoplasia. In our series, there were not *Candida* species resistant to amphotericin B.

Conclusion: Results in this study, consistent with those obtained by others' studies, suggest the prominent role of *C. albicans*, but also the importance of non-*C. albicans* species in bloodstream infections and the emergence of antifungal resistance among the *Candida* spp. For this reason, it has become prominent for diagnostic laboratories to perform susceptibility testing of yeast and to monitor trends, although adjustments regarding methodology and interpretation are still proposed with a relevant impact on the frequency of resistance reported. Continued monitoring of these trends is important as we strive to control and optimize therapy of BSI attributable to *Candida* species.

Molecular diagnostic methods of tuberculosis

O129 The DNA extraction method critically affects PCR sensitivity for the diagnosis of tuberculosis

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Objectives: Rapid diagnosis of *Mycobacterium tuberculosis* is of extreme clinical importance, however, culture techniques have a very long turnaround time and acid-fast stains (AFS) are not sensitive. PCR techniques provide fast and accurate results but are reported to lack sensitivity in AFS-negative specimens. A critical factor influencing PCR performance is the DNA extraction protocol applied. The purpose of the study was to evaluate three different methods of DNA extaction in culture-positive, AFS-negative clinical specimens.
Methods: Out of 1480 consecutive clinical specimens, 38 from 30 patients (31 sputum, 2 bronchoalveolar lavage and five gastric aspirate samples) were found to be culture-positive but AFS-negative, and were retrospectively tested using an in-house PCR protocol. This protocol amplifies a 123-bp sequence of the IS6110 segment of the *M. tuberculosis* complex and its analytical sensitivity corresponded to 89 fg DNA. Extraction was performed using two commercial kits, the QIAamp DNA Mini Kit, Qiagen, Germany with some modifications and IsoQuick, Orca, USA, as well as a modified in-house developed method, Boom's protocol. All DNA samples were additionally tested diluted 1/10 to detect PCR inhibition. The amplification products were electrophoresed in a 2% agarose gel and visualized under UV illumination.
Results: Thirty-two out of 38 samples were PCR-positive using the QIAamp method, 25 using the IsoQuick method and 22 with the Boom's protocol corresponding to assay sensitivities of 84.2, 65.8 and 57.9%, respectively. Testing the samples diluted 1/10 increased sensitivity with all methods; 1, 1 and 3 additional samples were found positive, respectively. All three methods evaluated were easy to perform but turn-around time was 1 h longer with QIAamp in comparison to IsoQuick and Boom (45 min only), owing to an additional enzymatic incubation step. Finally, the costs of the respective protocols corresponded to € 3.52, 2.06 and 1.08 per test.
Conclusions: Even in samples with low bacillary counts as characteristically are culture-positive and AFS-negative samples, molecular diagnosis of tuberculosis by PCR can provide very sensitive results. It appears that the DNA extraction method critically affects assay sensitivity. The modified QIAamp DNA Mini Kit proved to be the most sensitive of all methods.

O130 Comparison of the molecular identification of mycobacteria by use of the RIDOM, MicroSeq, GenBank and RDP-II databases

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Objectives: Molecular identification of mycobacteria provides two primary advantages to phenotypic identification: rapid turn-around time and improved accuracy. The information content of the 5′-end of the 16S rRNA gene is sufficient for identification of most mycobacterial species. The RIDOM service (http://www.ridom.de/) is in the process of making freely available a comprehensive database much like that of the high-quality commercial database MicroSeq.
Methods: An evaluation was performed to demonstrate the quality and accuracy of results provided by two specialised databases (RIDOM version 1.0 and MicroSeq 500 vs. 1.4.1) and the more general GenBank and RDP-II databases. The newly determined sequences from the ATCC *Mycobacterium*-type strains ($n = 79$) and from clinical isolates ($n = 94$) were analyzed by these databases.
Results: All of the type strain sequences analyzed by RIDOM were correct with 100% similarity. MicroSeq does not include all sequences of established species, such as *M. lentiflavum* as well as many of the most recently described species. Consequently, a few type strains were misidentified. In contrast, only 23 and 25% of species had a prefect match with sequences from GenBank and RDP-II, respectively. An overwhelming 39 and 34% of the type strain sequences were not given top scores against GenBank and RDP-II, respectively. Therefore, these strains would have not been correctly identified. Querying the different databases with the 94 clinical isolate sequences, RIDOM gave in 92.5% a perfect match, whereas MicroSeq yielded this result only in 73.4% of all cases. Only, 4.3% of all RIDOM results had a similarity equal or below 99%, which we regard as the threshold for the reporting criteria of a 'distinct species'. MicroSeq failed in 12.8% to surpass this threshold. GenBank and RDP-II again delivered results insufficient for reliable diagnosis of *Mycobacterium* species.
Conclusion: Whereas both the MicroSeq and RIDOM databases provide (especially in comparison to GenBank and RDP-II) excellent results for the majority of mycobacterial isolates, RIDOM is not only freely accessible but also is significantly more exhaustive. Therefore, the RIDOM service is an excellent tool for all laboratories with sequencing capacities.

O131 Use of real-time PCR for detection in clinical samples genetic polymorphisms causing resistance to isoniazid and rifampin in *Mycobacterium tuberculosis*

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Objectives: Development of a detection system in clinical samples of genetic mutations causing resistance (r) to isoniazid (H) and rifampin (R) in *Mycobacterium tuberculosis* (MTC).
Methods: From 1999 to 2001, we collected samples from patients with MTC isolates and studied the susceptibility pattern to first line antituberculous drugs (radiometric method). Based on a previous study describing the most common genetic mutations causing resistance to H and R in our geographic area, we designed probes with fluorescence molecules (Taqman probes) to detect point mutations in c315 of the *katG* gene, ribosome-binding site (RBS) of *inhA* locus and c432–458 of *rpoB* gene. A probe homologous to *IS6110* was designed as amplification control. We used a real-time PCR detection system (ABIPRISM 7700).
Standardisation: To determine the specificity of the method we used 61 strains with known mutations in the studied genes. The sensitivity was analyzed preparing samples with known inoculum of MTC.
Detection in clinical samples: The DNA of clinical samples was extracted and each gene was amplified. Target genes were sequenced in resistant strains.
Results: The study of previously typed strains showed that this method had 100% specificity in detection of known point mutations in each gene. The threshold of detection was about 1000 cfu/mL.
Detection in clinical samples: We analyzed 91 samples (52 patients) with a H-r strain, 27 samples (11 patients) with a R-r strain and 126 samples (126 patients) with a sensitive strain used as a control group. The overall detection sensitivity in positive Ziehl–Nielssen clinical samples was of 97%, being 40% in negative stain samples. This method detected directly from clinical samples: 78% (14/18) strains with c315 *katG* gene mutation, 68% (13/19) with an RBS *inhA* locus mutation and 67% (10/15) H-r strains without mutations in these positions, and 100% (11/11) strains with mutation in c432–458 of *rpoB* gene. These results were corroborated with sequenciation of studied genes. This study detected 52% of H-r-mutated strains and 100% of R-r-mutated strains.
Conclusions: Development of a methodology able to rapidly (48 h) detect in clinical samples the presence of the most frequent mutations causing H-r and/or R-r (detection of 60% of the possible mutated resistant strains). This methodology may thereby allow modification of antituberculous treatment to avoid relapses, transmision of resistant strains and improvement in the prognosis of patients.

O132 Use of multiple genetic markers to study evolution of the *Mycobacterium tuberculosis* Beijing family in North-western Russia

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Objective: To study genetical polymorphism and evolution of the *Mycobacterium tuberculosis* Beijing family in North-western Russia, where this genotype is prevalent in up to 50% of clinical strains.

Methods: PCR, PCR–RFLP and hybridization analysis of the direct repeat (DR) locus, *IS6110*, *IS1547*, *katG463*, *Rv3135-PPE*, and *mtp40* sequences.
Results: A total 195 of the 394 strains studied in 1996–2001 were of the Beijing genotype as defined by spoligotyping (signals 35–43). A total of 98% of these Beijing strains (typical) were closely related by *IS6110*-RFLP (DR > 0.8), five strains (two distinct profiles, 'atypical') were more distant from the rest (DR 0.6). The *IS6110*-RFLP-based NJ-tree showed the branchs' lengths considerably longer for the atypical strains.Typical strains had two IS1547 copies, one of them with forward left-arm *IS6110* insertion, and 1-kb *Rv3135* gene. Atypical strains had a single intact *IS1547* copy, and 1.9-kb *Rv3135* gene. The *IS1547* copies (both in typical and atypical strains) differed from the published *IS1547* sequences of *H37Rv, CDC1551* and the Beijing strains from Thailand. All the strains had *katG463Leu* allele and intact *mtp40*.
Conclusions: All the Beijing family *M. tuberculosis* strains currently circulating in the North-west of Russia are relatively ancient, and may present a particular separate subgroup within this genotype endemic for this region since the very evolutionary time. Atypical Beijing strains (2% of strains) are evolutionary older, but do not seem to be the ancestral; they might have a common (unknown) predecessor with typical Beijing strains. The DR locus structure of the Beijing-type is extremly conserved since the evolutionary distant time. Sequence changes in *IS1547, Rv3135* and *mtp40* are too deply rooted to correlate with recent evolution events in this genetic family. Recent and ongoing clonal dissemination of the drug-resistant Beijing strains with high copy number of *IS6110* could be owing to the *IS6110*-mediated genome rearrangements and could reflect one specific course of adaptation to the host.

New drugs

O133 Multivalent drug design: synthesis and in vitro analysis of an array of vancomycin dimers

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Objectives: The design, synthesis, and in vitro microbiological analysis of an array of 40 covalently linked vancomycin dimers is reported. This work was undertaken to systematically probe the impact of linkage orientation and linker length on biological activity against drug-resistant and drug-sensitive Gram-positive pathogens.
Methods: To prepare the array, monomeric vancomycin synthons were linked through four distinct positions of the glycopeptide (C-terminus (C), N-terminus (N), vancosamine residue (V), and resorcinol ring (R)) in 10 unique pairwise combinations. Peptide-based linkers of four different lengths (11, 19, 27, and 43 atoms) were employed.
Results: The in vitro susceptibility studies revealed several key results. First, both linkage orientation and linker-length substantially impact antibacterial potency in vitro. Second, the effects of linkage orientation are not monotonic; i.e. no linkage series displays activity that is either uniformly superior or inferior to that of vancomycin against all test organisms. For example, whereas all series have at least one compound that displays enhanced activity against enterococci and penicillin-resistant *Streptococcus pneumoniae*, only compounds in the V–V linkage series display even modestly enhanced potency against glycopeptide-intermediate-resistant *S. aureus*. Third, the C–C, C–V, and V–R series display the most promising broad-pectrum activity among those studied, whereas the N–N series is the least potent overall. Fourth, three categories of linker length effects on activity were observed—no effect, maximal potency at shorter lengths, or maximal potency at intermediate lengths.
Discussion: The results are interpreted in terms of a mechanistic model in which covalent dimerization can provide both gains and losses of function relative to vancomycin itself. Gains of function may accrue as a result of enhanced affinity for target (depsi)peptide ligands, while losses of function may result from disruption of antibacterial effects that do not involve association with these ligands.

O134 Antibacterial activity of two dimeric vancomycin analogues, AMI 462 and AMI 905: lack of correlation between MIC and bactericidal activity

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Objective: To evaluate and compare the antimicrobial activity of two dimeric vancomycin analogues with clinically utilized comparator agents against both vancomycin-susceptible and -resistant bacteria.
Methods: Minimal inhibitory concentration (MIC) and minimal bactericidal concentration were determined utilizing the broth microdilution method as per NCCLS guidelines against *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. Studies of resistance induction in VanB enterococci were performed by preincubation of bacteria with subinhibitory concentrations of vancomycin or the agent under study before determination of the MIC. Bactericidal activity was also determined by time-kill method. Post-antibiotic effect (PAE) following exposure for one hour at MIC was also determined.
Results: AMI 462 was more active against the staphylococci with lower MICs than vancomycin, however, it did not exhibit activity against the enterococci. AMI 905, while exhibiting similar to slightly higher MICs than vancomycin against the staphylococci and streptococci, was active against even VanA enterococci. Utilizing induction studies, the reason for the difference was partially elucidated. Growth of VanB enterococci in the presence of sub-MIC vancomycin or AMI 462 itself induced resistance to AMI 462 but not to AMI 905. It was also observed that while MICs of AMI 462 were lower against the staphylococci as compared to AMI 905, AMI 462 was bacteriostatic in contrast to the bactericidal AMI 905. Interestingly, AMI 905, while bacteriostatic against the VanA enterococci, exerted a substantial PAE.
Conclusion: Through a multivalent approach, introduction of a substituent group into an inhibitor molecule which may also bind to the same site or concomitantly another target, greater affinity, and thus the inhibitory activity might be expected. In the case of antibacterials, one may consider applying this principle to salvage agents to which resistance has developed. Our findings with AMI 462 and AMI 905 strongly suggest that antibacterials with novel properties can be created from known compounds and utility may be regained or improved.

O135 Equivalence of shorter course therapy with oritavancin compared to vancomycin/cephalexin in complicated skin/skin structure infections (CSSI)

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Objective: To determine the efficacy of oritavancin (O), an investigational semisynthetic glycopeptide with bactericidal activity in vitro against the Gram-positive pathogens, as compared with vancomycin/cephalexin in the treatment of complicated skin, soft-tissue infections.
Methods: Phase 3, double-blind, randomized study of 517 patients with CSSI caused by Gram-positive pathogens. Patients received O 1.5 or 3 mg/kg (3–7 days i.v., then oral placebo) or vancomycin (V) 15 mg/kg/dose (3–7 days i.v., then oral cephalexin) for total therapy of 10–14 days. Signs/symptoms of infection, blood and local culture specimens, and safety assessments (vitals, ECG, labs, adverse event (AE) monitoring) were assessed at baseline, on-therapy, and at early and late follow-up.
Results: Efficacy was equivalent between the two O doses in all analyzes. In an intent-to-treat analysis of dosed patients ($N=480$), 63% O and 65% V patients had successful clinical outcome (cure or improvement), 95% CI (−0.115, 0.076). Of the clinically evaluable patients ($N=384$), cellulitis, major abscess, or wound/burn/other was the diagnosis in 160 (42%), 143 (37%), and 81 (21%) patients, respectively. Of these 384 patients 76% O and 80% V patients were clinically successful, 95% CI (−0.139, 0.048). Of clinically evaluable patients with MRSA ($N=33$), 74% of O and 80% of V patients were clinically successful. In bacteriologically evaluable patients

($N=256$), 74% O and 76% V patients had successful bacteriologic outcome, 95% CI (−0.144, 0.097). *S. aureus* was the most common organism isolated, identified as the sole pathogen in 100 (39%) of baseline cultures. Relapse rates of qualified patients at late follow-up were 10, 4, and 5% for O 1.5, O 3, and V, respectively. The AE profile was similar between treatment groups. The mean total therapy length for O 1.5, O 3, and V was 5.3, 5.7, and 11.5 days, respectively.
Conclusion: Oritavancin i.v. therapy for 3–7 days was equivalent to vancomycin/cephalexin therapy for 10–14 days in a phase 3 study of CSSI caused by Gram-positive pathogens, including MRSA.

O136 Combination studies of short-chain cecropin A-melitin hybrid peptides with different antibiotics against multiresistant *Acinetobacter* spp. by killing curves

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Objectives: To study the combination of different peptides with antibiotics against *Acinetobacter baumannii* by using killing curves.
Material and methods: Two *A. baumannii* strains, a susceptible (ATCC 19606), and a multiresistant strain were studied. CA(1–8)M(1–18), a synthetic cecropin A-melittin hybrid peptide (KWKLFKKIGIGAVLKVLTTGLPALIS-NH_2), and three short-chain peptides: CRIS2 (KWKLFKKILKVL), CRIS3 (KWKLLKKIGAVLKVL) and CRIS7 (WKLLKKILKIL) were tested in combination with tobramycin (TOB), imipenem (IMP), fosfomycin (FOS) or colistin (CT). Peptides and antibiotics were tested alone and in combination at the following concentrations: 1 μM for CA(1–8)M(1–18) and CRIS2, 0.5μMn for CRIS3 and CRIS7, 8 mg/L for TOB, 40 mg/L for IMP, 130 mg/L for FOS and 8 mg/L for CT. Antibiotic concentrations were chosen as the peak concentration found in human serum after normal doses. Tube controls and tubes with one or two agents were inoculated simultaneously to achieve a final concentration of 10(5) to 10(6) cfu/mL. Viable colony counting was performed at 0, 1, 2, 6, and 24 h. Synergy was defined as a $\geq$2 log 10 cfu/mL decrease of combination compared with the most active single.
Results: Indifference was observed with CA(1–8)M(1–18) combined with TOB, IMP, CT and FOS in the susceptible strain. The same peptide studied in the resistant strains showed synergy in the combination with TOB and indifference with the others combination. CRIS2 showed indifference associated with TOB, IMP and CT in both susceptible and resistant strains. When associated with FOS antagonisms were observed in both strains. CRIS3 showed indifference associated with TOB, IMP, CT and FOS in the susceptible strain. The combination with TOB, IMP, CT showed synergy in the resistant strain. The combination with FOS was indifferent. CRIS7 showed indifference associated with TOB, IMP, and CT, but showed antagonism when associated with FOS in the susceptible strain. The combination with TOB, IMP, CT showed synergy in the resistant strain but the combination with FOS was indifferent.
Conclusions: By using killing curves, CA(1–8)M(1–18) showed synergy when combined with tobramycin in a multiresistant *A. baumannii* strains. Two short-chain derivatives (CRIS3 and CRIS7) showed synergy in the resistant strain when combined with tobramycin, imipenem and colistin, suggesting a future potential use for these peptides.

Linezolid

O137 Susceptibility of Gram-positive bacteria from ICU patients in UK hospitals to linezolid and other antimicrobial agents

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Objectives: To determine the prevalence of resistance to antimicrobial agents, including linezolid, among clinically significant Gram-positive cocci from ICU patients in the UK hospitals.
Methods: Microbiologists in 25 sentinel laboratories in the UK were asked to refer up to 100 consecutive, clinically significant, Gram-positive cocci isolated from ICU patients. Isolates were identified and subjected to MIC determinations by the British Society for Antimicrobial Chemotherapy method for a range of antimicrobial agents including linezolid.
Results: A total of 1613 isolates were collected, comprising predominantly *S. aureus* (47.6%), coagulase-negative staphylococci (CNS) (30.6%), enterococci (14.4%), streptococci (3.5%) and pneumococci (2.7%). The remainder comprised diptheroids, *Bacillus* spp. and a *Nocardia*. Among the *S. aureus* isolates, 60% were oxacillin-resistant, as where 81% of the CNS. Vancomycin-resistant *S. aureus* were not detected although three isolates (0.4%) were resistant to teicoplanin (MICs 8 mg/L). In contrast, 14% of the CNS were resistant to teicoplanin (MICs 8–32 mg/L), with 1% resistant to vancomycin. Among the enterococci, 72% were *E. faecalis* and 25% were *E. faecium*, the remainder comprising *E. casseliflavus* or *E. gallinarum*; 18% of the *E. faecium* were vancomycin-resistant compared to only 3% of the *E. faecalis*. Rates of high-level gentamicin resistance in *E. faecalis* *E. faecium* were 25 and 40%, respectively. Among the streptococci and pneumococci, 11 and 9%, respectively, were resistant to penicillin, whilst 13 and 7% were resistant to erythromycin. None of the isolates showed resistance to linezolid, with the MICs for the entire study population falling in the range 0.5–4 mg/L.
Conclusions: Resistance to first-line antimicrobial agents is a major problem among Gram-positive bacteria occurring in the UK ICUs. The dominance of methicillin-resistant staphylococci is, especially striking, as is the frequent vancomycin resistance in *E. faecium*. Although resistance to linezolid has been reported in enterococci and in one MRSA isolate, these data indicate that such resistance is still extremely rare in the UK, and that the drug is a potentially useful option for Gram-positive infections in ICU patients.

O138 Linezolid in the treatment of methicillin-resistant *S. aureus* infection

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MRSA infection is a serious problem associated with big epidemiological and economical impact. The development of new drugs like linezolid give new chances in MRSA infection management.
Objective: To analyze the effect of linezolid in the treatment of MRSA infection compared to glycopeptides and its influence on mortality rate, number of admissions and length of stay.
Methods: Prospective study including patients with MRSA infection admitted to our hospital between January 2000 and October 2001. Assimptomatic carriers and patients prematurely dead were not included. Periodical clinical and control cultures were performed during follow-up. We collected baseline characteristics, length of stay (from MRSA diagnosis to discharge), mortality rate and number of persistant carriers. Differences between patients receiving linezolid or glycopeptides were analyzed using ANOVA tests and bivariate correlation.
Results: Thirty-six patients were included, median age 73.9 years (48–95), 19 (52.7%) were female, site of infection: 66% soft tissues, 11% respiratory tract, 11% cardiovascular and 8% surgical wound. Nine patients received linezolid that was well tolerated. Few adverse events were observed. In this group, median length of stay was 10.3 days, and during the follow-up five patients were readmitted, four were found to be persistent carriers, none died and three needed social support. Linezolid reduced length of stay: 10.3 day versus 23.8 ($P=0.01$); reduced number of persistent carriers: 4 versus 12 ($P=0.045$); and showed a tendency to reduce mortality rate ($P=0.079$) compared to glycopeptides. Multivariate analysis disclosed that linezolid had an independent effect on the reduction of length of stay ($P=0.008$).
Conclusions: Linezolid reduced length of stay in MRSA infected patients, reduced the number of persistent carriers and showed advantages in the treatment of this infection compared to glycopeptides. Linezolid was well tolerated with few adverse events.

O139 Linezolid vs. vancomycin in nosocomial pneumonia: prospective surveillance for appearance of vancomycin-resistant enterococci in stool

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Objective: The emergence of vancomycin-resistant enterococci (VRE) has been associated with the use of vancomycin (vanco). To monitor for the appearance of VRE during vanco use, we prospectively cultured rectal swabs during a comparison of vanco versus linezolid (LZD) in the treatment of nosocomial pneumonia (NP).
Methods: Patients with NP were randomized to treatment with LZD 600 mg/12 h every day or vancomycin 1 g/12 h every day for up to 21 days. Patients could receive aztreonam for Gram-negative coverage. Rectal swabs were obtained at baseline and at EOT and were cultured for the presence of VRE.
Results: A total of 623 patients (321 LZD, 302 vanco) were treated; 585 (304 LZD, 281 vanco) had rectal swabs collected at both baseline and EOT. The incidence of VRE at EOT in prospectively cultured patients without VRE at baseline was 1/288 (0.3%) for LZD compared with 15/267 (5.6%) for vanco ($P < 0.001$). Average length of therapy in all treated patients was 9.5 and 9.4 days for LZD and vanco, respectively. The LZD-treated patient newly colonized with VRE at EOT was treated for 4 days. The 15 vancomycin-treated patients who became colonized with VRE were treated for an average of 11 days (range 6–21 days).
Conclusion: During this prospective NP study, rectal swabs collected before and after treatment revealed one LZD-treated patient who became colonized with VRE during treatment, while the conversion rate was significantly higher (5.6%) for vanco-treated patients. Conversion in the vanco group was associated with a longer duration of therapy.

O140 Comparison of hospital resource use between linezolid and teicoplanin for the treatment of Gram-positive bacterial infections: results of a multicentre trial

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Background: Linezolid (LZD), a novel oxazolidinone antibiotic available in intravenous (i.v.) and 100% bioavailable oral forms, is as effective as standard therapies, including vancomycin, in the treatment of Gram-positive infections. LZD has been shown to reduce hospital resource use when compared to vancomycin (Pharmacotherapy 2001; 21: 263–74).
Objective: Compare i.v. days of study medications (i.v. duration), length of hospital stay (LOS) and hospital-discharge rate (HDR) between LZD and teicoplanin (TEI), a widely used alternative to vancomycin, for the treatment of Gram-positive infections in an open-label, multicenter randomized trial, which showed that LZD had significantly better efficacy, especially in bacteremia (ICAAC 2001; poster L-1481).
Methods: In this trial, 430 hospitalized patients with pneumonia, complicated skin/soft tissue infection, or bacteremia caused by Gram-positive bacteria were treated with LZD (i.v. followed by optional oral) or TEI (i.v. followed by optional intramuscular). Patients received up to 4 weeks of treatment followed by up to 3 weeks of observation. Arithmetic mean, Kaplan–Meier survival function adjusted mean and median, and proportion were used for i.v. days, LOS, and HDR, respectively. Between-treatment differences in these measurements were tested with Student's *t*-, Wilcoxon's, and χ^2-test, respectively.
Results: See Table 1 below for the total intent-to-treat sample results. Though not shown in the table, bacteremia patients ($n = 32 + 33$) treated with LZD had much shorter LOS (KM mean: 18.8 vs. 23.7; KM median: 17 vs. 25) though the difference was not statistically significant owing to limited sample size.

Table 1 The total intent-to-treat sample results

			LOS		Patients discharged days (%)			
Study Median	*N*	i.v. day mean	KM mean (±SE)	KM median (95% CI)	7	14	21	28
LZD	215	6.2	13.3 (0.83)	9 (8–10)	35	67	80	87
TEI	215	9.4	14.7 (0.87)	10 (8–11)	27	63	74	83
P-value		0.000	0.20		0.08	0.31	0.14	0.22

Conclusion: LZD patients tended to have shorter LOS and higher HDR in the first week, especially for bacteremia, possibly due to LZD group's significantly shorter i.v. duration.

Experimental infections

O141 Rifampin + ceftriaxone decrease lipoteichoic acid *CSF concentrations and reduce neuronal damage compared* to ceftriaxone alone in experimental *S. pneumoniae* meningitis

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Objectives: Rifampin (RIF) releases smaller quantities of lipoteichoic acids (LTA) from *S. pneumoniae* than ceftriaxone (CRO). Owing to the rapid development of resistance, RIF cannot be used as a single agent for therapy of bacterial meningitis. For this reason, we studied the effect of an initial RIF therapy followed by the combination RIF + CRO in the rabbit model of meningitis.
Methods: NZW Rabbits were infected intracisternally with a *S. pneumoniae* type 3 strain. At 12 h after infection, treatment was started with RIF (10 mg/kg bolus, 5 mg/kg/h maintenance dose), 6 h later CRO was added (20 mg/kg followed by 10 mg/kg/h). Controls received CRO (20 mg/kg followed by 10 mg/kg/h). Bacterial titers, leukocyte densities (WBC), LTA, lactate and protein concentrations were determined at 12, 14, 18, 20, and 24 h. Density of neuronal apoptoses/mm^2 were quantified in the granule layer of the dentate gyrus by in situ-tailing.
Results: Bacterial titers were effectively reduced in both groups (Δ log cfu/mL/h ± SD = 0.64 ± 0.16 [CRO] vs. 0.42 ± 0.15 [Rif]). Release of LTA was lower in rifampin-pretreated animals. Median of Δ12–14 h (ng/mL) was −0.1 (from −6.8 to 3.6) vs. 0.7 (from −0.2 to 35) ($P = 0.01$). When CRO was added 18 h after infection, LTA concentrations in CSF did not increase (median of Δ 18–20 h: 0.1 [from −1.6 to 3.2]). Density of neuronal apoptoses was lower after combination therapy (median: 37.8/mm^2 [9.9–90.2] vs. 67.3/mm^2 [32.7–183.7] $P = 0.03$). The CSF lactate/protein concentrations and WBC increased during the course of the experiment (no differences between treatment groups).
Conclusion: Initial therapy with RIF followed by a combination of RIF + CRO, which is suitable to prevent rapid development of resistance, is capable of reducing the release of proinflammatory compounds and neuronal damage in bacterial meningitis.

O142 In vivo foreign body infection induces an exponential decrease of protein synthesis in *Staphylococcus epidermidis*

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Objectives: Coagulase-negative staphylococci (CNS) can cause persistent infections in association with foreign bodies. The exact cause of this persistent nature remains unclear, but a state of bacterial dormancy is hypothesised as a contributing factor. The aim of this study was to evaluate overall bacterial metabolical activity during in vivo foreign body infection. Therefore the expression of the *16S* gene, which is known to be the rate-limiting step in bacterial protein synthesis, was followed.
Methods: Catheter segments ($n = 175$) were inoculated in vitro with *S. epidermidis* in exponential growth phase and immediately implanted subcutaneously in rats. The catheters were removed after 15 min, 1, 2, 4, 6, 12 h and 1, 2, 7 and 14 days. Directly after explantation, an instant RNA and DNA isolation was performed using a FastPrep^{T}-based protocol. RNA (translated in cDNA) and genomic DNA (gDNA) were quantified with Taqman quantitative PCR to determine the expression of the *16S* gene given by the cDNA/gDNA quotient.
Results: *16S* expression decreased rapidly after implantation of the catheters in vivo (one-way ANOVA: $P < 0.0001$). The decrease in expression was exponentially and given by the formula: expression $= 525 \times \text{time}^{-0.7883}$, with $R^2 = 0.96$. Average expression was 48.4 (15 min), 27.4 (1 h), 20.1 (2 h), 4.2 (3 h), 6.5 (6 h), 2.7 (12 h), 1.09 (1 day), 1.3 (2 days) and 0.3 (1 and 2 weeks). Differences between early and late expression levels were significant (Bonferroni test: $P < 0.001$).
Conclusion: In vitro, early adhesion to foreign bodies provokes a significant increase in *16S* expression [ICAAC 2001 abstract 957]. In vivo, *16S* expression in CNS attached to foreign bodies declines exponentially directly after implantation of the foreign body. Thus, the in vivo environment induces a rapid decline in protein synthesis in CNS attached on foreign bodies, and very low levels are reached within 1 day. These findings add strong evidence to the hypothesis that during the in vivo foreign body infection the majority of adherent CNS enter a state of metabolical dormancy. This may explain the limited benefit of antibiotics that act on cell wall and protein synthesis for the eradication of long-standing CNS foreign body infections.

O143 Studying the role of *Staphylococcus aureus* coagulase in experimental endocarditis (EE) by transfer and expression in *Lactococcus lactis*

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Background: Studies with Coa-inactivated *S. aureus* failed to identify a role for this factor in EE. In these experiments, redundant *S. aureus* virulence factors could have masked the Coa defect. Here, we studied the intrinsic role of Coa by expressing it in the low pathogenic *L. lactis*. Coa + *L. lactis* were tested in vitro and in rats with EE, and compared both to the parent, and to *L. lactis* expressing the *S. aureus* fibronectin binding protein A (FnBPA).
Methods: The *coa* and *fnbA* genes were constitutively expressed in *L. lactis* using the pOri23 vector (Infect. Immun. 2000; 68:3516). Parent and recombinants were inoculated to rats with aortic EE. The minimal inoculum infecting >80% of rats (ID80) and the vegetation (Vgs) bacterial densities were followed for 48 h. Platelet-induced bacterial killing was determined by exposing *L. lactis* to platelet-rich or platelet-depleted rat plasma.
Results: The ID80 (in CFU) were 107 (parent), >109(Coa+) and 105 (FnBPA +). After inoculation, parent-infected Vgs maintained a low-grade infection (3 log cfu/g Vgs) over 48 h. In contrast, Coa + Vgs became rapidly sterile within 24 h, whereas FnBPA + Vgs became more heavily infected (>8 log CFU/g Vg). In in vitro, Coa + lactococci triggered plasma coagulation and lost >5 log cfu/24 h in platelet-rich plasma, compared to <2 log cfu/24 h in platelet-depleted plasma ($P < 0.0001$).
Conclusion: Constitutive Coa production in *L. lactis* triggered spontaneous eradication from the Vgs. The in vitro results suggest Coa-induced platelet-activation and consequent release of platelet microbicidal proteins. Thus, while FnBPA increased infectivity, Coa ws potentially deleterious for the microorganism. In *S. aureus*, this effect might be circumvented by the tight regulation of the *coa* gene, which is expressed only in during the early phase of bacterial growth.

O144 A murine model of *Chlamydia pneumoniae* infection

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Objective: *Chlamydia pneumoniae* (Cp) represents an emerging pathogen identified only 12 years ago. It occurs throughout the world and seroprevalence in the adult population is about 70% with presumed persistence of the intracellular pathogen in 80% of these. Increasingly, associations of Cp with chronic and degenerative disease such as asthma, multiple sclerosis and atherosclerosis are reported. The aim of this study was to establish and characterize a murine model of Cp infection, in order to study pathophysiology and putative treatment regimens.
Methods: Mice were infected intranasaly during anesthesia by ethylether with 106 Cp/mouse. At various time-points after infection, the bacterial burden in different organs was determined by a novel quantitative real-time PCR with a sensitivity of 0.1 genome equivalents per PCR sample. The development of anti-Cp antibodies was followed by microimmunofluorescence test, which was adapted for mice. Levels of TNF-a, IL-6 and IL-10 in bronchoalveolar lavage (BAL) and lung, as well as serum amyloid A (SAA) were determined by ELISA.
Results: Intranasal application of 106 Cp resulted in an asymptomatic infection characterized by a maximal bacterial burden in BAL on the day of infection and in the lung two days after infection. No bacterial load was detected in a variety of other organs suggesting no systemic spread. By day 95, Cp were completely eradicated from BAL and lung. Notably, the course of the established infection was very mild with no apparent symptoms, lack of acute phase response (SAA) and no induction of TNF-a, IL-6 and IL-10 in BAL and lung. In serum of all infected mice, anti-Cp IgG was detectable at day 18, indicating activation of the immune system.
Conclusions: So far, there is no evidence of persistent infection of mice following single infection by Cp. PCR detection of bacteria allows to monitor closely elimination of bacterial burden. The model is currently being employed to study the antibiotic and immunomodulatory treatment strategies.

Molecular diagnostics (Symposium organized by ESGMD)

S145 Clinical virology in real time

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The ability to detect nucleic acids has had and still has a major impact on diagnostics in virology. Both quantitative and qualitative techniques, whether signal or target amplification based systems, are currently used routinely in most if not all virology laboratories. Technological improvements, from automated sample isolation to real time amplification technology, has given the ability to develop and introduce test systems for most viruses of clinical interest, and to obtain clinically relevant information needed for optimal antiviral treatment options. Both PCR- and NASBA-based amplification technologies in combination with real time detection can be used currently to generate results in a short turn-around time and to determine whether variants relevant for antiviral resistance are present. These new technologies now enable the introduction of the individual patient disease management concept. Within our clinical setting, we have introduced this, e.g. for the quantitative detection of Epstein Barr Virus (EBV) in T-cell depleted allogeneic stem cell transplant patients. This enabled us to develop models

for pre-emptive anti B-cell immunotherapy for EBV reactivation, thereby effectively reducing not only the incidence of EBV-lymphoproliferative disease (EBV-LPD) but the virus-related mortality. Furthermore, additional clinically relevant viruses can now easily be detected. It also becomes more feasible to introduce molecular testing for those viruses that can be easily detected using more classical virological methods. Prospective studies are needed to evaluate the meaning of additional positive samples, for which it seemed that these classical testing already had full clinical relevance. It should, however, be made clear that a complete exchange of technologies are unlikely to occur, and that some complementary technologies should stay operational. The implementation of these molecular diagnostics technologies furthermore warrants the use and introduction of standardized materials as well as participation in international quality control programs. The use of an internal control not only ensures the accuracy of the results generated, but also is necessary to enable precise quantification of these results and to determine detection thresholds. Since so many targets do have clinical implications, laboratories have to use more universal internal controls before the in-house developed assays should be introduced in clinical virology.

S146 A polyphasic approach for identification of bacteria: the *Burkholderia cepacia* example

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Polyphasic taxonomy is not a new, nor a recent development in bacterial classification. It arose in the early 1970s and aimed at the integration of different kinds of data and information on microorganisms. Nowadays, it comprises three major elements. First, species demarcation is based on DNA–DNA hybridization experiments as described by Wayne et al. (1987). Second, bacterial phylogeny can be deduced from comparative sequence analysis of conserved macromolecules such as 16S rDNA. Third, it recognizes and uses the value of these and various other methods for distinguishing and describing bacteria at different taxonomic levels. The application of a polyphasic approach to unravel the taxonomic structure and relationships of *Burkholderia cepacia* exemplifies the strengths and weaknesses of a classification based on polyphasic taxonomy. It illustrates how and why different techniques were used to reveal the identity over 3000 *B. cepacia*-like isolates. Simultaneously, it highlights a painful consequence of a classification based on multiple parameters: identification of new or unusual isolates has become polyphasic too. *B. cepacia* is an extremely versatile organism that is truly considered friend and foe to humans. It is a genuinely ubiquitous organism that is now recognized as the most useful bacterium with a range of biotechnological applications including biocontrol, bioremediation and plant growth promotion. However, it has also become notorious as a naturally multiresistant and life-threatening pathogen in immune suppressed hosts such as cystic fibrosis patients. A polyphasic analysis including comparative 16S rDNA and RecA sequence analysis, DNA–DNA hybridization experiments, whole-cell protein and fatty acid analyses, various DNA fingerprinting methods, DNA base ratio determination, and biochemical characterization, was performed on over 3000 isolates. This study revealed the presence of various novel organisms that are regularly misidentified as *B. cepacia*. It also demonstrated that *B. cepacia* is a complex of at least nine closely related organisms each representing a distinct species. Most of these nine species occur in the environment and are associated with human infections. Potential candidates for biotechnological applications occur in all of these species. However, salient characteristics differ strongly between them. A thorough evaluation of cystic fibrosis related isolates demonstrated that correct species level identification is essential for patient management and infection control.

Old and new hot topics in infection control (Symposium organized by ESGNI)

S151 Infection control and modeling

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Little is known about the amount of cross-transmission, the risk factors involved and the relative effectiveness of infection control procedures when nosocomial pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) have become endemic in settings like intensive care units. Descriptive epidemiological tools such as molecular epidemiology and cluster statistics provide answers towards the amount of transmission that occurs in a given unit, whereas analytical approaches such as case-control and cohort studies address the risk factors involved. The success of interventions can be determined by carefully designed cross-over trials if ethical approval can be obtained. A fourth genre of epidemiological investigations consists of mathematical modeling. Models can be used as a conceptional framework for estimating the relative contribution of various factors involved in the dissemination of pathogens among susceptible hosts. Alternatively, mathematical models can be utilized to predict the success of interventions when parameters have been carefully ascertained during analytical and observational studies. Using observed parameters with only limited parameter estimation, the models provide further insights into the underlying epidemiology of nosocomial pathogens via estimation of the effective case reproductive number, R_n. Using cohort data from an adult intensive care unit in the UK the merits of various epidemiological approaches will be discussed.

Inhibitors of pumps and beta-lactamases in Gram-negative bacteria (Joint ESCMID/ICAAC symposium)

S160 Multidrug efflux in Gram-negative bacteria

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Efflux mediated resistance to antimicrobials in Gram-negative bacteria has been known for two decades. More recently, however, efflux systems of broad substrate specificity, accommodating and, thus, providing resistance to a variety of structurally unrelated antimicrobials have been described in a number of Gram-negative organisms. The most significant of these pumps are tripartite, comprised of an inner membrane proton-drug antiporter, an outer membrane- and periplasm-spanning channel-tunnel and a periplasmic membrane fusion protein that apparently links the membrane-associated components Homologous 3-component multidrug efflux systems have been described in *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*. These systems can accommodate most classes of antimicrobials as well as biocides, organic solvents, dyes, detergents, and inhibitors of fatty acid biosynthesis. In organisms such as *P. aeruginosa*, several of these multidrug efflux systems have been described where they play a role in intrinsic and acquired resistance to multiple agents. Five such tripartite multidrug efflux systems have been described to date in this organism, collectively providing resistance to most known classes of antimicrobials and expressed constitutively (MexAB-OprM), in response to antimicrobials (MexXY-OprM), or hyperexpressed as a result of mutation (MexCD-OprJ, MexEF-OprN, MexKL-OprM, as well as the other two). The corresponding efflux genes are organized in operons and linked to regulatory genes that are responsible for controlling efflux gene expression and are, in many instances, the target for mutations leading to efflux gene hyperexpression and acquired multidrug resistance. Despite their significance vis-à-vis antimicrobial resistance, the multiplicity of these multidrug efflux systems in a given organism and their broad distribution in Gram-negative bacteria raises questions about antimicrobial export as their intended or natural function. Indeed, it has recently been shown that MexAB-OprM operation is important for the pathogenesis of *P. aeruginosa*, possibly as a result of its export of virulence factor(s). Still, whatever the intended function, it is clear that targeting these efflux mechanisms therapeutically would be useful in countering intrinsic and acquired antimicrobial resistance in many Gram-negative pathogens.

Quality improvement in antibiotic prescription (Symposium organized by ESGAP & BSAC)

S162 Practice guidelines: different strategies, same goals

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The goals of practice guidelines are to improve the quality of care and the appropriateness of antibiotic prescription, to optimize cost-effectiveness and to serve as an educational tool. They are not a substitute for clinical judgment. The quality standards of guideline development must be followed to ensure scientific validity. Guidelines based upon clinical experience of experts or a consensus conference have their weaknesses. A systematic review of scientific evidence must be evaluated by the development group and translated into a clinical guideline. Specific issues concerning the topic and the purpose of the guideline, its target population and its target professional groups must be determined. The strength of the recommendations must be clearly linked to the grade of evidence. The AGREE instrument allows to assess the quality of the guideline and its recommendations.

The applicability can be tested in pilot hospitals. Effective and efficient dissemination and implementation strategies must be explored to induce changes in clinical practice and prescribing behaviour. Implementation is essentially a local activity and the multidisciplinary antimicrobial management team is the best forum to adapt the guideline and to organize its implementation and follow-up. Understanding patient, system and physician barriers to adherence may provide a framework of improvement.

S164 Antimicrobial drug list in countries with limited resources: a tool to promote rational use of antimicrobials and to contain the emergence of antimicrobials resistance

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The need for antimicrobials (AMs) is driven by the high incidence of infectious diseases. Death from acute respiratory tract infections (ARI), diarrhoeal diseases, measles, AIDS, malaria, and tuberculosis account for more than 85% of the mortality of infection worldwide (WHO, 2001). In Indonesia, the leading causes of infant and child mortality were infectious diseases: diarrhoea, ARI, neonatal tetanus, and measles. Anti-microbial resistance (AMR) is directly linked to the use of antimicrobial agents, and is common in the general community as well as in hospitals. The emerging problems of AMR cause a significant burden mainly of the poor population. AMR costs money, livelihoods and lives, as well as threatens the effectiveness of health delivery programmes especially in developing countries. Complex political, socio economical and behavioral factors are associated with the emergence of AMR which include overuse, misuse, and also underuse of AMs by health professionals, unskilled practitioners, patients/lay persons. Action to optimise prescribing patterns and to reduce inappropriate AMs use is thus crucial. Effective interventions must address the underlying causes of current systems, practice, and barriers to change. Limiting AMs choice by adopting the WHO essential drug concept can be implemented, however, antimicrobial drug list alone will not be effective in containing the emergence of AMR and on opportunities for improving economic efficiency. At community level, health centers are provided with basic or essential AMs against common infectious diseases, tuberculosis, leprosy based on morbidity pattern, standard diagnostic and treatment guidelines. In the hospital setting, limitation of AMs is formulated via the Formulary System based on the standard diagnostic and treatment guidelines, antibiotic guidelines, including the antibiotic policy. A strong Hospital Pharmacy and Therapeutics Committee is regarded important, and an active 'antimicrobial agent team' consisting of infectious disease specialists, infection control practitioners, clinical microbiologists, and clinical pharmacists has been found extremely important in implementing rational antimicrobial policies. Education should be considered as the cornerstone of the programme. Integrated interventions through multidisciplinary approach to reduce AMs use in the health centers and hospitals being proposed, as a solution will be presented.

Sepsis pathogenesis

O166 Late immunoneutralization of procalcitonin (ProCT) in septic pigs is as therapeutically effective as early therapy

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Objectives and methods: A model of highly virulent sepsis was developed in pigs (cecotomy followed by intraperitoneal instillation of 1 g/kg cecal contents plus 1012 cfu *E. coli*) Sepsis was induced in 25 castrated male Yorkshire pigs (25–30 kg), after which three treatment arms were studied: (1) early therapy with a 1-h intravenous infusion of rabbit antiporcine ProCT IgG given simultaneously with the induction of sepsis; (2) late therapy with the same peptide administered after the 3rd hour, when the animals were moribund; and (3) septic controls which received nonimmune rabbit IgG, either at the early ($n=8$) or the late ($n=5$) time period.

Results: The septic controls all died by 11 h. They exhibited progressive hypotension, oliguria, decreased cardiac output, and lactic acidemia. In contrast, 86% of animals receiving early therapy and 80% of those receiving late therapy survived until euthanasia at 15 h. Prior to the time of sacrifice ($t=15$ h), arterial blood pressure (ABP), cardiac index (CI), serum creatinine (Cr), urine output (UOP), pH and lactic acid (LA) were measured (Table 1).

	Number animals	ABP* (mmHg)	CI* (L/m/m^2)	CR* (mg/dL)	UOP* (mL/h)	pH*	LA* (mg/dL)
Early Rx	8	64 ± 30	2.81 ± 0.70	1.2 ± 0.4	58 ± 94	7.31 ± 0.11	3.0 ± 1.4
Late Rx	5	65 ± 17	2.25 ± 0.72	1.1 ± 0.2	51 ± 38	7.36 ± 0.02	2.7 ± 1.1
No Rx	12	†	†	†	†	†	†

*Mean ± SEM, not statistically significant by *t*-test at $P<0.05$.
†All animals died.

Conclusion: There were no significant differences in any parameters measured between those treated early versus late. Furthermore, there was a minor trend toward improvement in some parameters (Cr, pH and LA) in animals which underwent late therapy in comparison to those having early therapy. These findings further support the potential efficacy of such therapy in humans, even if sepsis is recognized late in the course of this disease, when physiologic and metabolic parameters begin to deteriorate.

O167 Bioartificial extracorporeal phagocytosis assistance in a pig-model of Gram-positive sepsis

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Objectives: One major reason for the development of a systemic bacterial or fungal infection is the impairment of the host immune system especially phagocytosis. Therefore, we investigated the possibility to establish an extracorporeal phagocytosis assistance system for the treatment of sepsis. The presented study shows results of bioartificial phagocytosis assistance in a pig-model of Gram-positive sepsis.
Methods: Human hematopoetic precursor cells have been expanded and stimulated to differentiate towards functional granulocytic cells. After stimulation the cells showed increased chemiluminesence, active intracellular killing and a high reduction rate of *E. coli*, *Staphylococcus aureus*, *Candida albicans* and Picornavirus. In an animal-model of sepsis, similar to the one described by Lee et al. (Crit Care Med, 1998, 26:730–737), 21 female immature landscape swines (7.5–12 kg) were given 8×10^9 cfu/kg living *S. aureus* i.v. and for 7 days clinical parameters and survival time were monitored. After a 1-h infusion of bacteria 14 pigs were treated for 4 h by an extracorporeal plasmaperfusion model containing a membrane-based bioreactor (Nylon) with in mean 6.2×10^9 cells. Plasmaseperation was carried out by centrifugation, plasma reflux to the animal was done through a 0.2 µm pore-size plasmafilter (PF 1000,GAMBRO AB). Group I was treated without cells (untreated-group = UG, $n = 7$), group II with cells (treated-group = TG, $n = 7$). Group III were given the bacteria without extracorporeal treatment after inoculation (septic control group = SCG, $n = 7$).
Results: All animals of the SCG died (between 31, 75 and 156 h, in mean: 70 h). Two out of seven pigs of the UG, but six out of seven pigs of the TG survived the whole observation time (survival-time TG between 165 and 168 h, in mean: 167.57 h; survival-time UG between 1.08 and 168 h, in mean: 75.19 h). Statistic significance (log rank test) were seen between UG and TG ($P = 0.0019$) and between SCG and TG ($P = 0.0001$) but which was not seen between UG and SCG ($P = 0.43$). The plasmaperfusion model showed a good compatibility (no great differences between SCG and UG in the course of disease). The other clinical parameters showed benefit for the cell-treated animals (TG) compared with the UG (see Table 1).

Table 1 Clinical parameters showing benefit for the cell-treated animals (TG) compared with the UG

Clinical parameter (expressed as mean)	Untreated group (UG) ($n = 7$)	Treated group (TG) ($n = 7$)	Septic control group (SCG) ($n = 7$)
Survival time (h) (observation time 168 h)	75.19	167.57	70
Bacterial count in blood (cfu/mL) after 168 h or death	$1.6 \times 10^4 \mp 4.2 \times 10^4$	$1.7 \times 10^3 \mp 4.2 \times 10^3$	$9.9 \times 10^3 \mp 1.5 \times 10^4$
Lactat after 168 h or death (mM/L)	5.53 ∓ 7.13	1.54 ∓ 0.93	10.6 ∓ 10.6
Creatinine after 168 h or death (µM/L)	110.3 ∓ 50.6	80.0 ∓ 47.5	86.3 ∓ 18.8
Urea after 168 h or death (mM/L)	9.4 ∓ 4.7	3.0 ∓ 2.9	10.2 ∓ 2.0
AT III after 168 h or death (%)	58 ∓ 8	75 ∓ 12	84 ∓ 34
Quick after 168 h or death (%)	81 ∓ 26	105 ∓ 8	96 ∓ 50
Thrombocytes after 168 h or death (µL)	163.000 ∓ 107.000	314.000 ∓ 259.000	115.000 ∓ 57.000

Conclusion: The cell-based extracorporeal immune support improves the outcome in a pig model of sepsis. Bioartificial phagocytosis assistance may be a new tool in the treatment of severe infection.

O168 Synergistic effect of muramyldipeptide with lipopolysaccharide and nonendotoxin-stimuli

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Objectives: The bacterial components serving for pattern recognition by the innate immune system are increasingly identified. However, little is known about the interplay of these components. Here, muramyldipeptide (MDP; *N*-Acetylmuramyl-L-alanyl-D-isoglutamine), i.e. a part of the peptidoglycan with a strong adjuvant effect, was characterized as to its synergistic effect with lipopolysaccharide (LPS) in monocytes and macrophages. Further, we investigated the structural requirements and besides LPS also other nonendotoxin-stimuli. We considered the receptor- and signalling-systems of MDP which are not yet completely known.
Methods: Employing a human whole blood cytokine release model or murine primary macrophages, 15 synthetic muropeptide-derivatives were tested as to their immunomodulatory effects.
Results: A number of these derivatives were potent synergists of lipopolysaccharide (LPS) and induced cytokine release, but did not induce an immune response on their own. MDP was the minimal bioactive structure of peptidoglycan. Further we observed a stereoselective recognition, i.e. other stereoisomers were not active. The LPS (*Salmonella abortus* equi) concentration–response curve with respect to TNF-α-release was shifted by a factor of 1000 when 20 pM MDP was added. In combination with nonendotoxin-stimuli like CpG-oligonucleotides or MALP-2 from mycoplasm we also observed synergistic effects of MDP, but not together with lipoteichoic acid (LTA) from *Staphylococcus aureus* or its exotoxin (SEB). Since receptor- and signalling-cascades have not yet been identified, toll-like receptor 2 (TLR-2) knock-out or C3H/HeJ LPS-nonresponder mice carrying a mutated TLR-4 were employed. In different macrophages from both mice, synergy of MDP with different immune stimuli was found. These findings suggest that MDP does not require TLR-2 or TLR-4.
Conclusions: MDP has a priming effect on the immune system. The results indicate that breakdown products of naturally occurring peptidoglycan components, which are ubiquitous in all bacteria, cause in the host stronger effects with other bacterial components than the substances alone. This potent synergy has implications for pyrogen testing, putative therapeutic strategies in infection and sepsis as well as for the development of novel immunostimulators.

O169 Lipoteichoic acid from *Staphylococcus aureus* is a strong stimulus for neutrophil recruitment

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Objectives: *Staphylococcus aureus* infections are often associated with pus formation. This implies that a major immune response to these bacteria is the recruitment and activation of neutrophils. Where lipopolysaccharide (LPS) is the major immunostimulatory principle of Gram-negative bacteria, lipoteichoic acid (LTA) is the equivalent molecule of the Gram-positive bacteria. Effects of highly pure bioactive LTA on neutrophil functions and chemokine induction were investigated.
Methods: The expression of tlr-2 on human neutrophils was investigated by means of flow cytometry. Isolated human neutrophils or human whole blood were incubated with LPS or LTA and release of degranulation products, cytokines or chemokines were measured by ELISA. Oxidative burst was measured by luminol-enhanced chemiluminescence induced by LPS, LTA or PMA alone or in combination.
Results: Neutrophils express the key receptors for LTA signaling, CD^{14} and tlr-2, however, LTA did not induce or prime for oxidative burst in whole blood or human neutrophils. Further, LTA did not induce degranulation of bactericidal permeability increasing protein (BPI) in whole blood at concentrations up to 10 µg/mL. LTA did not stimulate the release of significant amounts of TNF-α or leukotriene-B4 (LTB4) in purified neutrophils. However, LTA induced a very fast release of LTB4 in whole blood which could be attributed to the monocytes. The maximum of LTB4 release was similar to that induced by the same concentration of LPS from *S. abortus* equi. Four LPS (*E. coli*, *K. pneumoniae*, *B. pertussis*, *P. aeruginosa*) with different potencies were titrated to induce the same amount of TNF-α as 10 µg/mL LTA (*S. aureus*) in whole blood. All stimuli also induced equal amounts of IL-6. However, LTA induced more of the chemokine IL-8 than any of the LPS and induced comparable amounts of the chemoattractants MCP-1 and MIP-1α. LTA also induced more granulocyte colony-stimulating factor (G-CSF) release than the LPS.
Conclusions: Although LTA does not seem to activate neutrophils directly, it is a strong stimulus for the recruitment of neutrophils from the bone marrow via G-CSF induction and attraction of neutrophils to the site of infection via stimulation of chemokine release.

O170 Generation of fully human monoclonal antibodies specific to bacterial and fungal pathogens in a human/mouse radiation chimera: the Trimera system

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The emergence of drug resistant pathogens is becoming a global concern, which requires new therapeutic approaches. Antibody therapies for treating and preventing bacterial or fungal infections constitute about 25% of the current drugs in development.

Objectives: Generation of HmAbs for treating and presenting GNB and *Candida albicans* infection.

Methods: We have taken that approach to generate fully human monoclonal antibodies (HmAbs) directed against different pathogens such as *C. albicans*, Gram-negative bacteria (GNB) and *Staphylococcus aureus* (Staph A). These antibodies are being produced in the Trimera mouse system, which was shown previously to be a powerful tool to generate HmAbs against hepatitis B and hepatitis C viruses. This system consists of normal strains of mice that were rendered permissive for engraftment of human cells and tissues by lethal total body irradiation and radioprotection with SCID mouse bone marrow cells.

Results: We have employed the Trimera system to generate HmAbs against flagellin from GNB and against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *C. albicans*. Flagellin has a role in mucosal attachment and invasion and induces pro-inflammatory gene expression ultimately causing shock and mortality. GAPDH is a glycolytic enzyme. It is expressed on a fungal cell wall and has a role in *C. albicans* attachment to fibronectin and laminin adhesion molecules. These properties make these two molecules good candidates for immunotherapy. In order to produce HmAbs against GNB flagellin, Trimera mice were transplanted with lymphocytes from donors positive for antiflagellin antibodies. Immunization of these Trimera mice with the N-terminal part of flagellin presented on autologous dendritic cells resulted in amplification of the human specific immune response to GNB flagellin, up to 60-fold over the response of the donor. Similarly, immunization of Trimera mice with *C. albicans* GAPDH resulted in amplification of the human specific immune response to GAPDH, up to 40-fold over the response of the donor.

Conclusions: Hybridoma clones were generated from human B cells harvested from the spleens of the responding mice. These hybridomas secrete human anti-flagellin IgG and anti-GAPDH IgG. The properties of these mAbs are currently being investigated. Thus, the Trimera mouse system is an effective tool to amplify human immune responses in mice, leading to the generation of therapeutic human monoclonal antibodies.

O171 Curliated *Escherichia coli* and the sudden infant death syndrome

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Objectives: To explore the role of curliated *E. coli* and soluble curlin in the pathogenesis of SIDS.

Methods: Curlin production of a random selection of *E. coli* strains isolated from the intestines of 94 SIDS babies and 17 dead controls as well as 92 healthy baby stools was examined by culture on Congo red agar (CRA). Sera from these groups of babies were examined by dot-immunoblot and Western blot for soluble curlin (CsgA) using affinity purified rabbit anti-CsgA antibody.

Results: 100% of SIDS *E. coli* isolates (94 of 94 individual babies' isolates) produced curli when grown at 26° C for 48 h on CRA. Control isolates from non-SIDS deaths (13 of 17 individual isolates) had a significantly lower rate of curli production ($P = 0.00002$) than SIDS strains. 74 of 92 (80.4%) healthy baby isolates produced curli ($P = 0.00005$). The 15 kDa bands (matching CsgA control material) were seen on immunoblots of SIDS sera but not dead or healthy control sera.

Conclusion: Curliated *E. coli* and soluble curlin may be involved in the pathogenesis of SIDS. Physiological effects of curlin support this hypothesis.

O172 Alteration of numbers of circulating and lung-derived lymphocyte subsets after endotoxin challenge

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Objectives: A significant morbidity and mortality of intensive care patients is caused by secondary bacterial infections with bacterial pneumonia being the leading bacterial complication. The development of secondary infections has been associated with alterations of cell-mediated immunity including reductions of CD^{4+} T and NK cells in peripheral blood (PB). The aim of our study was to evaluate whether similar reductions of immune cells during sepsis also occur with lung-resident lymphocyte subsets.

Methods: Experimental sepsis-like condition was initiated in BALB/C mice with 10 µg of endotoxin given intraperitoneally; 24 h after the initial challenge animals were sacrificed by CO_2 anesthesia, exsanquinated, and circulation was perfused with 35 mL of cold physiologic solution. Lung-derived lymphocyte subsets were obtained by enzymatic digestion of lung tissue with collagenase and DNAse (both Sigma, Saint Luise, USA). The PB and lung-derived lymphocyte subsets were quantified with monoclonal antibodies purchased from Pharmingen (San Jose, USA) using Tru-Count TubesTM (Becton-Dickinson, San Jose, USA). Analysis was performed with three-color flow cytometer (FACScan, BD, San Jose, USA).

Results: The results are shown in the table.

	Peripheral blood (cells/mm^3)		Lung (cells/lobe × 10^3)	
Lymphocyte subsets	Control ($n = 8$)	Endotoxin ($n = 6$)	Control ($n = 8$)	Endotoxin ($n = 6$)
CD^{3+} cells	2845 ± 232	819 ± 286*	151.1 ± 29.2	76.3 ± 17.7#
CD^{4+} T cells	2294 ± 181	676 ± 205*	120.6 ± 18.3	58.4 ± 15.7#
CD^{8+} T cells	748 ± 52	192 ± 52*	47.4 ± 14.6	25.9 ± 7.1
B cells	943 ± 136	322 ± 123*	75.4 ± 27.2	50.1 ± 13.7
NK cells	346 ± 72	184 ± 75	59.9 ± 23.7	41.3 ± 14.4

*$P < 0.01$.
#$P \leq 0.05$ (statistical analyses employed Student's *t*-test). Data are presented as mean ± SE.

Conclusion: Our results demonstrate that experimental endotoxemia significantly reduced numbers of CD^{4+} T cells, CD^{8+} T cells, and B cells in PB as well as CD^{4+} T cells resident in the lungs. This suggests that an inflammatory response could establish an important predisposing condition for secondary bacterial pneumonia.

Acknowledgements: The study is supported by the grant no. 3D43TW00915 from the Fogarty International Center, NIH, Bethesda, USA.

O173 Monocyte and lymphocyte apoptosis resistance in patients affected by brucellae infection

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Objective: Brucellosis is endemic in southern Italy and its islands. Although antibiotic therapy quickly induces a clinical improvement, in several patients the infection persists and, after several months, induces relapses and become chronic. The bacteria infect monocytes and macrophages developing within these mononuclear cells. Recent data have shown that monocytes infected in vitro with brucellae become resistant to Fas-ligand-induced apoptosis. We studied the sensitivity of peripheral blood mononucleated cells (PBMC) to apoptosis and the expression of different immunologic and apoptotic markers in PBMC in 10 infected patients (two in chronic phase and eight in acute phase).

Methods: Apoptosis in monocytes and lymphocytes was evaluated in vitro after 24 h of CH11 (Fas-agonistic) treatment by flow cytometry using a three-color panel of fluorescent agents: annexine-V, propidium iodide and anti-CD^{61} or anti-CD^3. Moreover, the expression of Fas (CD^{95}) in CD^{4+} and

CD^{8+} lymphocytes and the percentage of CD^{4+}, CD^{8+} and CD^{38+} lymphocytes were also determined.
Results: Before the start of therapy, monocytes of all patients showed a high level of resistance to CH11-induced apoptosis. This resistance persisted 10–20 days after the start of therapy and then quickly decreased. Interestingly, lymphocytes were also more resistant to CH11-induced apoptosis compared with lymphocytes of healthy donors, although their resistance was lower than that observed in monocytes. The number of CD^{4+} and CD^{8+} cells was normal in all patients while the percentage of CD^{4+} lymphocytes expressing CD^{95} was increased in 50% of patients. No modification in CD^{95} expression was observed in monocytes. At the time of diagnosis all patients showed a high percentage of CD^{8+} lymphocytes expressing CD^{38}. This percentage decreased within one month from the start of therapy.
Conclusions: We demonstrated that PBMC of patients affected by brucellae infection were resistant to apoptosis despite the increase in CD^{95} and CD^{38} expression on lymphocytes. Our data indicate that the extrinsic apoptotic pathway (Fas/Fas-L) is not involved in CH11-resistance. Considering that lymphocytes are not infected by brucellae, their apoptosis resistance may be due to a soluble factor released by infected monocytes. The evidence of apoptosis resistance in PBMC of chronic patients suggests that the evaluation of sensitivity to CH11-induced apoptosis in monocytes may be used to test the effectiveness of therapy.

Pharmacokinetics of quinolones

O174 Kill kinetics of fluoroquinolones (FQ) in in vitro pharmacodynamic models of infection

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Background: Models that interpret both pharmacokinetics and bacterial response provide information about the bactericidal activity of the agents studied under clinically relevant experimental conditions. The aim of this study was to assess the speed of killing of various fluoroquinolones (FQ) against Gram-negative (GN) and Gram-positive (GP) bacteria.
Materials and methods: A one-compartment model was used. Serum concentrations following different oral doses of ciprofloxacin, moxifloxacin, ofloxacin, levofloxacin and gatifloxacin were simulated. Ciprofloxacin and ofloxacin were dosed twice, the others once daily. Three strains (varying in FQ susceptibility) of the following species were studied: *E. coli, K. pneumoniae, P. aeruginosa, S. aureus,* and *S. pneumoniae.* The antibacterial effect was quantitated by calculating the area under the cfu/mL curve and the time to 99.9% killing.
Results:
1 All FQs exhibited a dose-dependent effect against GNs however, only moxifloxacin and gatifloxacin exhibited a dose-dependent effect against GP.
2 Ciprofloxacin was the most active FQ against GN and reduced viable counts most rapidly.
3 *P. aeruginosa* exposed to ciprofloxacin was significantly affected and resistance did not develop. However, exposure to levofloxacin resulted in regrowth and resistance development.
4 Against *K. pneumoniae* both ciprofloxacin and moxifloxacin were significantly more active and reduced viable counts more rapidly than any other quinolones studied.
5 The speed of bactericidal kill against GP was greatest for moxifloxacin.
6 *S. pneumoniae* strains with gatifloxacin MICs of 0.25 and 0.5 mg/L regrew; postexposure isolates had gatifloxacin MICs of 0.5–2 mg/L
7 In contrast, moxifloxacin reduced cfu even of the borderline susceptible *S. pneumoniae* strain below the limit of detectability within 12 h and no resistance developed.
Conclusions: Amongst the quinolones tested, ciprofloxacin and moxifloxacin showed an enhanced rate of killing. Ciprofloxacin was the most active quinolone against GN and moxifloxacin the most active one against GP. Moxifloxacin demonstrated superior activity against borderline susceptible *S. pneumoniae* with no resistance development.

O175 Population pharmacokinetics of moxifloxacin intravenous in healthy subjects and patients with community-acquired pneumonia

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Background: Moxifloxacin (MXF) is an 8-methoxy-quinolone with excellent in vitro activity against a variety of respiratory pathogens.
Methods: The PK data obtained from a subgroup of patients with community-acquired pneumonia (CAP, 60 + 18 years and 76 + 22.5 kg, $N = 307$) participating in two multicentered, randomized clinical trials were compared with those from healthy subject (HV) studies ($N = 65$). An amount of 400 mg MXF was given once daily to the patients as intravenous (i.v.) drip infusion (median 60 min, range 45–137 min), whereas the HV received controlled short infusions (30 or 60 min) in the dose range of 100–400 mg SD and 400 mg once daily. For the CAP patients sparse plasma sampling was applied, whereas rich data sampling was obtained from the HV. Conventional and population PK (PPK) evaluations were used to analyze the data.
Results: PK of patients and HV were comparable. Mean peak concentrations were slightly higher for patients after the first dose (5.01 mg/L vs. 3.5 mg/L), whereas they were comparable to those of the HV at steady-state (4.5 mg/L vs. 4.1 mg/L). This effect was probably caused by a disease-induced reduction of the distribution volume (e.g. altered blood flow), which returned to normal upon recovery of the patient. Owing to the application of drip infusion the variability of C_{max} in the patients was ~50% relative to ~23% in HV. The dominant terminal half lives were comparable for patients and HV. Earlier findings in HV of a slight, but not clinically relevant gender effect on the PK of MXF were confirmed in patients (e.g. mean C_{max} 4.5 + 4.1 mg/L males; 5.6 + 2.8 mg/L females). PPK calculations estimated similar AUC for patients and HV; the patients' PPK estimates were biased owing to the complexity of the structural PK model and the necessity of sparse data sampling.
Conclusion: Overall, the PK data for MXF from CAP patients and those from healthy subjects were comparable and there was no evidence that any patient subset might require dose adjustments.

O176 Different potentials of fluoroquinolones to prevent bacterial resistance: can they be distinguished using in vitro dynamic models?

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Objectives: To examine the ability of moxifloxacin (MXF) and levofloxacin (LVF) to prevent the selection of resistant mutants of *Staphylococcus aureus,* multiple dose simulations were performed over a wide range of the ratio of area under the curve (AUC) to MIC.
Methods: A clinical isolate of methicillin-resistant strain of *S. aureus* was selected for the study. The MICs of MXF and LVF determined by multiple serial dilutions were 0.09 and 0.5 mg/L, respectively. The 'mutant prevention concentrations' (MPC) of MXF and LVF were 0.33 and 1.75 mg/L, respectively. A series of monoexponential pharmacokinetic profiles that mimic once-daily administration of MXF (half-life 12 h) and LVX (half-life 6.8 h) for 3 days were simulated in vitro. With each quinolone, these profiles were designed to provide peak concentration at levels equal to the MIC, between the MIC and MPC, i.e. within the 'mutant selection window', and above the MPC. The respective AUCT/MIC ratios varied from 12 to 15–222–242 h, where T is the 24-h dosing interval, and the starting inoculum was 108 cfu/mL. Changes in *S. aureus* susceptibility were examined daily with repeated MIC determinations.
Results: No MIC increase was seen at the peak concentrations equal to the MICs, i.e. at the lowest simulated AUCT/MIC ratios (13–15 h). Within the 'window' (AUCT/MICs of 27–29 and 53–58 h), significant increases in MICs were observed following treatment. With both quinolones, only minimal MIC increases were observed at AUCT/MICs of 107–116 h, and no MIC changes were seen at AUCT/MICs of MXF of 222 h and LFX of 242 h (trough levels above MPC). These 'protective' AUCT/MIC ratios correspond to 65% of the usual clinical dose of MXF and 220% of the clinical dose of LVF (Fig. 1).

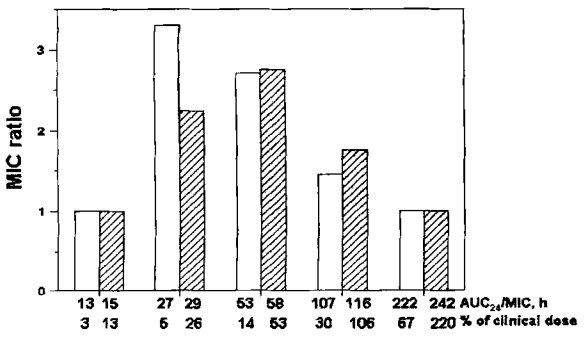

Figure 1 Ratio of final MIC to initial MIC for *S. aureus* exposed to different AUC_{24}/MICs of moxifloxacin (□) and levofloxacin (▨).

Conclusion: These data suggest that (1) in vitro dynamic models can be used to predict relative ability of quinolones to prevent mutant selection and (2) in this model MFX protects against resistance development at subtherapeutic doses whereas LFX provides a similar effect only at doses that exceed its clinical dose.

O177 Levofloxacin compensates with pharmacokinetics for less favourable pharmacodynamics towards *P. aeruginosa* compared to ciprofloxacin

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Objective: Ciprofloxacin has been regarded as the gold standard for the antimicrobial activity for infections with *P. aeruginosa*. Levofloxacin is less active if MICs are the measure of activity. However, levofloxacin has a more favourable pharmacokinetic. In an in vitro model, we simulated the pharmacokinetics of levofloxacin and ciprofloxacin after intravenous injection to follow the kill kinetics of *P. aeruginosa* wild type (5087 mL) and a low level resistant mutant (5087M1). Furthermore, the capacity of the drugs to select for resistant mutants was of interest.
Methods: We used an in vitro model according to Grassso, simulating the intra venous injection of 400 and 800 mg ciprofloxacin or 500, 750, and 1000 mg levofloxacin. The elimination of the drug out of the central compartment was regulated by a peristaltic pump, which added fresh Müler–Hinton broth. The samples were taken after 0.5, 1–6, 8, 10, 12, and 24 h, to measure the bacterial count on nutrient agar NI and the concentration of antibiotics by bioassay with *K. pneumoniae* as a test organism.
Results: The effect of levofloxacin on *P. aeruginosa* 5087 mL, the wild type strain, after the simulation of 500-mg i.v. dosage was less effective than a 400-mg dosage of ciprofloxacin for the first 6 h, when the reduction in cfus with both drugs was equal. Thereafter, ciprofloxacin allowed a slow regrowth, whereas levofloxacin kept a bacteriostatic activity. Doubling the ciprofloxacin dosage did not result in a marked increase of the effect, whereas a doubling of the levofloxacin dosage resulted in a further reduction of the bacterial count to nearly six orders of magnitude. With the efflux mutant M1 results were similar, however, regrowth occurred earlier. With none of the drug dosing, we saw a pronounced selection of resistant mutants. All isolates picked after the regrowth were sensitive. Resistant mutants always were a minor portion of the total count.
Conclusion: The favourable pharmacokinetic properties of levofloxacin seem to outweigh the less pronounced pharmacodynamics of this drug, as compared to ciprofloxacin. Because of the higher prolonged concentration of levofloxacin during the experiments, the overall effect of levofloxacin was slightly better than that of ciprofloxacin.

O178 Concentrations of moxifloxacin at the focus of infection of patients suffering from erysipelas after a 400-mg single dose

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Objective: The technique of microdialysis offers a powerful method to get more detailed PK information from infected lesions than 'classical' methods so far applied to patients. The aim of this study was therefore to apply this technique to determine moxifloxacin (MXF) concentrations at the focus of infection of in-patients suffering from soft-tissue infections and to compare them with those observed in noninfected tissue.
Methods: Six patients who were hospitalized for the treatment of soft tissue infections (erysipelas; mean age = 53.8 years) received a concomitant single 400 mg intravenous 1 h infusion. Blood samples were taken over 96 h to assess the pharmacokinetics of MXF. In addition microdialysate was collected for 8 h, in 0.5 h fractions after start of infusion in the infected and the noninfected tissue (contralateral extremity) to determine the unbound interstitial drug concentrations. Drug concentrations were measured using validated HPLC with fluorescence detection. The non-compartmental PK data were calculated; statistical assessment was done with ANOVA. To evaluate safety and tolerability, physical examination, haematology, clinical chemistry, urinalysis, and ECG were assessed, AEs were recorded.
Results: There were no serious adverse events in the study, the treatments were well tolerated. PK of MXF in plasma were comparable to those known from previous studies in healthy subjects. In the interstitial fluid of the tissues, geo. mean $AUC_{0\text{-}tn,u}$ and $C_{max,u}$ were increased by 133 and 161% comparing the infected with the not infected body site ($AUC_{0\text{-}tn,u}$ 6.84/2.5 mg h/L vs. 2.94/2.43 mg h/L, $C_{max,u}$ 1.78/2.78 mg/L vs. 0.68/2.45 mg/L, geo. mean/SD), with a within subject variability of about 120% for both parameters.
Conclusion: MXF was well tolerated in the study. The kinetic data related to the tissue indicate that MXF seems to accumulate in infected areas. This can be explained by the distribution characteristics of MXF itself and the pathophysiological changes at the focus of infection. The results of this study indicate that MXF has favorable distribution characteristics supporting its use to treat skin and soft tissue infections.

O179 Penetration of moxifloxacin into the infected tissues of patients suffering from diabetic foot infections determined by microdialysis

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Objective: Microdialysis has advantages compared to the 'classical' sampling techniques for kinetic studies. In the present study, microdialysis was used to compare the concentrations of moxifloxacin (MXF) at the focus of infection with those at the noninfected contralateral site in 'diabetic foot' patients.
Methods: Six hospitalized patients treated for diabetic foot infections (mean age = 64.3 years) received a concomitant single 400 mg i.v. 1 h infusion of MXF. Blood samples were collected up to 96 h after start of infusion to determine the PK of MXF. Microdialysate was sampled for 8 h after start of infusion in 0.5 h fractions to determine the unbound interstitial drug concentrations in the infected and the noninfected tissue (contralateral extremity). Drug concentrations were measured with a validated HPLC method and fluorescence detection. The non-compartmental methods were applied for the PK evaluation; for statistical analysis ANOVA was used. Physical examination, haematology, clinical chemistry, urinalysis, and ECG were assessed and AEs were recorded to evaluate safety and tolerability.
Results: There were no serious AEs in the study, the treatments were well tolerated. PK of MXF in plasma were comparable to those known from previous studies in healthy subjects. At the focus of infection the $AUC_{0\text{-}tn,u}$ and $C_{max,u}$ were decreased by 38 and 24% compared to the not infected body site ($AUC_{0\text{-}tn,u}$ 3.77/1.92 mg/h/L vs. 6.07/1.88 mg/h/L, $C_{max,u}$ 0.83/2.38 mg/L vs. 1.10/1.81 mg/L, geo. mean ± SD). The within-subject variability was ~74% for $AUC_{0\text{-}tn,u}$ and 96% for $C_{max,u}$. The half-lives were 4.85/01.17 and 6.06/01.22 h (infected vs. not infected, geo mean ± SD).
Conclusion: MXF was well tolerated in the study. The tissue PK data for MXF reflect the pathophysiological changes at the focus of infection of the diabetic

foot patients. Owing to the deterioration of perfusion caused by the disease related microangiopathies MXF concentrations in the infected area are lower than in the noninfected tissues. The results of this study are in line with previous findings for other anti-infectives studied in this population.

O180 Pharmacodynamics of levoxacin in the treatment of infective endocarditis

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Objective: Infective endocarditis (IE) is a severe and life-threatening infection, where standard treatments often failed. Levofloxacin (L) has been reported as an attractive option in animal models, but clinical data are lacking. Our aim is to evaluate the clincal outcome of therapeutic regimens including high dose levofloxacin, whose pharmacodynamics (PD) was investigated.
Methods: Five patients with uncomplicated community-acquired left-sided and two patient with 'late' prosthetic valve IE received L 500 mg/day i.v., Ceftriaxone (CX) 2 g/day i.v. and netilmicin (N) 300 mg/day i.v. At the end of the 2nd week of therapy, CX and N were suspended and L switched to oral administration. For each patient, eight blood samples were collected from 0 to 12 h post-dosing after the 6th i.v. and oral administration, to compare L serum levels, measured through standard HPLC technique. Blood cultures were taken at baseline, 5th and 28th day. Because, L had been given twice daily, in the PD analysis we only correlated the 24 h-area under the curve (AUC_{24}) with the L minimum inibitory concentration (MIC) of the isolate.
Results: Viridans Streptococci with respective L-MIC of 1, 0.5 and 0.5 mg/L were isolated in three patients, oxacillin-sensible *S. aureus* with L-MIC of 0.125 mg/L in 3, *K. oxytoca* with L-MIC of 1 mg/L in one. Overall, the duration of treatments was 28 days. No treatment-related side effect has been recorded. All patients had a favourable outcome, confirmed at 90-days follow-up. The serum values and pharmacokinetic profiles of L (i.v. and *per os*) were as expected, and overlapped in all patients evaluated. The AUIC (ratio AUC_{24}/MIC) were markedly over 220 in all cases.
Conclusion: Our findings, although very preliminary, suggest that L may have a role in the treatment of IE, providing an AUIC greater than 220. Should those findings be confirmed by larger trials, the equivalence of the intravenous and oral administration of L may be useful for both early switch programs and discharge.

O181 Efficacy and pharmacodynamics of human-like treatment with moxifloxacin (MOX) compared to levofloxacin (LEV) on experimental pneumonia due to penicillin-resistant pneumococci (PRSP) with decreasing susceptibilities to fluoroquinolones (FQ)

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Dijon, F

Background: The increasing frequency of PRSP with reduced susceptibility to ciprofloxacin (CIP) raises the question of the therapeutic efficacy of the new FQ on infections due to such strains.
Objectives: To investigate the in vivo efficacy of MOX and LEV on experimental pneumonia due to strains with various levels of susceptibility to FQ.
Methods: 1-PRSP pneumonia in rabbits treated with human-like treatment of MOX 400 mg/day, LEV 500 mg/day for 48 h; 2-pharmacokinetic data was obtained for each animal; 3-bacterial content (log cfu/g) in lung and spleen. The susceptibility profiles of the strains for CIP/LEV/MOX were (MIC, mg/L; genotype): strain SSS: 0.5/0.5/0.125, strain RSS: 8/1.75/0.25 (*parC* mutation), strain RRS: 64/16/0.5 (*parC* mutation), strain RRR: 64/16/4 (*parC* and *gyrA* mutations), respectively.
Results: 6–12 animals per group were tested. Lung bacterial reductions (log cfu/g) for LEV and MOX were: strain SSS: 4.2 ± 2 vs. 4.8 ± 2; strain RSS: 2.4 ± 2 vs. 1.3 ± 2 ($P = 0.1$); strain RRS: 0.9 ± 0.9 vs. 3.1 ± 0.7 ($P < 0.01$); strain RRR: 0.3 ± 2 vs. 0.8 ± 0.3. MOX was more effective in spleen than LEV ($P < 0.01$). Mutants were detected in lungs for RSS and RRS strains for both antibiotics. C_{max}/MIC, AUC/MIC and $T >$ MIC were strongly correlated and associated to in vivo antibacterial efficacy. A CART analysis identified discriminant PK–PD values for >2 log cfu bacterial reduction on MOX: C_{max}/MIC * 6.4, AUC/MIC * 32 and $T >$ MIC * 30 ($P = 0.04$). When MICs are normalized, in vivo mutants appeared when AUC/MIC_n were between 7 and 31 for both antibiotics ($P = 0.03$).
Conclusion: In this in vivo pneumococcal pneumonia model with human adapted treatment, MOX is (i) effective against CIP- and LEV-resistant PSDP, (ii) mutants appear in vivo when PRSP harbor *parC* mutation for both LEV and MOX; (iii) mutants did not appear in vivo for AUC on normalized MIC ratio >31.

Surgical infections: solutions to the emergence of antibiotic resistance (Symposium arranged by Pfizer)

S185 Definitions related to surgical infections

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A surgical infection is generally regarded as one that requires anatomic ("surgical") intervention to ensure its resolution, and which normally will not resolve upon antimicrobial intervention alone. The intervention may be as simple as opening a recent incision to drain a surgical-site infection or placing a percutaneous catheter in an abscess, or as complex and invasive as the debridement of infected retroperitoneal necrosis associated with pancreatitis. Thus surgical infections encompass a wide range of infections. Many terms have been applied to postoperative infections. To simplify communication, the Centers for Disease Control and Prevention in the United States of America, in collaboration with the Society for Healthcare Epidemiology of America and the Surgical Infection Society, has recommended that all such infections be called surgical-site infections (SSIs). These can be divided into superficial incisional infections, which occur above the muscular fascia and involve skin and subcutaneous tissues only, deep incisional infections, which penetrate as far as the muscular fascia, and "organ space" infections, which involve the body cavity or organ space of the operation away from the site of incision (peritoneal cavity, pleural space, mediastinum, joint space, etc.). All SSIs are, by definition, postoperative. The risk of an SSI is often estimated using the National Nosocomial Infections Surveillance risk index. Organ-space infections may occur without prior surgical intervention, and are variously termed peritonitis, intra-abdominal abscess, emphysema, pericarditis, septic arthritis, etc. Peritonitis can be divided into primary (usually not surgical), secondary, and tertiary forms. In some instances, the words sepsis and infection have been used interchangeably. However, we usually reserve the term sepsis to indicate a particular systemic response to infection. Many patients have a systemic inflammatory response that cannot be attributed to infection. This condition is called systemic inflammatory response syndrome (SIRS). When a systemic inflammatory response is caused by infection, the condition is termed sepsis or SIRS with infection. Two other terms, severe sepsis and septic shock, refer to sepsis associated with organ failure or hypotension, respectively. Severe sepsis and septic shock can occur in response to infections due to any pathogen and are not defined by Gram-negative, Gram-positive, fungal, or other pathogen characteristics, although certain pathogens (such as *Streptococcus pyogenes* and *Clostridium* spp.) are more likely to cause sepsis than others.

S186 The increasing threat of resistance in surgical infections

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Despite developments in surgical techniques and antibiotic prophylaxis, postoperative infection remains a considerable cause of morbidity and mortality among surgical patients. It is also responsible for considerable increases in the duration and cost of hospital stay. Indeed, it has been estimated that a single surgical wound infection accounts, on average, for 7 days of additional hospitalization and an increase of more than US$ 3000 in hospital charges.

Overall, in the USA, the annual direct cost of postoperative infection is probably well in excess of US$ 1.5 billion. The choice of appropriate antimicrobial agents for prophylaxis and treatment of postoperative infections requires an understanding of the microbiology of surgical infections, available antimicrobial agents, susceptibility and resistance patterns among likely pathogens, and individual risk factors for infection. The organisms most frequently associated with superficial (incision) wound infection are Gram-positive cocci, in particular *Staphylococcus aureus*. In the case of infectious complications, following clean surgical procedures that involve the implantation of prosthetic devices, it is coagulase-negative staphylococci and *S. aureus* (quite often methicillin-resistant strains) that predominate. Intra-abdominal and pelvic postoperative infections are most often characterized by a mixture of aerobic and anaerobic flora. The most common aerobes are Enterobacteriaceae (*Escherichia coli*, *Proteus* spp., and others) and enterococci, while among anaerobes *Bacteroides fragilis* group species prevail. The involvement of fungi (such as *Candida* spp.) also needs to be considered. Infections in patients who have been hospitalized for a long time or who have received antibiotics previously are more likely to involve resistant bacterial strains. Adequate drainage, surgical control of the source of infection, and effective adjunctive antimicrobial therapy are important factors in the successful treatment of postoperative infections. In the case of intra-abdominal and pelvic infections, antimicrobial therapy should be directed against both Gram-negative enteric and anaerobic bacteria. Combinations of aminoglycosides with clindamycin or metronidazole have been widely used with great success. In recent years, monotherapy with either a carbapenem or a β-lactam/β-lactamase inhibitor combination has proved effective in both clinical trials and clinical practice. Furthermore, β-lactam/β-lactamase inhibitor combinations have been shown to be effective options for perioperative prophylaxis during abdominal and pelvic surgery.

S187 General principles in surgical prophylaxis

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Antimicrobial prophylaxis can decrease the incidence of infection, particularly wound infection, after certain operations, but this benefit must be weighed against the risks of toxic or allergic reactions, the emergence of resistant bacteria, drug interactions, the selection of bacteria, and superinfection. Antimicrobial prophylaxis is recommended only for procedures with high infection rates, i.e. those involving the implantation of prosthetic material and those for which the consequences of infection are especially serious. An effective prophylactic regimen should be directed against the most likely infecting organisms but need not be active against every potential pathogen. Regimens that decrease the total number of endogenous and exogenous bacteria generally permit host defenses to resist clinical infection. Infection is prevented when therapeutically effective concentrations of an antibiotic are present in the blood and the tissue during and after the procedure, when the risk of contamination is maximal. Therefore, antimicrobial prophylaxis should commence just before the operation. The initiation of prophylaxis earlier is less effective, and its initiation after the operation is meaningless. A single dose given just after the induction of anesthesia is sufficient to achieve therapeutic drug levels at the time of maximal endogenous or exogenous contamination without selecting bacterial strains resistant to the drug. If the surgical procedure is delayed or prolonged (more than 3 h), or if major blood loss occurs, a second dose might be advisable, especially if the chosen agent has a short elimination half-life. For this reason, the use of a long-acting antibiotic agent in long-lasting procedures is to be preferred. The postoperative administration of prophylactic drugs is usually unnecessary (except in the case of heavy bleeding associated with the development of new hematomas) and, furthermore, is considered harmful. Prophylaxis that continues for more than 24 h will result in the selection of bacterial strains and the emergence of drug-resistant bacteria. Despite the existence of several consensus guidelines, the selection of an antibiotic prophylaxis regimen must take into consideration the local resistance patterns, the time of preoperative hospitalization, and, in particular, the presence of risk factors for infection, including those related to antibiotic use, the surgical procedure, and the environment. These factors are associated with significant increases in morbidity and mortality, and may indicate the need to modify an antimicrobial prophylaxis scheme in high-risk patients.

S188 The role of beta-lactam/beta-lactamase inhibitor combinations in surgical infections

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Many surgical infections are characterized by synergistic polymicrobial mixed infection, for which broad-spectrum antimicrobial therapy is usually administered on an empiric basis. Until relatively recently, standard empiric therapeutic regimens have involved the use of two or more antibiotics in combination, such as an aminoglycoside and an antianaerobic agent (clindamycin or metronidazole), to achieve adequate aerobic and anaerobic coverage. Evidence from a number of clinical studies suggests that single-agent therapy with a β-lactam/β-lactamase inhibitor combination is a clinically effective and cost-effective alternative to multidrug regimens, as well as to monotherapy with cephalosporins or carbapenems, in the treatment of mixed infections. It has been shown that β-lactam/β-lactamase inhibitor combinations can be used effectively in the treatment of intra-abdominal infections, gynecological pelvic, diabetic foot, and other mixed infections. The β-lactam/β-lactamase inhibitor combinations are also suitable for use in perioperative prophylaxis, and may offer benefits over other agents in terms of reduced incidence of surgical wound infections and lower costs. A β-lactam/β-lactamase inhibitor combination has been shown to be a cost-effective alternative for the prophylaxis of intra-abdominal infections.

Treat the infection or follow the protocol? An interactive debate

S193 Follow the protocol: keep a fallback choice in the therapy of infections in the critically ill

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Infections in the critically ill are a major source of morbidity and mortality, but are increasingly due to multiresistant organisms. These include resistant Gram-negative bacteria (*P. aeruginosa*, *Acinetobacter* species) and resistant Gram-positive organisms (methicillin-resistant *S. aureus*, vancomycin-resistant enterococcus). Although data show that initially adequate empiric therapy can lead to improved outcomes, the exact method for achieving such therapy is uncertain. One argument is that the uncontrolled and empiric use of broad-spectrum antibiotics in the intensive care unit can only add to the problem of resistance and have the end result of making initial therapy even more likely to be ineffective. In order to treat patients with effective antibiotic therapy it is necessary to have tight control over the use of antibiotics. This means no unrestricted use, and using the appropriate drug for each specific patient. Thus, we need protocols for empiric therapy of the critically ill that define the likely organisms, and take into account local susceptibility patterns. In this context, we should use the most focused therapy regimen possible, while still covering all likely etiologies. The use of a more broad-spectrum approach has no added clinical benefit and can only provide more selection pressure for antibiotic resistance. In addition to empiric therapy being focused, it is necessary to stop antibiotics or narrow the spectrum of therapy, once bacteriological and clinical response data become available. In this way, we will not use our most potent antibiotics excessively, and will have active agents, such as the carbapenems, the oxazolidinones, and advanced β-lactams as fallback choices for patients who really need them.

S194 Cover all possibilities – eliminate the risk of resistance

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Patients suspected of having an infection are usually treated according to a standard protocol that follows the hospital formulary or other guidelines; this usually includes guidance on the choice of antibiotic as well as dosing regimen

that should be used. However, guidelines are usually based on antibiotic efficacy and coverage of whatever microorganisms most frequently cause infections in a particular setting. Until now, few dosing regimens have been aimed at decreasing the chance of emergence of resistance (resistance counter-selection) and only a few studies, most of them involving the use of fluoroquinolones, have been designed to elucidate the underlying mechanisms of resistance. The simplest approach to study the risk of resistance developing is to determine the mutation prevention concentration (MPC) of a drug *in vitro*; the MPC is defined as the lowest concentration of an antibiotic that is needed to prevent the growth of resistant mutants using a high inoculum. It appears that the MPC is not the same for all fluoroquinolones. In addition, it has been shown that the emergence of resistance is dependent on the magnitude of the pharmacodynamic indices AUC/MIC and peak MIC, and may be different across various quinolones and causative species. Thus, both the choice of drug as well as the dosing regimen should be tailored for each bacterial species and in some cases even specific strains, not only for improved efficacy but also to decrease the risk of the emergence of resistance.

S195 Follow the protocol – save powerful antibiotics for targeted use

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Antibiotic guidelines are nationally, or internationally agreed strategies for the treatment of a specific infection, or infectious disease syndrome, using a standardized approach based on evidence-based criteria, epidemiological data and/or consensus-derived opinion. Protocols are local recommendations that are derived from these guidelines. The purposes of protocols are twofold: to ensure that the individual patient receives the most appropriate care, and that any consequential effects at a population level are minimized, i.e. that the same best treatment option remains available for the next patient. Protocols are developed for several reasons (a) to apply evidence-based criteria to guide practice (b) to assist in the management of complicated cases, and (c) to control antimicrobial use and to limit the development of resistance. There are a number of examples of protocol/guidelines in common use

- Pneumonia
- Endocarditis
- Febrile neutropenia
- VRE management
- HAART
- Meningococcal meningitis
- Tuberculosis
- Antifungal therapy
- Malaria
- Helicobacter infection

Despite the widespread acceptance of these protocols, there are surprisingly few data that show how well they are adhered to, or the consequences of avoidance. Within Europe, the Copenhagen declaration made important statements in respect of the threat of emerging resistance, and this remains an important justification for protocols and one argument in favor of reserving certain antibiotics for specific use. In addition, examples will be discussed in which protocols can have a beneficial effect on patient outcome. In my opinion, protocols should be followed, rather than the choice being left to individual clinicians, as this is an evidence-based approach that maximizes the chance of clinical success and thus reduces the risk of antibiotic resistance development.

S196 Cover all possibilities – right first time saves lives

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The cornerstone of any antibiotic management program is the optimization of antibiotic therapy so that patient outcome is improved. Optimization of therapy requires knowledge of local antibiotic susceptibility data. Hospital-wide antibiograms may be insufficient for this purpose, but antibiograms specific to the particular intensive care unit (ICU) involved are more useful. Unfortunately, in many ICUs, antibiotic resistance is so widespread that high percentages of antibiotics are resistant to many commonly used 'workhorse' antibiotics (such as extended-spectrum cephalosporins or β-lactam/β-lactamase inhibitor combinations). What is the impact of the incorrect choice of empiric antibiotic therapy? Several studies have now demonstrated that if empiric therapy does not cover the infecting organism, patient mortality rises. Inadequate empiric coverage is largely because of antibiotic resistance. In Gram-negative bacilli this resistance may be mediated by extended-spectrum β-lactamases; enzymes that inactivate extended-spectrum cephalosporins. Additionally, Gram-negative bacilli may produce multiple β-lactamases, thereby overcoming the effects of β-lactamase inhibitors. The carbapenems are not inactivated by extended-spectrum β-lactamases and AmpC β-lactamases. Because Gram-negative bacilli are such common causes of serious infection in ICUs, it is necessary for clinicians to be aware of the antibiotic susceptibility profile of these organisms in their units. The Empiric therapy should be chosen so as to maximize the likelihood that such therapy will cover the probable pathogens infecting their ICU patients, and not be based on national guidelines or cost considerations.

Electronic communication of microbial typing data: feasibility and added value? (Symposium organized by ESGEM)

S210 The gene project: construction and use of on-line databases based on automated ribotyping

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Objectives: Among the available methods for genetic fingerprinting, ribotyping (ribosomal RNA restriction fragment length polymorphism) is the only one that is fully automated, using instruments called RiboPrinters (DuPont-Qualicon, Wilmington, DE, USA). It remains to be fully evaluated.
Methods: A network of 18 (mostly European) laboratories, eight of which use a RiboPrinter and 10 of which use other fingerprinting techniques (including PFGE, AFLP, MLST and manual ribotyping), is currently (i) standardizing ribotyping procedures (restriction enzyme choice) and evaluating the interlaboratory reproducibility of automated ribotyping; (ii) creating an on-line database of electronic fingerprints; and (iii) evaluating the performance (discriminatory power, identification capacity) of automated ribotyping for 15 bacterial species of public health importance, including the major nosocomial, food-borne and community-acquired pathogens.
Results: Reproducibility of automated ribotyping was found to be higher than 95% in all species tested when the automated analysis of the fingerprints (categorization into 'ribotypes') is manually refined after visual inspection of the patterns. Comparative studies show that ribotyping can generally discriminate a high proportion of 'epidemic' clones previously identified based on other techniques (for example, 14 out of the 16 major multidrug resistant *Streptococcus pneumoniae* international clones have distinct PvuII ribotypes), but the ability of ribotyping to discriminate these clones from closely related, nonepidemic strains varies among species. Automated ribotyping was so far found to be useful for the identification of epidemic clones of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecium*, and the *Burkholderia cepacia* complex. A database of electronic fingerprints built using the software BioNumerics (Applied Maths, Belgium) has been made available on the Internet (see http://www.ewi.med.uu.nl/gene), allowing remote pattern matching and identification using BioNumerics or a web browser.
Conclusions: Automated ribotyping proves to be a powerful and reliable technology for international networks for the exchange of bacterial fingerprint data. However, automated ribotyping analysis generally needs to be complemented by other typing techniques, and its wider applicability to

clinical microbiology and molecular epidemiology is currently limited by its relatively high cost.

S211 Problems and solutions for electronic communication of DNA fingerprints — the ENEMTI experience

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Rapid molecular typing techniques are becoming essential tools for monitoring the geographical spread of particular virulent, epidemic or antibiotic-resistant bacteria. However, in many cases, such techniques either lack standardization and reproducibility, or their use is restricted to central reference laboratories. The European Network for Exchange of Microbial Typing Information (ENEMTI) is funded by the European Science Foundation (ESF) to provide a forum in which to evaluate initiatives designed to allow local strain analysis and identification, combined with centralized data comparison and exchange. Central to this approach is the concept of highly standardized typing protocols used in conjunction with a central interactive database. Advanced software for the construction of databases is now available, and various different typing approaches (AFLP, PFGE, RAPD, REP-PCR and molecular serotyping) have been evaluated by ENEMTI for their reproducibility between different participating laboratories with four groups of organisms: *Acinetobacter, Escherichia coli, Legionella* and *Staphylococcus aureus*. To date, PFGE and AFLP have shown the most promise, but these are also the most time-consuming of the techniques examined. Rapid PCR-based fingerprinting techniques had shown promise in preliminary experiments using standardized reagents, but recent problems in the manufacturing process have illustrated the dangers of relying on quality control procedures at a single commercial supplier. To allow easier comparison between fingerprints generated in different laboratories with different electrophoresis equipment, it may be advantageous to base the comparison on the extrapolated molecular sizes of fingerprints. However, once again there are problems with the lack of precision found with some commercial molecular size markers. It is clear that rigorous standardization is absolutely mandatory if interlaboratory exchange of electronic typing data is to be achieved. Further, if such typing databases are to be used widely in Europe and elsewhere in the future, a mechanism will probably be required to allow some form of practical training in the standardized protocols for members of participating laboratories. Details of all the protocols and activities of ENEMTI can be found at the ENEMTI website (http://www.rivm.nl/enemti/).

Pregnant women and neonatal infections (Joint symposium with IDSA)

S216 Towards elimination of perinatal HIV – an incomplete success story

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Since the time when AIDS was first reported in 1981, more than 50 million people, including at least 23 million women and 5 million children, have been infected with HIV across the globe according to the 1999 estimates of the World Health Organization. With the development of a test for HIV infection in 1985, transfusion-mediated disease was almost completely eliminated, leaving mother-to-child transmission the main mode of acquisition of pediatric HIV infection. Before preventive interventions became available, transmission rates of 14–33% and of 40–50% were reported by prospective studies from industrialized and developing countries, respectively. However, in 1994, the completion of the AIDS Clinical Trial Group 076 trial initiated a new era of hope and progress in the pediatric AIDS epidemic. The widespread introduction of this three phase regimen with ZDV into clinical practice resulted in a two third reduction of vertical HIV transmission in the industrialized world. Vertical transmission rates have steadily continued to decrease since due to the introduction of additional interventions like elective cesarean section delivery and refraining from breast-feeding. The application of a combination of these three interventions is now widespread and transmission rates as low as 1% have been achieved. More recently, complete suppression of maternal viremia by means of HAART has also been shown to result in similarly low transmission rates. Although many questions still remain partly unanswered, a more than 90% reduction in vertical transmission rates has been achieved over a period of less than 10 years in the industrialized world. Nevertheless, due to the enormous costs and infrastructural needs, we have not managed to translate this success story to those countries who would need such interventions to reduce vertical HIV transmission most desperately. Even though recent trials have demonstrated benefits from more abbreviated and less costly interventions and thus raised hopes that transmission rates can also be ameliorated in relatively impoverished countries in the future, our globe is still left with at least 1500 new pediatric HIV infections, which occur every single day, mostly in such countries.

Malaria: selected aspects

S223 Modern diagnostic strategies to avoid misdiagnosis of malaria

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In most endemic countries limited economic resources mean that malaria is usually diagnosed on clinical grounds alone. However, microscopy may be valuable as it could also be used in the diagnosis of other diseases, like tuberculosis. In countries with imported malaria the judicious use of the rapidly performed RDTs may especially help places with, and/or times of limited expertise in microscopy, like during on-call-hours. PCR methods are unlikely to find a widespread use in the routine diagnosis of malaria due to long turn-around-times. They may be useful in selected cases, mainly in confirming positive cases. Yet, as many as 50% of imported malaria cases may be missed at first presentation. The detection of hemozoin, during routinely requested full-blood-count, is a novel way to diagnose malaria even in the absence of clinical suspicion.

Antibiotic treatment of intra-abdominal infections Antibiotic prescribing policies

O224 Excess mortality associated with inappropriate initial empiric antibiotic therapy in patients undergoing surgery for intra-abdominal infection

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Objective: To assess the effect of inappropriate initial empiric antibiotic therapy on the risk of in-hospital death among patients undergoing surgery for intra-abdominal infections (IAI).
Methods: Retrospective analysis of patient records from two acute-care hospitals in Spain. The initial empiric therapy is classified as inappropriate if one or more baseline pathogens are resistant to all antibiotics in the initial regimen in case of positive culture or not according to guidelines in case of negative/missing culture. Multivariate logistic regression was performed to estimate the effect of inappropriate therapy on the risk of in-hospital death while adjusting for patient age, gender, infection site and type, and comorbid conditions.
Results: A total of 365 adult patients who underwent surgery for IAI between 1998 and 2000 were retrospectively identified. Mean age was 56.7 years; 41% were female. The most common sites of infection were appendix (39.7%) and upper GI (29.6%). A total of 293 patients (80.3%) had peritonitis; 93 patients (25%) received inappropriate initial empiric antibiotic therapy. A total of 52 of the 365 patients (14.2%) died; 18 (4.9%) required re-operation to resolve index infection; 58 (15.9%) required additional parenteral antibiotics to resolve index infection. The rate of in-hospital death among patients who received inappropriate initial empiric therapy was higher (30.0% vs. 9.6%, $P = 0.001$) compared with patients who received appropriate initial empiric therapy. In multivariate logistic regression, the risk of death was independently associated with inadequate initial empiric therapy (Odd's ratio (OR) = 3.7; 95% CI: 1.7–8.1). Other risk factors included age ≥ 65 (OR = 8.9; CI: 3.4–23.4), peritonitis from duodenal, gastric, biliary, or pancreatic source (2.3; 1.0–5.4, relative to appendicitis with peritoneal abscess), neoplasm (3.4; 1.3–9.0), diseases of the respiratory system (5.3; 2.4–11.4), diseases of the genitourinary system (2.6; 1.1–6.4), and infectious and parasitic diseases (4.6; 1.8–11.9).
Conclusions: Among patients undergoing surgery for IAI, the OR of in-hospital death are 3.7 times higher in patients who received inappropriate initial empiric antibiotic compared to those who received appropriate initial empiric therapy. Age ≥ 65, peritonitis from duodenal, gastric, biliary, or pancreatic source and certain comorbid conditions are also associated with higher risk of death. However, other unobserved confounding factors may still exist.

O225 Association between inappropriate initial empiric antibiotic therapy and the need for re-operation and second-line therapy among German patients undergoing surgery for community-acquired intra-abdominal infections

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Objective: To assess the association between inappropriate initial empiric antibiotic therapy and need for re-operation and second-line therapy among patients undergoing surgery for community-acquired intra-abdominal infections (IAI).
Methods: Patients who underwent surgery for community-acquired IAI after January 1999 were retrospectively identified at 20 German hospitals. Medical records were reviewed to confirm IAI and to collect data. Initial empiric therapy is classified as inappropriate if at least one pathogen is resistant to all antibiotics in initial regimen in case of positive culture or not according to guidelines in case of negative/missing culture. Multivariate logistic regressions were performed to estimate associations between inappropriate therapy and need for re-operation and second-line therapy while adjusting for age, gender and comorbid conditions such as neoplasm, congestive heart failure, diabetes, and cardiovascular, renal and respiratory diseases.
Results: A total of 423 eligible patients were included. Mean patient age was 56.4 ± 18.6 years; 42.8% were female. Most common sites of IAI included appendix (133 patients) and colon (94). IAI processes included perforation (117), abscess (59), and other secondary peritonitis (169). Pathogens were documented in 199 patients (47.6%); most frequently identified pathogens included *E. coli* (147 isolates), *Bacteroides* species (26) and *Streptococci* (19). A total of 21 patients died (5%); 12 due to infection and nine due to other causes. Sixteen patients (3.8%) required re-operation and 59 (14.0%) required second-line parenteral antibiotics to resolve infections. Fifty-one patients received inappropriate initial therapy. Compared with patients who resolved their index infection with initial therapy, those requiring re-operation and second-line therapy and those who died due to infection were more likely to receive inappropriate initial therapy (8.9% vs. 37.5, 23.7, and 16.7%, respectively). After controlling for covariates, patients who received inappropriate initial therapy were more likely to undergo re-operations (OR = 4.8, 95% CI = 1.7–13.9) and to require second-line parenteral antibiotic therapy (2.9, 1.4–5.8). The association between inappropriate therapy and infection-related death was not statistically significant.
Conclusion: Among patients undergoing surgery for community-acquired IAI, inappropriate initial antibiotic therapy increases the use of re-operation and second-line parenteral antibiotic therapy.

O226 Outcomes of initial empiric antibiotic therapy and hospital resource use in patients undergoing surgery for community-acquired intra-abdominal infections in three Swiss hospitals

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Objectives: To estimate outcomes and resources use in patients undergoing surgery for community-acquired intra-abdominal infections (IAI) and to assess the relationship between outcomes and resources use.
Methods: Patients with community-acquired IAIs were retrospectively identified at three hospitals in Switzerland using ICD10 codes. Medical records were reviewed to confirm diagnosis and to collect data. All patients had surgical control of infection in addition to parenteral antibiotic therapy. Patients with nosocomial infections were excluded. Empiric therapy was defined as successful if the index infection was resolved without additional surgical intervention or second-line parenteral antibiotic therapy. Multivariate linear regressions were performed to estimate the association between outcomes of initial empiric antibiotic therapy and total parenteral antibiotic days and hospital length of stay (logarithmic scale) while adjusting for patient age, gender, APACHE II score and comorbid conditions.
Results: A total of 370 patients had confirmed, community-acquired IAIs without exclusion criteria or missing data. Mean patient age was 51 ± 18 years; 48% were female. The most common sites of infection were appendix (45%), colon (34%) and biliary tract (10%). The infection processes included abscess (52%), perforations (9%), and other secondary peritonitis (36%). A total of 284 patients (77%) resolved the index infection with the initial empiric antibiotic therapy without additional surgery or second-line antibiotics, 33 (8.9%) required additional surgical intervention and 38 (10.3%) required second-line therapy to resolve infection, nine patients relapsed after discharge and six (1.6%) died in hospital due to IAI. The results of multivariate linear regressions indicate that patients who required additional surgery or second-line antibiotic therapy to resolve the index infection had significantly longer duration of parenteral antibiotic therapy (12.5 and 9.9 days vs. 6.5 days, $P < 0.05$) and hospital length of stay (24.1 and 15.1 days vs. 10.7 days, $P < 0.05$) compared to patients who resolved their infection with the initial empiric antibiotic therapy. Higher APACHE II score, infection of the colon and cardiovascular disease were also associated with longer LOS.
Conclusion: In patients undergoing surgery for community-acquired intra-abdominal infection, the need for re-operation or second-line antibiotic therapy results in significant increases in hospital resource use.

O227 Treatment outcomes, hospital resource use and cost of care for patients undergoing surgery for intra-abdominal infections in Northern Italy

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Objectives: To describe the treatment outcomes, detailed hospital resources use and cost of care among patients hospitalized for intra-abdominal infection (IAI).
Methods: A prospective observational study was conducted in five hospitals in Northern Italy. Adult patients hospitalized for IAI between January 2000 and September 2001 were enrolled. Patients with nosocomial infections were excluded. Clinical evaluation was done on day 3 and at discharge. Direct medical costs were assessed from the hospital perspective: antibiotic acquisition and administration (including materials and nursing time estimated by time-motion methods), laboratory and radiological procedures and hospitalization.
Results: A total of 112 patients were included in this preliminary analysis. Mean age was 57.1(SD 18.3) years; 42 were female. The most common types of infections were perforated appendicitis (32.1%), secondary peritonitis (25.9%) and cholecystitis with abscess (15.2%). Sixty-seven strains were isolated from 97 cultures from blood, abdominal cavity, or other intra-abdominal sites. *Escherichia coli* (17) and *Enterococcus faecalis* (10) were the most frequently isolated organisms. The average simplified acute physiology score (SAPS II) at baseline was 18.7 (10.6). At discharge, 95 (84.8%) patients were cured, 10 (8.9%) had improvement and seven (6.3%) failed (six died). The most commonly used parenteral antibiotics included metronidazole (573 patient-days), ceftriaxone (346) and cefotetan (232). Average number of parenteral antibiotic days and length of stay were 7.3 (15.5) and 15.3 (21.3) days, respectively. Average time to defervescence was 3.3 (2.6 days). The most frequently performed laboratory tests included blood cell count (2.6 per patient), serum chemistry (2.5), ECG (1.2) and cultures of blood and other sites (0.87). X-ray, ultrasonogram and CT were performed 1.2, 0.2, 0.14 times per patient, respectively. For each i.v. infusion, the average cost of materials and nursing time estimated by time-motion studies were 0.84 and 4.21 Euros, respectively. Average cost of antibiotic acquisition, administration, laboratory and radiology examinations, and hospitalization were 355.5, 125.7, 206.3, and 4575.2 Euros per patient per hospitalization, respectively.
Conclusion: Patients undergoing surgery for IAI result in significant resources use and cost. It is important to understand treatment outcomes, resources use and cost in order to improve the cost-effectiveness of patient management.

O228 Antimicrobial drug use and resistance pattern in *S. pneumoniae*: a correlation study in Italy

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Objectives: We compared regional data on antibiotic consumption with patterns of antibiotic resistance of *S. pneumoniae* as obtained within the EARSS project in 1999–2000 (European Antimicrobial Resistance Surveillance System), in order to evaluate the relationship between antibiotic use and resistance prevalence.
Methods: The Italian EARSS network comprised 64 laboratories, serving 70 hospitals. Participating labs were asked to collect and test for antimicrobial susceptibility all first isolates of *S. pnuemoniae* from blood and CSF. All isolates were sent to the Reference Laboratory, where confirmatory susceptibility testing were performed. Data on antibiotic use in 2000 were obtained from the Ministry of Health. National and regional level of antibiotic use were expressed both in terms of defined daily doses (DDDs) prescribed per 1000 inhabitants per day, and expenditure. The geographic relationship between resistance to macrolides and penicillins, and consumption of macrolides and β-lactams, was assessed by linear regression analysis (SPSS 10.0).
Results: A total of 322 strains were obtained from 50 participating laboratories. A total of 72.7% of the isolates were collected from blood and 27.3% from CSF. The level of resistance was 12.1% for penicillin, and 28.6% for erythromycin, with a marked geographic trend. Penicillin resistance was higher in southern than in northern Italy (21.4% vs. 9.5%, $P < 0.05$), a similar difference was observed for erythromycin (45.7% vs. 23.8%; $P < 0.05$). Macrolides and β-lactams represented 22.7 and 40.9%, respectively, of the overall level of antibiotic use (22 DDD/1000 inhabitants die), with large regional variation. A geographic correlation was found between total macrolides consumption and erythromycin resistance ($R^2 = 0.55$; $P < 0.05$), and between total β-lactams consumption and penicillin nonsusceptibility ($R^2 = 0.68$; $P < 0.05$).
Conclusions: In Italy, the resistance of *S. pneumoniae* to penicillin and macrolides follows a geographic trend, with higher level of resistance in southern Italy. A similar trend is observed for antibiotic consumption, which suggests that resistance pattern of *S. pneumoniae* could depend, at least in part, on the use of antibiotics. Further analysis is requested for a better understanding of these results.

O229 Influence of an infectious disease consulting service on quality and costs of antibiotic prescriptions at a university hospital

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Background: An infectious disease consulting service was established at the Department of Internal Medicine at the University Hospital Aachen, Germany in September 1999 in order to assess and optimize the quality of antibiotic usage.
Methods: Written guidelines relating to the most common community-acquired and nosocomial infectious diseases were implemented. Instructions on signs, symptoms and diagnosis of infectious diseases was given to physicians by infectious disease specialists. All patients admitted to the hospital via the emergency ward were enrolled the study.
Results: During the 8-month intervention period (September 1999 to April 2000) 20.7% (155/748) of the patients received antibiotics in comparison to 28.5% (146/513) during the 6-month control period (November 1998 to April 1999) without an infectious disease service. Demographic data of the patients and distribution of infections were comparable. In both groups, therapy was started in the emergency ward in about 85% of cases; the major indication for treatment were lower respiratory infections (69%). Inadequate prescriptions could be reduced significantly from 30% (44/146) to 17% (27/155). The route of administration was changed from i.v. to p.o. after 6.7 and 4.5 days, respectively. The mean length of therapy and mean length of stay in the hospital decreased form 11.6 to 9.3 days and from 16.4 to 14.2 days, respectively. There was no significant difference in mortality rates: 3.4% (5/146) in the control group and 5.8% (9/155) in the intervention group. Costs per patient on antibiotics could be reduced by 32% from 159 to 109 Euro, thus resulting in a total saving of about 10.500 Euro. The cost of the infectious disease specialist with an average weekly workload of about 4 h was approximately 4000 Euro.
Conclusions: (1) Establishing an infectious disease service improved the prescription behavior significantly and decreased the duration of therapy and of hospitalization without interfering with the quality of medical care. (2) Hospitals with limited financial and personal resources are advised to focus on the admitting unit. (3) The total cost saving of 6500 Euro for the intervention period shows that this service is indeed cost-effective.

O230 Antibiotics prescribing in the emergency department: a prospective study in 34 French EDs

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Background: In the ED the prevalence of infectious diseases (ID) is high and their antibiotic treatment (AB) unknown. Our purpose is to identify AB prescribing, defined as a de novo introduction (ABN), an interruption (ABI), a modification (ABM) or the pursuit (ABP) of an AB previously prescribed.
Methods: Consecutive patients aged 18 years or more are included if (1) ABN, ABI, ABM or ABP are decided by, or with the agreement of, the attending physician (2) they consent to be followed up by telephone for at least 3 days. They are classified in five classes according to pretest evaluation of acuteness of illness (PTA): I = no test required, II = I + tests ordered, III = functional prognosis unstable, IV = vital prognosis involved but no CPR needed, V = IV + CPR required.
Results: From December 11 through 24, 2000, 33 EDs reported 38 859 visits (97 ± 37/ED/day). Of 21 909 non-trauma patients. A total of 2468 (11.2%) were potentially ID, 80% of whom received an AB. All 34 participating EDs included 1981 patients (4.74 ± 0.96/center/day [1–21]) aged 52 ± 24 years, sex ratio 52%M with ABN 77.3%, ABI 3.2%, ABM 14.5%, and ABP 5%.

Main IDs are respiratory (37.4%), urinary (20.4%) and skin infections (18.6%) and AB classes used are penicillins (57.2%), 3GC (10.4%) and fluoroquinolones (20.8%). Patients' PTA is similar to that of the common French ED-patient: I, 19.8%; II, 45.8%; III, 22.3%; IV, 11.1%; V, 0.9%; 1089 (55%) were hospitalized. A 3-day follow-up was obtained for 1829 (93%), of whom 1.4% were reported dead, 3.9% did not observe AB and 23.2% had an AB change.
Conclusions: IDs are a motive for consultation in 10–20% of non-trauma ED patients. As our study population is fairly representative of French EDs, we would expect that of the 6 000 000 non-trauma patients seen in 2000, 672 000 had an ID and 537 000 were treated with at least one AB. The adequacy of AB with current guidelines is unknown.

O231 Modeling the temporal relationship between hospital use of macrolides, third generation cephalosporins and fluoroquinolones (FQ), and the percentage of methicillin-resistant *Staphylococcus aureus* isolates: a time series analysis

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Objective: To study the temporal relationship between hospital use of antimicrobials and %MRSA of all *S. aureus* isolates.
Methods: The study was performed in a Scottish 1200-bed tertiary referral, teaching hospital during the period January 1996 to December 2000. Monthly hospital data were obtained on the percentage nonduplicate, non-surveillance MRSA, and the use of antimicrobials in Defined Daily Doses (DDD) per 1000 patient-days. An Autoregressive Integrated Moving Average (ARIMA) model was adjusted to each of the series. A dynamic model with polynomial distributed lags was then adjusted to assess the relationship between %MRSA and use of various antimicrobials.
Results: The average monthly %MRSA was 14.9% but showed large variations from 0 to 41.5%. Examination of the %MRSA series showed a global increasing trend but included three seasonal cycles in April to July 1998, April to October 1999, and February to September 2000 (maximum to minimum level). The monthly no. of *S. aureus* isolates overall, i.e. susceptible + resistant isolates, did not show this seasonal pattern. The average monthly use of MAC, 3GC and FQ was 90.2, 62.5, and 51.9 DDD/1000 patient-days, respectively. However, there were also large variations during the study period, e.g. for MAC from 32.7 to 177.9. Examination of the series showed: (1) for MAC, a sustained increase over the whole study period and a seasonal pattern with maximum levels of use during winter months (November 1995, December 1996, December 1997, December 1998 to February 1999, December 1999 to January 2000, December 2000) (2) for 3GC, an increase for the overall period with a sharper increase from mid-1999 to December 2000 and a seasonal pattern with peaks in December months (3) for FQ, a less marked increase without an evident seasonal pattern. When adjusting the dynamic model to capture the relationships between these series, we found that the monthly %MRSA was explained by: (1) the %MRSA observed 3 and 6 months before (2) use of MAC up to 3 months before (3) use of 3GC 4 months before and (4) use of FQ between 3 and 9 months before. This model explained 93% of the variations in the %MRSA observed during the study period.
Conclusion: By using time series analysis, we demonstrated a dynamic relationship between the use of MAC, 3GC and FQ, and the %MRSA in our hospital. These results confirm previous hypotheses that the %MRSA observed in a hospital is related not only to the MRSA colonizing pressure, but also, and with a certain delay, to the use of several antimicrobial classes to which the Aberdeen MRSA are resistant.

Fungal infections

O232 Antifungal drug susceptibility of *Aspergillus* spp. isolated from nosocomial departments with high-risk patients

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Objectives: Invasive aspergillosis (IA) causes increased morbidity and mortality in immunocompromised hosts. Nosocomial transmission of airborne conidia has been considered as an important mode of fungal acquisition. We aimed to study antifungal drug susceptibility of aspergilli from hospital departments having patients with increased risk for IA.
Methods: Fifty-four *Aspergillus* isolates (19 *A. fumigatus*, 17 *A. flavus* and 18 *A. niger*) were evaluated for minimal inhibitory concentrations (MIC) of two current and two newer antifungal agents. The strains studied were randomly chosen from a total of 470 isolates collected from four units (Solid Organ Transplantation, Hematology, Paediatric Oncology, Paediatric Intensive Care) at 12-monthly air samplings during 2000. The in vitro susceptibilities to amphotericin B (AmB), itraconazole (ITC), voriconazole (VRC) and posaconazole (PSC) were tested by NCCLS M38-P micromethod. The endpoints for MIC (mg/L, no growth) were read visually.
Results:

spp.		AmB	ITC	VRC	PSC
A. fumigatus	MIC_{50}	0.5	2	0.5	0.125
	Range	0.6–2	0.06–16	0.25–1	<0.015–0.5
A. flavus	MIC_{50}	1	2	0.5	0.25
	Range	0.125–4	0.06–4	0.25–2	0.031
A. niger	MIC_{50}	0.125	2	0.5	0.5
	Range	0.6–0.25	0.25–4	0.25–4	0.06–1

Conclusions: *Aspergillus* spp. isolated from hospital departments with high-risk patients were found to have low MIC for currently used antifungal drugs. Susceptibility to AmB was *A. niger* > *A. fumigatus* > *A. flavus*. All three *Aspergillus* spp. were generally more susceptible to the newer azoles than ITC. Although *Aspergillus* spp. can be frequently isolated in nosocomial environments and pose a significant threat to immunocompromised patients, they do not appear to possess unusual patterns of drug resistance.
Acknowledgement: Supported by PENED99

O233 Multicentre evaluation of inoculum preparation methodologies for antifungal susceptibility testing (AST) of *Aspergillus* species

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Objectives: The procedure of NCCLS M38-P recommends inoculum preparation by a spectrophotometric method. However, color and size of spores can have an influence on the optical density (OD) values. Alternatives as spore enumeration with a hematocytometer could be a more reliable procedure for inoculum preparation (JCM 39: 1345–47, 2001). We have evaluated the interlaboratory reproducibility of a microscopic spore enumeration method.
Methods: Three labs participated in the study. A total of 40 *Aspergillus* strains including 10 each of *A. fumigatus* (AF), *A. flavus* (Afl), *A. terreus* (AT), *A. niger* (AN) were tested. Inoculum size were adjusted between 1×10^6 and 5×10^6 cfu/mL by spore enumeration with a Neubauer improved chamber (NB), and quantified by plating aliquots onto Potato Dextrose agar (colony counting, CC). OD at 530 nm of each inoculum suspension was also measured by spectrophotometrical reading (SP). Analysis: (i) agreement (AGR):% of CC within 1×10^6 to 5×10^6 cfu/mL; (ii) correlation: intraclass correlation coefficient (ICC), over a maximum value of 1.
Results: The total AGR for the three labs was 89.2%. By species the AGR were: (i) AF, 96.7%; (ii) Afl, 80%; (iii) AT, 90%; (iv) AN, 90%. For all participants, the average ICCs between CC versus NB and CC versus SP were as follows:(i) AF, 0.37 and −0.19, respectively; (ii) Afl, 0.69 and 0.39; (iii) AT, 0.52 and 0.44; (iv) AN, 0.81 and 0.58; (v) total, 0.73 and 0.15, respectively.

Conclusions: (i) The inoculum preparation by spore enumeration is a reproducible method with high interlab AGR, >90%, with the exception of Afl (AGR of 80%). (ii) The spore enumeration exhibits significant higher correlation with colony counting than that of SP procedure. (iii) Inoculum size has a great influence on the MICs for filamentous fungi and a reliable procedure for inoculum preparation is mandatory.

O234 A retrospective observational study of nosocomial candidemia

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Objective: Aim of the present study was to evaluate, in an Italian tertiary care hospital, the incidence of nosocomial candidemia together with causative pathogens, treatment, and risk factors for death.
Materials and methods: A retrospective, observational study was conducted in an Italian tertiary care hospital. Data were collected between January 1998 and August 2001. Candidemia diagnosis was defined as the detection of at least two positive blood cultures yielding *Candida* spp. during the same hospital admission.
Results: During the study period a total of 55 episodes of candidemia occurred in 52 patients, accounting for a global incidence of 4.17/10 000 in-patients. Median age was 55 years and both sexes were equally represented. Fungemia occurred a mean of 30 days after admission. The most common reasons for hospitalization were solid neoplasia (27%), abdominal surgery (19%), trauma (11%) and hematologic neoplasia (8%). Twenty-four (46%) patients were hospitalized in medical wards, 17 (33%) patients in surgical wards and 11 (21%) patients in intensive care units (four patients in neonatal intensive care units). *C. albicans* was the most frequently isolated pathogen, accounting for 50% of fungal isolates, followed by *C. parapsilosis* (14%), *C. tropicalis* (13%), *C. glabrata* (11%), *C. zeylanoides* (4%), *C. lusitaniae* (2%), *C. pseudotropicalis* (2%), *C. guillermondi* (2%) and *C. sake* (2%). Thirty-three (63%) patients received adequate antifungal therapy. Eighteen, adults (43%) of the 42 patients, with central venous catheters underwent line removal; five of them had catheter-related candidemia. Neonatal fungemia episodes were observed only until 1998 and patients neither underwent central line removal nor died. The 30-day crude mortality rate was 31%. Hematologic neoplasia, inadequate antifungal therapy and retention of central lines were significantly associated with poor outcome.
Conclusions: Candidemia is still a frequent and life-threatning complication in patients hospitalized and with a severe underlying disease. Adequate antifungal therapy and central line removal independently reduced the high mortality of the disease. In our case-record, no neonatal case was detected after 1998 indicating that there could be an improuvement in the patient care. Moreover, neonatal patients, a small sample size, showed a favourable outcome despite the persistence of the central-line.

O235 Ninety-four cases of candidemia in a Belgian hospital: a retrospective study on outcome

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Objectives: To describe the epidemiology of candidemia in our hospital, and its prognostic determinants.
Methods: A retrospective study of 94 patients with a proven candidemia between January 1993 and October 2001 in Stuivenberg Hospital Antwerp, Belgium.
Results: The average age was 62.3 years, ranging between 8 and 95 years and the male/female ratio was 1.35. We measured an average candidemia-related mortality of 44.6% without any specific trend during the study period. *C. albicans* was isolated in 53% of the cases, followed by *C. glabrata* in nearly 30% and *C. parapsilosis* in 7% of the patients. We noticed a decrease of the absolute number of candidemias with a shift towards the non-*C. albicans* species from 1/3 to 1/2 of the isolates. Among all the *Candida* species, *C. albicans* had the highest mortality (54%). Eighty-five percent of our patients were colonised with *Candida* spp. prior to candidemia (sputum (40%), urine (35%) and catheter (23%)). There was a poor outcome if patients were colonised with *Candida* spp. in urine ($P < 0.0001$, RR = 3.8), skin swabs ($P < 0.0001$, R = 2.8) or catheters ($P < 0.0001$, R = 2.75). The number of colonised sites was correlated with an adverse outcome as well ($P = 0.037$). Patients receiving antibiotics or a combination of antibiotics with steroids or TPN did not influence the outcome, whereas the combination of antibiotics, steroids and TPN would worsen the outcome with a factor 2.2 ($P < 0.0001$). The SOFA score on the first day of sepsis correlated well with the outcome ($P = 0.034$), were the APACHE II score didn't on admission. There was no significant correlation with age or immunodeficiency.
Conclusion: Candidemia in our patients was associated with a high mortality. This was related to: the species, the prior colonisation and the number of colonisation sites, the combination of antibiotics, steroids and TPN and the SOFA score on the onset of the sepsis. These may be important parameters and could guide decisions on treatment.

O236 Caspofungin treatment of candidal esophagitis in HIV-infected patients

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Objectives: Caspofungin is a new broad-spectrum antifungal drug of the novel echinocandin class. We analyzed the clinical efficacy of caspofungin 50 mg/day in the treatment of HIV-infected adults with endoscopically proven *Candida esophagitis* from four Phase II/III trials.
Methods: Symptoms were assessed daily; a favorable outcome required complete resolution of all esophageal symptoms assessed at the time of discontinuation of therapy. Relapse was defined as recurrent symptoms during the subsequent 2 weeks. A multivariate logistic regression model was developed to identify potential factors [including severity of presenting symptoms, CD^4 count on entry, extent of disease assessed endoscopically at baseline, causative *Candida* species, duration of therapy (total and after resolution of symptoms), time on treatment prior to symptom resolution, and antifungal prophylaxis] that might predict symptomatic relapse in the 2 weeks following completion of caspofungin therapy.
Results: The 95/123 evaluable patients (77%) were men. Median CD^4 count was 31/mm^3. *C. albicans* was isolated from 109/110 cases where a pathogen was cultured and constituted the sole isolate in 77% of these cases, 55% had extensive esophageal disease at the time of pretreatment endoscopy. Duration of therapy ranged from 7 to 20 days (median:12 days). At the completion of therapy, symptoms had completely resolved in 117/123 patients [95%; 95% confidence interval: 90–98%]; median time to resolution of symptoms was ~4 days. Response rates were 43/46 (93%) and 70/73 (96%) for patients with more or less than 50 CD^4 cells/mm^3, and 80/85 (94%) and 23/24 (96%) in infections caused by *C. albicans* alone or in association with non-*C. albicans* isolates. Symptoms recurred within 2 weeks of stopping caspofungin in 19/115 evaluable patients (17%), including 3/16 (19%) receiving antifungal prophylaxis. Relapse rates were similar for patients with more or less than 50 CD^4 cells/mm^3. In this small number of patients, only symptom severity and extent of disease judged endoscopically at baseline were significantly ($P < 0.10$) associated with early relapse in the multivariate model.
Conclusions: Caspofungin 50 mg/day appeared to be rapidly effective in eradicating symptoms of *Candida esophagitis* in HIV-infected patients. As expected, symptomatic relapse occurred commonly in the 2 weeks after discontinuation of therapy. Response and relapse rates were similar in patients with more or less than 50 CD^4 cells/mm^3.

O237 Caspofungin (CAS) versus α Amphotericin B deoxycholate (AmB) in the treatment (Rx) of invasive candidiasis (IC) in neutropenic (N) and non-neutropenic (NN) patients (Pts): a multicentre, randomized, double-blind study

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Objectives: AmB remains the gold standard for the Rx of IC, but toxicity and lack of efficacy often limits its use. Although well tolerated, fluconazole (F) is often limited to NN pts, and non-*C. albicans*. *Candida* infections are relatively more resistant to F. CAS, an echinocandin fungicidal against *Candida*, was compared to AmB for primary Rx of IC.

Methods: Patients with IC were randomized to CAS (70 mg × 1, then 50 mg/day) or AmB (0.6–1.0 mg/kg/day). Inclusion required a (+) blood culture or culture from a sterile invasive site within 4 days of study entry (SE) and evidence of infection (fever/hypothermia, hypotension, or local signs of infection) within 2 days of SE. Patients were stratified by Apache II & N status. Patients were treated for 14 days after the last (+) culture but could be switched to F after 10 days IV Rx. The primary analysis was the MITT assessment (met major entry criteria & received >1 day IV Rx) at the end of IV Rx. A predefined secondary analysis was the evaluable (EP) assessment (met MITT & received at least 5 days IV Rx). Success required both symptom resolution & microbiological clearance. A blinded clinician assessed eligibility & response.
Results: Of 239 pts enrolled, 224 (CAS 109; AmB 115) met MITT. Baseline characteristics, including percentage of N pts (CAS 13%; AmB 9%, $P=0.32$) & Apache II scores (mean CAS 14.8; AmB 15.4, $P=0.46$), were similar. Infections were primarily candidemia (83%; CAS 92; AmB 94) & *Candida peritonitis* (10%; CAS 12; AmB 10). Infections were caused by *C. albicans* (45%), *C. parapsilosis* (19%), *C. tropicalis* (16%), & *C. glabrata* (11%). Success at end of IV Rx in MITT was 73.4% in the CAS group and 61.7% in the AmB group. For EP, success was 80.7% in CAS and 64.9% in AmB. A summary of the results for both MITT and EP is in the table below.

	CAS		AmB		
	n/m	%	n/m	%	Difference adjusted for strata (%)
MITT	80/109	73.4	71/115	61.7	12.7
	(65.1, 81.7)		(52.8, 70.7)		(−0.7, 26.0)
EP	71/88	80.7	63/97	64.9	15.4
	(72.4, 89.0)		(55.4, 74.5)		(1.1, 29.7)

Values in parentheses are at 95% CI.

At 6–8 week follow-up, there was no difference in relapse or survival between groups. Fewer pts in the CAS group had drug-related (DR) clinical AEs (CAS 29%; AmB 54%) or lab AEs (CAS 24%; AmB 54%); one (1%) CAS & 16 (13%) AmB pts had DR SAEs. Three (3%) CAS & 29 (23%) AmB pts were discontinued (DC) for a DR AE. Nephrotoxicity (CAS 4%; AmB 23%) was also less frequent in CAS pts.
Conclusions: In the primary analysis (MITT), CAS was equivalent to AmB in the Rx of IC. In a predefined secondary analysis (EP), CAS was more effective than AmB. CAS was better tolerated than AmB as assessed by DR clinical AEs, lab AEs, SAEs, & AEs leading to DC.

O238 Bactericidal and fungicidal activity of *N*-chlorotaurine, a product of human leukocytes

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Objectives: *N*-chlorotaurine ($ClHN–CH_2–CH_2–SO_3–$) is a long-lived oxidant produced by neutrophilic and esinophilic granulocytes as well as monocytes during the oxidative burst.

Methods and results: By quantitative suspension tests, a 1% aqueous solution (55 mM) of the synthetic sodium salt demonstrated strong killing activity against gram-positive and gram-negative bacteria (e.g. *Staphylococcus aureus* including methicillin-resistant strains, *Streptococcus pyogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa*) within 10–30 min as well as against yeasts (*Candida* spp.) and moulds (*Aspergillus* spp., *Alternaria alternata, Fusarium moniliforme, Penicillium commune*) within 1–4 h. As a special feature, the microbicidal activity can be increased significantly in the presence of organic material leading to killing of fungi within minutes. This phenomenon is explained by transhalogenation, i.e. transfer of the active chlorine to other amino compounds, especially to ammonia generating monochloramine.
Conclusions: Since 1% *N*-chlorotaurine sodium proved to be very well tolerated by application to the human eye, urinary bladder, ear and paranasal sinuses, it is a promising new antimicrobial agent for topical treatment of infections in different body regions.

O239 Use of real-time PCR and ELISA for rapid diagnosis of invasive fungal infections in immunocompromised hematology patients

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The early diagnosis of invasive fungal infection (IFI) remains a significant clinical problem in immunocompromised hematology patients. This prospective blinded study aimed to validate the use of real-time PCR and new generation ELISAs to detect IFI in patients with hematological malignancy undergoing intensive chemotherapy/stem cell transplantation. A total of 56 patients (86 neutropenic episodes) recruited between December 2000 and August 2001 are currently evaluable. Peripheral blood samples taken on admission and twice weekly to hospital discharge or until immunosuppression discontinued, have been analyzed using PCR to detect 18S ribosomal DNA and by ELISA for mannan (*Candida* spp.) or galactomannan (*Aspergillus* spp.). In addition, standard clinical and laboratory data have been collected. A total of 39/56 patients had positive PCR results: of these, 13 were positive on a single occasion, 12 were intermittently positive and 14 (four high risk and ten high-intermediate risk) had sequential positive results (two or more). A total of 20/56 patients had positive ELISA results: 15 were positive on a single occasion, one was intermittently positive and four were sequentially positive. Correlating clinical and standard laboratory data for 86 neutropenic episodes, there were one proven, four probable, four possible and 77 episodes with no evidence of IFI, based on EORTC criteria. For the 14 patients with sequential positive PCR results, seven had clinically proven, probable or possible IFI based on EORTC criteria and of these, 6/7 were PCR positive 1–55 days (mean 22 days) prior to commencing empiric antifungal therapy. The remaining seven patients had no evidence of IFI and did not receive empiric antifungal therapy. Of these patients, 5/7 showed resolution of PCR positivity coincident with neutrophil recovery. There were no cases of proven/probable IFI in the 17 patients consistently PCR negative. One patient with intermittent positive PCR results had probable IFI. The ELISA results showed poor correlation both with PCR and clinical criteria. These early results provide evidence that molecular techniques are likely to become important diagnostic tools for the early detection of IFI.

Appropriate use of antibiotics and its impact on development of resistance (LIBRA symposium supported by an educational grant from Bayer)

S240 Globalization of antimicrobial resistance – epidemiological challenges

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The steady increase in antimicrobial resistance among many common bacterial pathogens has markedly reduced the initial empiric options of the primary care physician. Taking *S. pneumoniae* as an example, macrolides, tetracyclines, and cotrimoxazole can no longer be considered as first line antibiotics for most respiratory tract infections in many European countries. Likewise, the dosages of β-lactams need to be readjusted. Thus, prescribers can no longer confidently reach for their "tried and trusted" agents choice, since many conclusions of the clinical trials which support their choice may simply no longer be valid if older than a few years. Even recent agents are losing their momentum (e.g. the recent reports from Hong Kong highlighting resistance rates to so-called "new fluoquinolones" of >10% [Ho et al. JAC 2001]). Similarly, difficult situations may arise for many other important pathogens. It is, therefore, essential

- for the prescriber to remain informed about the true level of sensitivity of the organisms causing the infections she/he wants to treat within her/his local environment, and to act accordingly;

- for the Health Authorities to collect sensitivity data, to publish them on a regular basis, and to review regularly national and local guidelines according to these surveys;
- for both Industry and Public Health authorities to promote the appropriate use of antibiotics by agreeing on conditions of usage (as specified in the SPC and other official documentation) that will reduce the risk of rapid emergence of resistance. These rules should not only apply to new drugs but also to older ones, including generics, which need to be regularly re-evaluated in view of the changes in patterns and extent of resistance.

S241 Infectious disease/population dynamics perspective: consequences of action and inaction

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Recognition and acceptance of a problem is the most important step in changing the situation. The crisis in antimicrobial resistance has now reached epidemic proportions in some countries with respect to certain key bacterial pathogens. The ability of physicians to empirically prescribe is diminishing daily but unfortunately it seems only the "authorities and experts" recognize the situation rarely do primary care physicians acknowledge the new circumstances of treating infection. The consequences of positive action may be the slowing or reversal of antimicrobial resistance, conversely the continuity of the status quo in terms of today's prescribing will only serve to exacerbate the problem. Previous attempts to control macrolide resistance in Finland (Seppala NEJM 1995) is a good example of how effective antimicrobial control can reverse the growing situation. However there are many examples of continued use, especially with poorly active class agents, has driven the resistance growth. The use of different dosage regimen, shorter durations of therapy, combination treatment or just less antimicrobials have all been suggested as ways of taking action, but the most important step is convincing the GP and others that there is a problem and it needs action. The consequences of nonacceptance will be catastrophic as resistant clones become globally disseminated and fewer agents are viable.

S242 PK/PD perspective: does dose matter?

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Paris, F

The recent advances in the understanding of drug dosing and frequency have improved the use of some antimicrobials, e.g. quinolones. However the implementation of specific pharmacodynamic ratios such as AUC:MIC or C_{max}:MIC have usually been to the least amount of drug needed to ensure an outcome such as clinical cure in a specific patient type or bacteriological eradication with a certain pathogen. However to date the use of such ratios to prevent bacterial resistance emergence has not been effectively developed. The fluoroquinolones, particularly the newer class members have been designed to achieve minimum levels to not only achieve clinical cure but also exceed the MIC of most common pathogens such as pneumococci thereby endeavoring to slow or prevent resistance emergence. However, the application of these approaches to other commonly used classes has been inconsistent and clearly requires expansion if classes such as the macrolides and β-lactams are to maintain reliable levels of activity. As recommended by the World Health Organization in 2000 one of the most rationale approach is the "use of the most PD potent member of a class in order to maintain efficacy". The specific PD ratio used to measure PD potency is essentially dictated by the mode of antibiotic bactericidal activity (concentration or time dependent), but it is now admitted that use of less active class members or even misuse of highly active antibiotics could contribute to emergence of resistance which although not immediately clear and obvious to frontline prescribers must dictate their future use if we are to keep what we currently possess. Dose does matter as does frequency and duration, it is now essential that this is explained and promoted to all antimicrobial users.

S243 Physicians' perspective: life at the sharp end of prescribing

W. Holmes
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Although most patients who present with acute respiratory infection are previously well and have uncomplicated disease, many primary care physicians nonetheless find the management of such problems difficult. With only a few minutes to assess their patients' complaint, provide treatment and information, consultations are always full, and often rushed. Professional and government pressure to limit unnecessary prescribing, to use drugs deemed "cost-effective", and to deal with rising microbial resistance adds further to the primary care doctor's problems. In UK primary care, as in most countries, access to investigations is limited, and patients demands and litigation are rising steadily. Faced with this mix of difficulties, many doctors struggle with this common clinical problem. Yet the key features of respiratory infection: cough, chest pain, sputum and breathlessness, can be quickly evaluated and appropriate clinical decisions made. Research into the management of acute cough in primary care has provided useful information to help primary care physicians provide better care—identifying those patients who can be reassured, and those who require treatment. Primary care doctors need to understand the natural history of acute respiratory infection, and develop confidence in its management.

Controversies in the management of LRTIs (Symposium arranged by Pfizer)

S244 *Chlamydia* and *Mycoplasma*: do they matter?

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Milan, I

The term "atypical pathogens" is used to describe a number of microorganisms that can cause so called "atypical pneumonia", and other respiratory and probably nonrespiratory diseases. For the past decade, the frequency of pneumonia due to atypical pathogens has varied considerably in different clinical series, but these pathogens as a group have become accepted as relatively common causes of pneumonia in both outpatient and inpatient settings. The most important bacteria included in this group are *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella* species. *M. pneumoniae* infection occurs worldwide throughout the year, without significant seasonal fluctuations and is both endemic and epidemic. Peaks of incidence occur every 5–7 years and epidemics of *Mycoplasma* infection last 6–8 months. Children and young adults are most often involved in *M. pneumoniae* infections, and this agent is the most common cause of CAP in the 5–20 years age groups. *C. pneumoniae* is considered the most common nonviral intracellular human respiratory pathogen. It is involved in a wide spectrum of infections of the upper (pharyngitis, sinusitis and otitis media) and lower respiratory tract (acute bronchitis, exacerbations of chronic bronchitis, asthma, and CAP) in both immunocompetent and immunocompromised hosts. Recently, evidence of *Mycoplasma* and *C. pneumoniae* involvement in asthma attacks has been reported. These pathogens are also involved in chronic asthma, and both in vitro and animal model studies indicate that atypical agents may play a role in the pathogenesis of the disease. The finding of a relationship between wheezing episodes and acute *M. pneumoniae* or *C. pneumoniae* infection is intriguing and suggests a potential role for these pathogens in the exacerbation of childhood asthma. It is likely that *M. pneumoniae* and *C. pneumoniae* can trigger the "wheezing process" in subjects who are predisposed either by their genetic background or by events that have "primed" their immune systems and lungs. Persistent *C. pneumoniae* infection is common in chronic bronchitis. It is thought to contribute to disease progression by increasing the level of chronic inflammation through pro-inflammatory cytokine production and by its toxic effect on bronchial epithelial cells. The preliminary data on *C. pneumoniae* chronic infection in COPD patients and its interaction with host cells indicates that this agent is a plausible candidate for the modulation of the natural history of chronic bronchitis, emphysema and asthma. There is also convincing evidence that *C. pneumoniae* is associated with atherosclerosis and acute cardiovascular events.

S245 What is the impact of pneumococcal resistance? Pharmacological evidence

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Based on theories that have historically been utilized with β-lactams and aminoglycosides, the serum concentrations of some of the macrolides suggest that not only should some of them be ineffective against infections caused by sensitive pneumococcal strains, but they should also breed resistant strains of these pathogens. While the increasing volume of in vitro pneumococcal resistance reports appears to bear out these thoughts, the complete lack of reports of concurrent clinical failures does not. This stark contrast between plentiful in vitro data and absent in vivo data forces us to remember that not all drugs are pharmacokinetically and pharmacodynamically similar, and, though helpful, data from a test tube cannot always explain what will happen in a human. Although extracellular, tissue site concentrations of the β-lactams and aminoglycosides are in relative equilibrium with those measured in serum, those of the macrolides and quinolones are not. Extracellular, tissue site concentrations of macrolides and quinolones are always higher than those measured in serum and are even higher at sites of inflammation. Highest concentrations are measured within the acute reactant cells that will actually be clearing the bacteria, regardless of whether the pathogen is in the blood or at the infection site. The concentrations within neutrophils and monocytes are at least a log-fold higher than those demonstrated at the tissue site. It is this high intracellular level of drug that not only allows them to be bactericidal rather than static in some situations, but is also the explanation for the lack of clinical failures due to resistance. Speculation as to whether the intracellular activity of these drugs is only applicable to intracellular pathogens has been dismissed by a recent report by Mandell et al. [1] which showed good intracellular activity of macrolides against intracellular pneumococci. The concepts discussed above also suggest that drugs with relatively lower or less sustained intracellular concentrations will become clinically ineffective much sooner than compounds like azithromycin, that concentrate to a high degree and maintain these high white blood cell concentrations for prolonged periods.

Reference

1. Mandell GL, Coleman EJ. Activities of antimicrobial agents against intracellular pneumococci. *Antimicrob Agents Chemother* 2000; 44: 2561–3.

S246 What is the impact of pneumococcal resistance? Clinical evidence

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Antibiotic resistance is increasingly prevalent among the common bacteria causes of LRTI's, the pneumococcus, *Haemophilus influenzae*, and *Moraxella catarrhalis*. In patients with CAP, pneumococcus is the most common pathogen, and multiple drug resistance is present in up to 40% of all isolates, although most is intermediate and not high level resistance. Drug resistant pneumococcus (DRSP) is more likely in certain at-risk populations including those >age 65 years, and those with a history of alcoholism, multiple medical comorbidities, therapy with a β-lactam in the past 3 months, immune suppression, and exposure to a child in day care. The many guidelines for management of CAP all deal with DRSP differently, but the ATS guidelines advise modification of empiric therapy in at-risk populations in an effort to keep current levels of resistance from increasing. DRSP includes not only penicillin resistance, but also multidrug resistance, including to macrolides. For this reason, macrolide monotherapy of CAP should be limited to inpatients and outpatients with none of the risk factors for infections with DRSP or gram-negative bacteria. However, in this population, use of a focused therapy (macrolides) may help to prevent selection pressure for resistance that could emerge if broad-spectrum agents were used. The paradigm for CAP therapy in at-risk populations is to use a selected β-lactam with a macrolide, or alternatively to use monotherapy with a new quinolone. If a quinolone is used, the most active agents are gatifloxacin, moxifloxacin or gemifloxacin, and these may have advantages now that there are reports of levofloxacin resistant pneumococci, especially in COPD patients. There are some data indicating that the higher levels of penicillin resistance (MIC of = 4 mg/L) are associated with increased mortality, but fortunately few patients have this level of resistance present. For the time being, it is important to identify patients at-risk for infection with DRSP and to target them with highly effective therapy to prevent the selection of more resistance in the future, while using focused and targeted therapy in patients without risk factors.

S247 RTI guidelines — Europe versus North America

H. Lode
Berlin, D

Guidelines for respiratory tract infections have been published or endorsed by a number of learned societies around the world. The intention of these documents is to guide and educate physicians, so that antibiotic prescribing is both clinically effective and cost effective, and antibiotics are used in a rational way. The information should further ensure that patients receive optimal disease management and that society benefits by making the best possible use of all resources within the healthcare system. Guidelines are most usefully viewed as a hypothesis that physicians should modify based on the collection of local data, such as epidemiology and susceptibility of potential pathogens. For community-acquired pneumonia (CAP) in Europe, early national guidelines published in France, the United Kingdom, Italy, and Spain have been followed by guidelines from the European Respiratory Society, and in 2001 updated guidelines from the British Thoracic Society. In North America, the first guidelines were published by a Canadian Conference Consensus Group and the American Thoracic Society (ATS), to be followed by guidelines from the Infectious Disease Society of America (IDSA). More recently, the Canadian Infectious Disease Society and Canadian Thoracic Society (CIDS/CTS) jointly released guidelines and the IDSA and ATS issued updates, all of which were evidence-based documents. The Centers for Disease Control and Prevention (CDCP) published recommendations for antimicrobial therapy but not disease management per se. There are fewer published guidelines for the treatment of acute exacerbations of chronic bronchitis (AECB). The Asia Pacific Region Consensus Group made recommendations for empiric AECB therapy in 1998, the same year as the European Thoracic Society published guidelines for recommended treatments. Therapy recommendations for both CAP and AECB vary between the guidelines and these differences will be discussed in more detail.

Emerging challenges in the treatment of resistant Gram-positive infections: new therapeutic options (Symposium arranged by Gilead)

S248 Antibiotic resistance in Gram-positive pathogens: the European experience

I. Phillips
Malaga, E

During the first decades of the antibiotic era, multiple-resistant *Staphylococcus aureus* (the hospital *Staphylococcus*) ravaged our hospitals, being succeeded by methicillin-resistant *Staphylococcus aureus* and multiple-resistant coagulase-negative staphylococci as medical advances produced an increasing pool of vulnerable patients. Hospital infection control and antibiotic prescribing policies were largely developed in response to the difficulties posed by these organisms. Resistance to glycopeptides, quinolones, streptogramins, and linezolid (the last two still relatively uncommon) has occurred among these staphylococci, giving rise to increased multidrug resistance. Meanwhile, threatening these same patients, glycopeptide-resistant enterococci have appeared, perhaps selected by the increased use of cephalosporins and quinolones in general and of glycopeptides in specific for staphylococcal

and *Clostridium difficile* infections. Staphylococci can also acquire resistance to streptogramins and linezolid. Initially, *Streptococcus pyogenes* seemed likely to mimic the staphylococci as tetracycline and macrolide resistance developed. However, while these resistances have waxed and waned, perhaps in relation to the patterns of use of the drugs and to clonal spread of different strains, resistance to β-lactams has never developed in any of the β-haemolytic streptococci. *Streptococcus pneumoniae* has behaved similarly to *S. pyogenes* until recently; over the past 2 decades it has developed low-level resistance to β-lactams, which, although low-level, has considerable clinical importance. Largely in the background, alpha-haemolytic streptococci in the normal respiratory flora have provided a ready store of transferable genes mediating resistance, particularly to β-lactams and macrolides. Of note, however, is that these organisms have become increasingly prevalent in bacteremias in immunocompromised patients. Thus, although there have been periods during which the Gram-positive pathogens have been eclipsed in importance by the Gram-negative bacteria, and although the geographic distribution of resistance in Europe has been uneven, often affecting southern more than northern Europe, the development of multiple antibiotic resistance in virtually all the important Gram-positive pathogens has been inexorable. Furthermore, many of these multiple-resistant bacteria infect the most vulnerable patients – the very young, the old, and the immunocompromised.

S249 Serious Gram-positive infections: clinical challenges of the new millennium

P. Munoz
Madrid, E

Gram-positive infections have, for the first time, outnumbered Gram-negative infections in clinical microbiology laboratories. Their increase in number and the relentless development of antimicrobial resistance have focused the attention of clinicians and the pharmaceutical industry towards finding new therapeutic tools. *Staphylococcus aureus* and coagulase-negative staphylococci account for the vast majority of infections related to prosthetic materials including intravascular catheters, heart valves, articular prosthesis, etc. Their related morbidity and mortality is high and therapy usually requires the parenteral administration of glycopeptides. *Streptococcus pneumoniae* remains the most common cause of pneumonia and severe meningitis, and has also become an important nosocomial pathogen. The reduced susceptibility of this microorganism to penicillin has greatly complicated the management of central nervous system infections in which its participation is suspected. *Enterococcus* spp. is the second-leading cause of nosocomial urinary tract infections and is also commonly implicated in wound infections, bacteremia, and endocarditis. The widespread diffusion of multiresistant Enterococci occasionally leaves the clinician without well-established bactericidal therapies. Group A *Streptococcus* has caused significant social alarm due to the increasing number of cases of necrotizing fascitis in previously healthy patients. Group B *Streptococcus* is associated with severe episodes of bacteremia and endocarditis, as well as urinary tract infections and osteomyelitis. *Streptococcus viridans* has become a significant cause of bloodstream infections in hematologic patients with neutropenia and mucositis, as well as empyema and other purulent collections in critically ill patients. Other Gram-positive microorganisms that are becoming a cause of concern include *Clostridium difficile, Listeria monocytogenes, Nocardia farcinica*, and different species of *Corynebacterium*. Due to the increasing number of clinical challenges, there is a pressing need for effective new bactericidal therapies for the treatment of serious Gram-positive infections.

S250 Emerging challenges in the treatment of resistant Gram-positive infections: new therapeutic options

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Athens, GR

MRSA, MRSE, VRE, PRSP, and, to a much lesser extent, GISA, represent the majority of pathogens currently implicated in serious Gram-positive infections for which therapeutic decisions are increasingly difficult. Vancomycin, although active in vitro against multiresistant staphylococci and pneumococci, possesses pharmacokinetic and pharmacodynamic disadvantages necessitating drug monitoring and the addition of rifampicin and/or gentamicin to overcome its slow bactericidal activity. Furthermore, variable kinetics are responsible either for subtherapeutic antibiotic levels or for potential vancomycin toxicities. Teicoplanin lacks extensive use in "difficult to treat" infections such as staphylococcal endocarditis and osteomyelitis, and dosage schedules are difficult to manage. On the other hand, inappropriate extensive empirical use of vancomycin, particularly in ICU settings and in febrile neutropenic patients, has led to the emerging threat of increased VRE infections. PRSP in lower respiratory tract infections appears to respond to high-dose IV penicillin G; however, PRSP meningitis is a potentially lethal infection necessitating intrathecal vancomycin. New therapeutic modalities active against Gram-positive pathogens include linezolid, quinupristin/dalfopristin (Q/D), daptomycin, oritavancin, and glycylcyclines, with the newer ketolides and "respiratory" quinolones being principally active against PRSP. Only linezolid and Q/D are on the market, and the in vivo data appear promising. However, there are several considerations: (1) linezolid is not bactericidal against staphylococci, and prolonged therapy is limited by concerns for anemia and thrombocytopenia; and (2) Q/D requires central venous routes, it is not bactericidal against *Enterococcus faecium* and MRSA with ermB resistance phenotypes, and several adverse drug interactions have been described. Despite the different site of action and the promising in vitro activity and kinetic profiles of these 2 new compounds, the in vivo emergence of resistance during therapy is already a fact, signaling the necessity for prudent prescribing practices by clinicians.

S251 Daptomycin: a new agent to treat serious Gram-positive infections

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Utrecht, NL

Daptomycin is a novel lipopeptide antibiotic with rapid, concentration-dependent bactericidal activity against all clinically relevant Gram-positive bacteria, including multiresistant strains of *Staphylococcus, Enterococcus*, and *Streptococcus pneumoniae*. Spontaneous acquisition of resistance occurs rarely. The mechanism of action appears to be at least partly due to bacterial membrane depolarization. Daptomycin exhibits linear pharmacokinetic disposition, has a prolonged elimination half-life (~9 h), and is largely excreted unchanged in urine. Efficacy has been demonstrated in a variety of preclinical animal models including bacteremia, soft tissue/thigh infection, endocarditis, pneumonia, and osteomyelitis. Preclinical toxicology has identified skeletal muscle as the primary organ for toxicity; there are no effects on cardiac or smooth muscle. Daptomycin-induced myopathy was characterized by minimal degeneration with regeneration in the absence of fibrosis. The myopathy is easily monitored with creatinine phosphokinase (CPK) levels and is minimized by once-daily dosing. Preliminary phase II data suggested efficacy in bacteremia and in skin and soft tissue infections. Phase III data available to date include 2 large prospective, randomized evaluator-blinded studies of the efficacy and safety of daptomycin vs. standard therapy (semisynthetic penicillin or vancomycin) in the treatment of complicated skin and soft tissue infection. Results demonstrate that daptomycin is as effective as standard therapy based on clinical and microbiologic endpoints. However, patients receiving daptomycin required significantly shorter duration of therapy than patients receiving comparator agents. Based on clinical trials to date, daptomycin appears to have a safety profile comparable to conventional therapy. Adverse effects such as local venous irritation, hypersensitivity, and gastrointestinal toxicity have been uncommonly observed with daptomycin, without evidence of nephrotoxicity, ototoxicity, or hepatotoxicity. Overall incidence and severity of CPK elevations have been similar to standard therapy. Ongoing studies include the treatment of moderate to severe community-acquired pneumonia, staphylococcal endocarditis/bacteremia and infections due to vancomycin-resistant enterococci. Daptomycin appears to be a promising new agent for the treatment of serious Gram-positive infections.

Interactive grand rounds: braving the storms of hospital infection (Symposium arranged by AstraZeneca)

S252 Hospital-acquired pneumonia: a complex killer

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Vienna, A

Hospital-acquired pneumonia (HAP), defined as a pneumonia that develops at least 48 h after hospital admission, is the second most frequent nosocomial infection (after urinary tract infection) and the one with the highest death rate. Diagnosis and treatment of patients with HAP remains a difficult and complex undertaking. Diagnosis is difficult due to the absence of typical symptoms like fever, purulent tracheal secretions or cough — this is particularly true for older patients or patients with immune deficiency. Effective treatment relies on rapid identification of infected patients and accurate selection of antimicrobial agents for initial treatment. The selection of initial antibiotic therapy, however, is based on the presumed causative pathogens and their patterns of antimicrobial susceptibility. The decision making process is complicated by the diversity of pathogens responsible for HAP; these are selected by local or systemic host factors, including the underlying disease of the hospitalized patient, the virulence of the organism, and prior exposure to antimicrobials. Of particular importance are the Gram-negative organisms (*Escherichia coli, Klebsiella* spp., *Serratia* spp., *Proteus mirabilis, Pseudomonas* spp.) and *Staphylococcus aureus*. To add to the complexity of diagnosis and treatment, other factors to be considered are prior duration of hospitalization, previous usage of antimicrobial agents, and intrinsic activities of antimicrobial agents. This case study will address the challenge of diagnosing and treating a patient with HAP.

S253 Suspected intra-abdominal infection: approaching the unresponsive patient

U. Schoeffel
Freiburg, D

Suspected intra-abdominal infection consistent with laboratory data but without localizing signs at abdominal examination is a typical surgical problem. The clinical presentation of unresponsive patients is often non-characteristic and leaves the clinician in a dilemma of how best to proceed. With no clear-cut signs of diffuse peritonitis or other intestinal catastrophe, questions arise over which diagnostic tests to perform and/or which therapy to begin. A stepwise approach to some key questions is imperative and should ensure the most appropriate course of action is taken. Firstly, does an intra-abdominal infection seem probable? There may be clues such as bowel paralysis, distension, some reaction to palpation, or the occurrence of intra-abdominal fluid. Secondly, should a directed or a more wide-ranging search for the presence of infection be adopted? If, in turn, a suspected intra-abdominal infection is confirmed, the third question should address the urgency and approach to source control. In parallel to this stepwise approach, empirical anti-infective therapy should be initiated as soon as severe bacterial infection is suspected. If there is no obvious source of contamination, then therapy should be directed against indigenous enteric bacteria. The case study presented will tackle the problem of an unresponsive patient and suggest how best to address the treatment of intra-abdominal infection.

S254 Candidaemia: diagnosis and treatment challenges

T. Calandra
Lausanne, CH

Candida is a frequent cause of nosocomial infections with significant morbidity and mortality. Along with an increasing incidence, these infections remain difficult to diagnose. Clinical symptoms and signs are usually non-specific, and standard culture techniques and serological methods lack sensitivity. Amplification of genomic DNA sequences by polymerase chain reaction may improve our diagnostic ability, but is not available on a routine basis. The most frequent clinical manifestations of *Candida* are candidaemia, intra-abdominal candidiasis, candidal urinary tract infections, and disseminated candidiasis. Candidaemia, associated with a high crude and attributable mortality, will be examined in this case study. Most episodes are caused by *Candida albicans*, however, nonalbicans *Candida* infections are increasing and may predominate in some institutions. Risk factors for candidaemia include treatment with antibiotics, use of intravascular devices, parenteral nutrition, surgery, and colonization with *Candida* at multiple sites. In light of this, intravascular devices should be removed or exchanged whenever possible in patients with candidaemia. Regarding treatment, studies of non-neutropenic patients with candidaemia have shown that azoles are as effective but less toxic than amphotericin B. New antifungal agents, such as the echinocandins, look promising for the treatment of patients with candidiasis, including bloodstream infections, and are currently being evaluated for candidaemia. This case study will highlight some of the issues faced when treating patients with this serious infection.

Chlamydia and *H. pylori* in vascular diseases

S259 *H. pylori*: a headache for the third millenium?

M. R. Gismondo
Milan, I

Despite our current understanding that *H. pylori* is confined to gastric mucosa, new interest is generated by the link between *H. pylori* and disorder outside the alimentary tract. An increasing number of extra-digestive conditions have been reported to bear association with *H. pylori*: coronary heart disease, vascular disorders, inflammatory and immune-mediated disorders, etc. Our results, according to those of other AA., shown that *H. pylori* infection is common in patients with headache and with migraine without aura. We have carried out two studies: an epidemiological investigation and a clinical study. Epidemiological results have shown that 42% of infected patients suffer from imigraine or headache. The number of positive CagA strains was significantly higher than those of VacA positive strains. Patients treated by using "Lactobacillus" significantly reduced symptoms. These results encourage futher investigations to undestand the "poliedric" role of *H. pylori* in human disease.

Clostrium difficile (Symposium organised by ESGCD)

S261 Diagnostic methods and testing protocols for *Clostridium difficile* infections in Europe: an overview

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In recent decades, *Clostridium difficile* has emerged as an important enteropathogen responsible for antibiotic-associated diarrhea and/or colitis. The recognition of *C. difficile* as a leading cause of nosocomial diarrhea also contributed to the development of a large number of tests for the diagnosis of *C. difficile*-associated diseases (CDAD). These tests include fecal culture on selective media, detection of toxins A and/or B with "classic" or "rapid" enzyme immunoassays, or detection of the glutamate dehydrogenase (GDH), a marker antigen of *C. difficile*. The lack of guidelines in Europe can make the choice of testing protocols difficult.

Under the auspices of the ESCMID Study Group on *C. difficile* (ESGCD), we undertook a European-wide survey aimed at obtaining an overview on the current practices in bacteriology laboratories. A standardized questionnaire

about diagnostic methods and testing protocols was sent to one co-ordinator in each country participating in this survey. This co-ordinator was in charge of forwarding the questionnaire to hospital laboratories arbitrarily selected on the basis of 1 lab per 10 000 beds of hospitalization. This questionnaire covered different fields about CDAD diagnosis: circumstances of request, criteria used for undertaking *C. difficile* investigations, methods used for the diagnosis (culture, toxin detection, GDH detection) ... antimicrobial susceptibility testing and experience in typing *C. difficile* strains were also evaluated. The questionnaire also focussed on the strategies that are currently used in labs for CDAD diagnosis. Data about the number of *C. difficile* cases detected in 2000, the size of hospital (number of beds and number of admissions) were also collected in order to approximately estimate the incidence CDAD in different care settings. Global results and differencies among countries will be discussed.

Community-acquired pneumonia

O264 Severity of airflow obstruction and bacterial etiology of acute exacerbation in COPD – data from a prospective study

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Berlin, D

Introduction: Retrospective data from a previous analysis of our study-group suggests a relationship between severity of airway obstruction in COPD, and the pattern of causative bacterial pathogens in acute exacerbations (AECB) [Eller et al. Chest (1998)]. This hypothesis was tested in a prospective study.
Methods: From January 1997 to April 2001, patients with COPD and worsening of respiratory symptoms were evaluated for inclusion into this investigation. Radiographic and other procedures were performed as required to exclude other conditions but AECB as cause of respiratory distress. Sputum samples were obtained whenever possible, and semiquantitative microscopic and cultural bacteriologic testing was performed. For all subjects, spirometric data from an infection-free interval was required. Patients were classified into three stages of severity of airflow obstruction, according to ATS criteria. Stage I, FEV1* 50% predicted; stage II, FEV1 35–50% predicted; and stage III, FEV1 *35% predicted. Bacteria were divided into three groups. Group 1 included *Streptococcus pneumoniae, Staphylococcus aureus*, and other Gram-positive cocci, group 2 consisted of *Haemophilus influenzae* and *Moraxella catarrhalis*, and group 3 included Gram-negative enteric bacteria and *Pseudomonas aeruginosa*.
Results: A total of 292 subjects were evaluated for inclusion. Sixty-seven patients were treated as outpatients, 225 subjects were inpatients. In 76 individuals, bronchiectasis or conditions other than AECB (e.g. pneumonia, carcinoma) disqualified from inclusion. In 115 patients, an increase of purulent sputum was present (Type Winnipeg I or II), and relevant bacteria were isolated from valid sputum samples (AMS-criteria). Sixty-eight subjects (59.1%) were male, mean age was 62 years (range 54–68 years). Half of the individuals were present smokers ($n = 57$, 49.6%), 18 were never-smokers (15.7%). Thirty-two (27.8%) isolated bacteria belonged to group 1-organisms, 48 (41.7%) were group 2-bacteria, and 35 bacteria (30.4%) belonged to group 3. There was a significant relationship between extent of airway obstruction prior to onset of AECB and the pattern of isolated bacterial pathogens in an acute exacerbation ($P = 0.01$).

	Bacteria, *n* (%)			
COPD	Group 1	Group 2	Group 3	*n*
Stage I	19 (40.4)	22 (46.8)	6 (12.8)	47
Stage II	10 (27.0)	16 (43.2)	11 (29.7)	37
Stage III	3 (9.7)	10 (32.3)	18 (58.1)	31
n	32	48	35	115

Conclusion: These findings confirm our previous results from retrospective analysis. They might be valuable in guiding initial antibiotic therapy in patients presenting with AECB presumptively caused by bacteria (Type Winnipeg I or II).

O265 Efficacy and safety of moxifloxacin versus ceftriaxone in the treatment of AECB (SMART)

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Pavia, Perugia, Genoa, Milan, I

Objective: Moxifloxacin is a broad spectrum 8-methoxy-fluoroquinolone that covers all the common community-acquired Gram-positive and Gram-negative respiratory tract pathogens. The aim of this study was to compare the efficacy and safety of moxifloxacin (MXF) orally given for 5 days, to ceftriaxone (CFX), intramuscularly given for 7 days in the treatment of patients suffering from AECB.
Methods: A total of 476 patients suffering from AECB (Anthonisen I and II) were enrolled in a nonblinded, randomised, multicenter Italian clinical trial comparing moxifloxacin (400 mg OD p.o.) to ceftriaxone (1 g OD i.m.). A total of 423 patients were valid for efficacy (PP), 213 (MXF) versus 210 (CFX). The two groups were comparable in terms of demography and clinical status.
Results: The clinical success rate at the test-of-cure visit (Day 10 post-therapy) for MXF (90.6%) was statistically equivalent to that of CFX (89.5%) [95% CI 0.60; −2.158]. However, in the MXF group, patients experienced a lower percentage of relapses compared to those in the CFX group (71.3% vs. 76.7%). Relapse was recorded for patients successfully treated at the test-of-cure visit and followed for up to a 6-month period. The incidence of adverse events (AEs) was similar in both groups.
Conclusions: Five-day oral MXF is as effective and safe as 7-day intramuscular CFX in patients with AECB. In addition, MXF may have a potential benefit in prolonging the exacerbation-free period.

O266 Risk factors for adverse outcome in hospitalized patients with pneumococcal pneumonia

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Objective: To identify the risk factors for death and respiratory failure in hospitalized patients with pneumococcal bacteremia and pneumonia, we study retrospectively all the available data from our patients medical charts.
Patients and methods: During the last 18 months 204 adults (mean age 59 ± 6 STD years) admitted to our Department for treatment of pneumococcal pneumonia with bacteremia.
Results: From these patients, 25 (12.5%; 95% confidence interval [CI], 12–28%) died and 34 (16%; 95% CI, 16–31%) survived after short mechanical ventilation for acute respiratory failure. In univariate analyzes, patients with various pre-existing lung diseases (relative risk [RR], 2.5; 95% CI, 1.8–4.4), initial body temperature >39 °C (RR, 2.0; 95% CI, 1.6–4.6), or nosocomial infections (RR, 2.2; 95% CI, 1.9–3.9) or who were ^{3}55 years old (RR, 2.8; 95% CI, 1.8–5.9) were at greater risk for adverse outcomes than patients without these risk factors. From 75 patients without these risk factors, only three (4%; 95% CI, 0.6–2.0%) died, and the remaining 72 patients did not require admission to the Intensive Care Unit (ICU). Calculating these risk factors with a multivariate logistic model, death or acute respiratory failure would have been predicted in 55% of patients. Better outcome was accurately predicted in 79% of our patients. In multivariate analysis, nosocomial infection was the greatest risk factor (adjusted odds ratio, 16.6; 95% CI, 2.9–8.9).
Conclusions: In hospitalized patients with pneumococcal pneumonia and bacteremia, risk factors determined at hospital admission can easily predict the final outcome. If we were able to identify in time these risk factors ICU or

more aggressive medical treatments would benefited these patients. Independent of age, patients who acquired nosocomial infections, during their hospitalization, were at the greatest risk factor for death or respiratory failure.

O267 Community-acquired pneumonia guidelines: evaluation of antibiotics, outcomes and costs

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Introduction: The most appropriate empiric antibiotic choice will depend upon local antibiotic resistance patterns, patient demographics, epidemiological data, and cost. The Ohio State University Medical Center (OSUMC) developed a practice guideline for CAP. Azithromycin ± ampicillin/sulbactam was recommended for empiric coverage unless the patient was critically ill or had modifying factors such as suspected or documented penicillin resistant streptococcus pneumonia (PRSP).
Objective: To assess the clinical and economic outcome of patients who receive azithromycin for CAP, with a secondary goal to determine the appropriateness of the guidelines.
Methods: This was a retrospective analysis of all patients who received azithromycin between September 1999 and May 2000, for the treatment of CAP. Demographic and epidemiologic data included age, gender, and environment of the patient prior to hospitalization. Prior antibiotic use was recorded. Clinical and microbiological outcomes were recorded. Adverse drug reactions (ADR) and discharge antibiotics were identified. Hospital length of stay (LOS) and total cost of care were calculated.
Results: Azithromycin was administered to 124 hospitalized patients with CAP; 40 as monotherapy, 41 in combination with ampicillin/sulbactam, 35 in combination with antipseudomonal antibiotics, and eight in combination with others. Mean age = 63.9, 53% male. Environment prior to hospitalization was 80% from home, 9% transfer from other hospital, 5% nursing home, and 6% prison. Prior to hospitalization, 21% patients failed or had intolerance to oral outpatient antibiotics. Microbiologically evaluable patients included 5/109 with bacteremia; two *Streptococcus pneumoniae* (1 PRSP), one β-hemolytic streptococcus, one *Enterococcus faecalis*, one *Staphylococcus aureus*, and 22/77 with a positive sputum culture (1 PRSP). Microbiologic success was 98%. Clinical success was 98%. Mortality was <1%. ADR's were diarrhea (1%). Most patients (91%) were discharged on an oral antibiotic; 38% azithromycin, 33% amoxicillin/clavulanate, 20% others. Mean hospital LOS = 4.6 days. Mean cost = US$ 4850.
Conclusion: Compliance to the OSUMC practice guideline for CAP show that patients have successful clinical outcomes with an acceptable LOS and cost. The low rate of PRSP (<2%) supports the use of azithromycin as the preferred antibiotic unless modifying factors are present.

Ertapenem

O268 Ertapenem is highly active against common clinical bacterial pathogens

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Objectives: Determine the in vitro activity of ertapenem, a new once a day parenteral β-lactam, against common clinical bacterial pathogens and relate MIC_{90} values to plasma ertapenem concentrations.
Methods: Isolates were obtained from adults enrolled in Phase II and III clinical trials of ertapenem therapy. These studies enrolled patients with community-acquired and mixed infections, including complicated intra-abdominal, skin and skin structure, and urinary tract infections, acute pelvic infections, and community-acquired pneumonia at 425 sites worldwide. A total of 3221 aerobes and anaerobes were tested at Merck Research Laboratories for susceptibility to ertapenem by microtiter dilution. In separate studies, ertapenem concentrations in plasma and urine were measured for up to 24 h following 1-g dose in 68 healthy adult volunteers.
Results: Ertapenem MIC_{90} values (mcg/mL) for the most common pathogens isolated during clinical trials were: *Escherichia coli* ($n = 759$), 0.016; *Klebsiella* spp. ($n = 149$), 0.03; *Enterobacter* spp. ($n = 56$), 0.25; other *Enterobacteriaceae* ($n = 124$), 0.06; *Staphylococcus pneumoniae* ($n = 113$), 0.25; methicillin-susceptible *S. aureus* ($n = 187$), 0.25; *S. pyogenes* ($n = 37$), 0.03; *S. agalactiae* ($n = 48$), 0.125; *Haemophilic influenzae* ($n = 59$), 0.125; *Moraxella catarrhalis* ($n = 9$), 0.016; *B. fragilis* group ($n = 479$), 1; all other anaerobes ($n = 805$), 1; methicillin-resistant *S. aureus* ($n = 27$), 16; enterococci ($n = 169$), 16; *P. aeruginosa* ($n = 86$), 16. The mean plasma ertapenem concentrations after a 1-g dose were 9–11 μg/mL at 12 h and 1.2–1.9 μg/mL at 24 h.
Conclusions: Following 1-g dose, ertapenem plasma concentrations exceed MIC_{90} values for common clinical isolates, except enterococci, *P. aeruginosa*, and methicillin-resistant *S. aureus* for at least 24 h. These data suggest that ertapenem, 1 g once a day, is likely to be highly effective against most aerobic and anaerobic bacteria responsible for community-acquired and mixed infections.

O269 Efficacy of ertapenem in the treatment of community-acquired pneumococcal pneumonia

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Objectives: Determine the efficacy of ertapenem, a new once a day parenteral β-lactam, for treatment of adults with serious CAPP and compare it with ceftriaxone (CRO) therapy.
Methods: A prospective, double-blind (with in-house blinding), multicenter study was conducted in adults with community-acquired pneumonia requiring initial parenteral therapy. Patients were stratified based on Pneumonia Severity Index (PSI 1–3 or >3) and age (65 year or less or >65 year) and randomized in a 1 : 1 ratio to intravenous (i.v.) ertapenem, 1 g once a day, or i.v. CRO, 1 g once a day. Patients whose sputum or blood cultures grew *Streptococcus pneumoniae* were included in this subgroup analysis. Switch to oral amoxicillin-clavulanate was allowed after at least 3 days of i.v. therapy. Clinical and microbiologic efficacy were assessed 7–14 days post-therapy (i.v. + oral).
Results: Serious CAPP was diagnosed in 48 (26%) of the clinically evaluable patients in the ertapenem group and 60 (30%) in the CRO group. Baseline demographics were similar in the two treatment groups. In the ertapenem and CRO groups, respectively, 19 and 32% of patients had a PSI > 3 and 38 and 43% were >65 year of age. Of patients who received ertapenem, six were bacteremic, as were 17 of those treated with CRO. Median duration of total therapy was 10.5 days in the ertapenem group and 13 days in the CRO group; 98% of the patients in the ertapenem group and 92% in the CRO group received oral therapy. Ertapenem versus CRO clinical cure rates were 92% (44/48) versus 93% (56/60) overall, and by penicillin (pen) susceptibility (S, susceptible; NS, nonsusceptible; UNK, unknown; R, resistant) were: pen-S, 88% (28/32) versus 91% (32/35); pen-NS, 100% (11/11) versus 92% (12/13); pen-UNK, 100% (5/5) versus 100% (12/12); pen-R, 100% (1/1) versus 100% (3/3). Bacterial eradication rates were 98% for ertapenem and 98% for CRO; rates were not affected by penicillin susceptibility. Both ertapenem and CRO were generally well tolerated.
Conclusions: In this subgroup analysis, ertapenem 1 g once a day, with an oral switch option, was highly effective for treatment of serious CAPP, including infections with pen-NS isolates, and was as effective as CRO 1 g once a day. The outcome of CAPP treated with ertapenem or CRO was not influenced by the degree of penicillin susceptibility. Ertapenem had a safety and tolerability profile similar to CRO.

O270 Ertapenem versus ceftriaxone (CRO) for treatment of community-acquired pneumonia (CAP): the European experience

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Objectives: Determine the efficacy of ertapenem, a new once a day parenteral β-lactam with the potential to be used for community-acquired infections, for treatment of adults with serious CAP and compare it with CRO. We report here on the clinical experience of 188 patients from six European countries.

Methods: In two prospective, double-blind (with in-house blinding), multicenter studies conducted worldwide, including six European countries, adults with serious CAP were stratified based on Pneumonia Severity Index (PSI 1–3 or >3) and age (65 year or less or >65 year) and randomized to ertapenem, 1 g once a day, or CRO, 1 g once a day, both given i.v. or i.m.. Switch to oral amoxicillin-clavulanate was allowed after at least 3 days of i.v. therapy and clinical improvement. Respiratory specimens were collected for culture and susceptibility testing of isolated pathogens. Clinical and microbiologic efficacy was assessed 7–14 days post-therapy (i.v. + optional oral).
Results: Of the 866 patients randomized worldwide, 118 (14%) were from European sites; 90 (76%) were clinically evaluable; 52 (44%) were microbiologically evaluable. The two treatment groups were similar with respect to age, gender, and ethnicity. Median total therapy was 10 days in both treatment groups; 67% of patients in the ertapenem group and 69% in the CRO group received oral therapy. Clinical cure rates overall and by stratum are shown in the table below.

Drug	PSI > 3	PSI 1–3	>65 years	≤65 years	Overall
Ertapenem	94% (15/16)	90% (38/42)	91% (21/23)	91% (32/35)	91% (53/58)
CRO	82% (9/11)	86% (18/21)	80% (12/15)	88% (15/17)	84% (27/32)

Eradication rates for *Streptococcus pneumoniae*, the most common pathogen, were 92% for ertapenem and 100% for CRO and were not influenced by susceptibility to penicillin. Both ertapenem and CRO were generally well tolerated.
Conclusions: In this subgroup analysis of European patients, ertapenem 1 g once a day, with an oral switch option, was highly effective for serious CAP, including elderly patients and patients with concomitant diseases, and was as effective as CRO. The safety and tolerability profile of ertapenem was similar to CRO.

O271 Safety and tolerability of ertapenem administered intramuscularly (i.m.)

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Objectives: Ertapenem is a new once a day β-lactam antimicrobial agent that can be administered by intravenous (i.v.) infusion or i.m. injection. The objective of this study was to evaluate the local tolerability and safety of i.m. ertapenem, which in human subjects is >90% bioavailable, compared with i.m. ceftriaxone.
Methods: In a prospective, double-blind (also with sponsor blinding), multicenter study, adults ($n = 117$) with lower respiratory tract infection, skin infection, or urinary tract infection requiring initial parenteral therapy were randomized in a 3:1 ratio to i.m. ertapenem, 1 g once a day, or i.m. ceftriaxone, 1 g once a day. Both agents were reconstituted in 1% lidocaine without adrenaline. Patients who improved clinically could be switched to oral amoxicillin-clavulanate after at least 2 days of i.m. therapy. Tolerability and safety were evaluated in the treated population; efficacy was assessed in the modified-intent-to-treat population.
Results: Eighty-seven patients received i.m. ertapenem therapy for a mean of 4.1 days, and 30 patients received i.m. ceftriaxone for a mean of 3.8 days. 31/87 patients (36%) treated with ertapenem and 13/30 (43%) treated with ceftriaxone experienced one or more symptoms at the local injection site; the most common were tenderness or pain. Local symptoms were generally mild, and were judged to be moderate to severe in only one (1%) patient in the ertapenem group and three (10%) in the ceftriaxone group. Clinical drug-related adverse experiences were reported during i.m. therapy in 14 (16%) patients in the ertapenem group, most commonly mild gastrointestinal symptoms, and five (17%) patients in the ceftriaxone group. The efficacy of i.m. ertapenem appeared comparable to that of i.m. ceftriaxone.
Conclusion: An i.m. ertapenem therapy, 1 g once a day, was generally well tolerated. The safety and tolerability profile of i.m. ertapenem therapy was comparable to those of i.m. ceftriaxone therapy.

Epidemiological resistance of Gram-negative bacteria

O272 Chromosomal, plasmid and β-lactamase gene heterogeneity among *Salmonella enterica* enteriditis PT4 isolated in southern Italy

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Objectives: The aim of this study was to determine the mechanisms and vectors of resistance to expanded spectrum β-lactams observed among six *Salmonella enterica* ssp. enterica serotype Enteritidis isolates from southern Italy during 1990–1998.
Methods: Isolation, species characterization, serotyping and phage typing were performed according to standard procedures. Susceptibility to 15 antibiotics was determined by a disk diffusion method and evaluated using NCCLS guidelines. Resistance plasmids were transferred by conjugation to an *Escherichia coli* recipient strain, isolated and digested with EcoRI prior to agarose gel electrophoresis. β-Lactamase content was determined by isoelectric focusing from salmonella and *trans*-conjugant cell lysates, PCR and DNA–DNA hybridization with SHV and ampC primers and probes, and nucleotide sequencing. Genomic DNA fingerprints were generated by *XbaI* restriction and pulsed field gel electrophoresis.
Results: Of the 1889 S. Enteritidis isolates characterized during 1990–1998 at the Centre for Enteric Pathogens of southern Italy, only six—five of which were of phage type PT4—were resistant to expanded spectrum cephalosporins. Of these, one, of PFGE type A, harbored a nontransferable *ampC* gene, whilst the remaining five, of PFGE type B, contained a transferable, plasmid-borne *SHV12* gene. However, two distinct types of *SHV12*-bearing plasmids were observed, one of which also contained a type 1 integron including *aacC4*, *aadA1* and *catB2* gene cassettes.
Conclusions: Resistance to expanded spectrum cephalosporins in food-borne S. Enteritidis PT4 strains constitutes a potential clinical problem. An unusual heterogeneity with respect to plasmid and gene content, as well as genomic background, was observed among such strains from southern Italy.

O273 Population structure and antibiotic resistance of Acinetobacter DNA group 2 and 13TU isolates causing nosocomial infections in United Kingdom hospitals

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Objectives: To examine the population structure of isolates of Acinetobacter DNA groups 2 (*Acinetobacter baumannii*) and 13TU (unnamed) obtained from clinical specimens in UK hospitals. These are the two DNA groups of Acinetobacter associated most frequently with outbreaks of nosocomial infection. A subsequent aim was to determine whether resistance to particular antibiotics could be correlated with the ability of individual genotypes to cause outbreaks of infection.
Methods: A total of 595 Acinetobacter isolates, collected consecutively from 54 hospitals in UK, Wales and Scotland, were identified to the DNA group level by tDNA fingerprinting. Of these, 287 consecutive isolates belonging to DNA groups 2 and 13TU obtained from 46 hospitals throughout the UK were analyzed by RAPD-PCR using primers DAF-4 and ERIC-2. Isolates with RAPD fingerprints showing >72% similarity with both primers were considered to be closely related and to belong to the same genotype. Antibiotic susceptibility testing was performed on all isolates examined.
Results: In contrast to previous European studies which have suggested a limited number of circulating genotypes, RAPD typing indicated a heterogeneous population, with 37 genotypes of Acinetobacter DNA groups 2 and 13TU circulating within and between UK hospitals. However, 42% of isolates belonged to four predominant genotypes found in 15, 12, 12 and eight different hospitals, respectively. Resistance was widespread to most of the major antibiotic classes, except carbapenems and polymyxins. However, in general, sporadic isolates were more susceptible than outbreak isolates, and possession of multiple antibiotic resistance was associated with the ability of strains to cause outbreaks.

Conclusions: The study demonstrated that there is a heterogeneous population of Acinetobacter DNA group 2 and 13TU isolates causing infections in UK hospitals. Preliminary evidence suggests that possession of multiple antibiotic resistance may be a useful indicator of outbreak potential.

O274 Evolution of TEM ESBLs in Polish hospitals 1995–2000

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Objectives: To follow the evolution of TEM extended-spectrum β-lactamases (ESBLs) in Poland.
Methods: Seventeen ESBL-producing *Klebsiellae* and *Escherichia coli* isolates recovered in 1998–2000 in five hospitals were investigated. The isolates were subjected to mating, and, together with the *trans*-conjugants, to the IEF analysis of β-lactamases. ESBL activity was detected by the bioassay approach. ESBL-encoding genes were amplified by PCR and sequenced together with their promoters. Some of the isolates were compared by PFGE and plasmid fingerprinting with 10 *K. pneumoniae* and *E. coli* isolates from 1995 to 96 from 4 other centres, identified before as producers of TEM-47, -48, -49 or -68 ESBLs.
Results: Apart from other β-lactamases the isolates were found to produce enzymes with pIs of 5.5 or 6.0, which showed the ceftazidime- and cefotaxime-hydrolysing activity in vitro. All these enzymes were identified as TEM β-lactamases and in the majority of the isolates their genes were located on transmissible plasmids. Sequencing has revealed that seven different TEM ESBLs, TEM-4, -29, -47, -85, -86, -93 and -94, were expressed by the isolates, out of which the TEM-85, -86 and -94 are novel variants of ESBLs, and TEM-4, -29 and -93 were identified for the first time in Poland. The sequence analysis of *blaTEM* genes and their promoters suggested that the evolutionary tree of TEM ESBLs in Poland consists of at least two separate branches, one of which, with the single *blaTEM-93* gene, originates from the *blaTEM-1* A precursor gene. The second branch, containing all the remaining variant genes, originates from the *blaTEM-1F* gene and is highly structured with at least three major and several minor subbranches. The PFGE and plasmid fingerprinting analyzes revealed a remarkable diversity of *K. pneumoniae* clones and plasmids containing the related *blaTEM* genes. The only similarities were observed among *TEM-47* producers from 1995 to 96 from three different centres.
Conclusions: Ten different variants of TEM ESBLs identified between 1995 and 2000 in Poland, including seven unique types, indicate that Polish hospitals have been a place of rapid evolution of this family of β-lactamases. Identification of the same or closely related *blaTEM* gene variants in numerous hospitals together with typing data suggest that both convergent evolution as well as spread of ESBL producers between different centres may have contributed to the situation observed nowadays.

O275 Outcomes of infections caused by extended spectrum β-lactamase (ESBL) producing strains of *E. coli* and *Klebsiella* spp. treated with cefepime

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Objectives: The NCCLS currently recommends that Microbiology Laboratories and Clinicians consider that organisms carrying an ESBL to be resistant to all cephalosporins. However, the susceptibility of such strains to 3rd and 4th generation cephalosporins has been reported to be between 40 and 90%, depending on the source of the strains and the agent studied. Among the cephalosporins, FEP, a 4th generation agent with increased stability against most betalactamases, shows very high susceptibilities using the conventional break point of 8 mg/L but clinical data on outcomes with the use this agent have been lacking.
Methods: In order to obtain such data, case report forms were provided to selected clinicians in six countries to obtain information on patients with ESBL *E. coli/Klebsiellae* treated with FEP before susceptibilities were available. They were also asked to provide the strains that were considered to be ESBL producers for verification of the MICs against cephalosporins. All strains were retested by tube dilutions to obtain their MIC for cefepime and other agents.
Results: Sixty-eight cases were collected. Twelve patients were excluded for a variety of reasons. Forty-four FEP treated patients had isolates confirmed as ESBL bearing strains of *E. coli/Klebsiellae*. Twelve patients treated with imipenem also had isolates confirmed as ESBL *E. coli/Klebsiellae*. Of the 44 patients receiving FEP as initial empiric therapy, six also received an aminoglycoside. 68% of the 44 isolates had an MIC of * 8 mg/L of FEP whereas 97% of those tested had MICs of * 8 mg/L of ceftazidime. Of the FEP in vitro susceptible isolates, 80% (24/30) were eradicated or presumably eradicated with FEP therapy. Of the in vitro resistant isolates, 35.7% (5/14) were eradicated or presumably eradicated with FEP therapy. Cure rates by site of infection were: urinary tract 90%, lower respiratory tract 76%, bacteremia 45% (60% for strains with MIC's of * 8 mg/L). Of these patients, 12 case report forms were obtained from a study of empiric imipenem versus FEP for nosocomial pneumonia in which an additional 12 patients with ESBL *E. coli/Klebsiellae* were also treated with imipenem. The bacterial eradication rates were 83 and 70% for FEP and imipenem, respectively.
Conclusion: These data suggest that FEP may be effective therapy for ESBL producing strains of *E. coli/Klebsiellae* which fall within the conventional susceptibility range. Such information may be used in designing empiric therapy in institutions where the susceptibilities of the resident ESBL clones are known.

Influenza and other viruses

O276 The development of a new national surveillance system in the UK for 'influenza-like illness'

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NHS Direct is a nurse led national telephone helpline for health advice.
Objectives: To describe the evolution of a new surveillance system designed to detect 'influenza like illness'. Assess the usefulness of this surveillance system for early detection of 'influenza like illness' in the community. Report the results of the surveillance undertaken during three winters (1999–2002).
Methods: NHS Direct is open 24 h a day, 365 days a year, organized into 23 call centres and takes over 5 million calls a year. During the first winter of the surveillance (1999/2000), call data relating to respiratory symptoms were received from three NHS Direct sites (population 6.6 million). The surveillance has been extended to all 23 sites (population 52 million) during the winter of 2001/2002. Age-specific data are received every weekday and disseminated to public health colleagues in UK and Wales. Nurses at all NHS Direct sites use the Clinical Assessment System (CAS) to handle calls. The CAS is based around 200 computerized clinical algorithms. For surveillance during 2001/2002 the 'cold/flu', cough and fever algorithms were used to monitor respiratory symptoms in the community.
Results: The data are accurate indicators of influenza activity during English winters characterized by both high (1999/2000—predominantly Influenza A H3N2) and low influenza activity (2000/2001—predominantly Influenza B). However, analysis of data is complicated by seasonal trends in other respiratory viruses and the availability of other primary care services. The proportion of NHS Direct calls for which the 'cold/flu' algorithm is used usually fluctuates between 1 and 3% but peaks over Christmas (when other primary care services are less available), as well as during periods when respiratory viruses are known to be circulating in the community. During periods of peak influenza activity NHS Direct data reflects the age distribution of influenza shown by laboratory data. This paper will be updated with NHS Direct surveillance data from the winter of 2001/2002.
Conclusions: The benefits of the NHS Direct based surveillance system compared to currently used routine surveillance systems for influenza are its national coverage and timely nature. The complex and flexible nature of CAS provides an opportunity to improve the specificity of the definition of influenza like illness and to detect syndromes rather than primary symptoms.

O277 The incidence of influenza-associated hospitalizations in children in Germany

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Objectives: Since new vaccines and antiviral drugs for influenza have become available, collation of actual and country-specific epidemiological data is essential. Since respiratory syncytial virus (RSV) is a well-known pediatric airway pathogen and some epidemiologic data exist already, a comparison between influenza and RSV is performed.
Methods: From July 1996 to June 2001, the naso-pharyngeal aspirates (NPA) of children from birth to 16 years of age, admitted to one of the two pediatric hospitals in Kiel, Germany, were investigated by a 9-valent multiplex reverse transcriptase PCR assay.
Results: NPA were investigated in 60.5% of 3469 children admitted with an acute respiratory tract infection. Community-acquired (nosocomial) infections due to influenza A were diagnosed in 122 (10), due to influenza B in 14 (2) and due to RSV in 329 (14) cases. Patients with influenza A (median 752 days) and influenza B (median 966 days) were older than patients with RSV (median 168 days). The spectrum of disease presentation was broader in influenza than in RSV. In each winter, admissions with influenza were less common than with RSV. Influenza B only occurred in 2 years. The cumulative, population-based incidences per 100 000 children 0–16 (0–5; >5–16) years of age were 53 (123; 22) for influenza A, 16 (30; 9) for influenza B and 165 (453; 4) for RSV. Cardiac conditions and asthma were the major risk factors in influenza A (RR 9.8; 4.1) and in RSV (8.5; 2.1) infections. Underlying conditions were most common in influenza B. Low gestational age doubled the risk for influenza A infection, but did not show a dose-effect relationship as in RSV.
Conclusions: The importance of influenza-positive hospitalizations is about one third that of RSV. The incidence is similar to reports from the USA. Targeting children with underlying conditions, especially cardiac conditions and asthma in the German immunization program is appropriate, as long as no policy for vaccination of the general pediatric population exists.

O278 Persistence of Picornaviridae in human neural stem cells: an in vitro model for central nervous system damages and viral etiologies

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Background: Multiple in vivo and in vitro studies suggest that persistent infections due to Picornaviridae are not uncommon. It has now been proved that Poliovirus can persist in human central nervous system (CNS), and it is now hypothesized that this enteroviral persistence may be related to neurological diseases.
Objectives: To investigate the possible links between Picornaviridae persistent infections and CNS damages using an in vitro model developed with human neural and glial cells.
Material and methods: The human cell line Dev, established from a primitive neuroectodermic tumor is similar to neural precursors and retains the capacity to differentiate towards astrocytes. A total of 25 000 cells were inoculated with calibrated suspensions of three $TCID_{50}$/25 µL of each virus strain: Cytomegalovirus, Herpes Simplex Virus, Adenovirus, Mumps virus, Measle virus, Enterovirus type 70, Coxsackievirus B3, Poliovirus type three sabin strain, ECHOvirus type 6 and 7, and Human Parechovirus type 1. After a contact period of 1 h 30 min, the inoculum was discarded and cells cultivated with 5 mL Dulbeccos MEM supplemented with 10% Fetal Calf Serum at 37 °C in a 5% CO_2 incubator. Cytopathic effect was observed daily, culture passages performed each 72 h. When no CPE was observed, total DNA or RNA was extracted from the monolayer and tested with the respective specific PCR. Eventually, when a persistent infection was suspected, supernatants of the cell monolayers were inoculated onto sensitive cells and titrated according to the Reed and Muench method.
Results: A cytopathic effect was observed with all viruses except human parechovirus type 1. After cytopathic effect, the monolayer was spontaneously restored in ECHOvirus 6 and ECHOvirus 7 infected cells only. Despite the lack of cytopathic effect, human parechovirus type 1 established a persistent infection in Dev cells, as confirmed by a specific RT-PCR. Supernatants from HPEV1, E6 and E7 persistently infected Dev cells titrated 104 $TCID_{50}$/25 µL on HRT cells, 105 $TCID_{50}$/25 µL on MRC-5 diploid cells and 104 $TCID_{50}$/25 µL on MRC-5 diploid cells, respectively.
Conclusion: This in vitro model confirms that several Picornaviridae can be responsible for persistent infections in neural and glial human cells. In agreement with the results described with poliovirus, this in vitro model may be helpful to investigate the role of Picornaviridae in CNS damages and in neurodegenerative diseases.

O279 Epidemiology of varicella infection to assess the burden of disease in Germany

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Objectives: Varicella is a common but not always benign disease which may place a great economic burden. To provide baseline estimates of varicella epidemiology in Germany, a retrospective study was conducted.
Methods: A representative nationwide sample of 1334 unvaccinated varicella cases was obtained from randomly selected pediatricians and general practitioners/internists. Each physician contributed five randomly selected varicella cases occurring in 1999. Epidemiological data and data on resource utilization were collected from patient files via telephone interviews. Because pediatricians were over-represented in the sample, results were weighted according to the true relationship of diagnoses by pediatricians (0.6) and general practitioners (0.4) on the basis of the German prescription index for 1999.
Results: Mean age was 7.4 (median 5 years) with 90% of cases younger than 12 years. Highest incidence was in 5–6-year-olds. The treating physician assessed for 16.3% of varicella cases a severe course. Overall incidence of complications was estimated to be 5.7%. Complications accounted for an average of 0.09 hospital days per case. Certificates of sick leave were issued for 1.31 days per case. Of these, 0.63 days were paid by sickness funds for parents caring for their sick children. Using the incidence of 760 000 cases in 1999 according to the German prescription index, we estimated almost one million days of work loss. The overall costs for society were about 150 million Euro, thereof 81% for indirect costs.
Conclusion: The findings of the study indicate that varicella is a serious disease, especially for adults resulting in substantial medical and societal costs in Germany. The most important economic indicator is work loss. Costs could be saved essentially by introducing universal varicella vaccination.

AIDS and HIV infection

O280 Ritonavir boosted protease inhibitor (PI) regimens show superior virological efficacy compared to single PI regimens

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Background: Low dose ritonavir boosting of a further PI can result in beneficial pharmacokinetic interactions that allow BID dosing while maintaining higher drug trough concentrations. These regimens are, therefore, a promising strategy to improve both HAART-associated tolerability and efficacy. We retrospectively analyzed the virological responses of HIV patients receiving either single PI- or boosted PI-based HAART as first PI-containing treatment in the Cologne–Bonn patient cohort.
Methods: Data of 387 patients were available for short-term virological response analysis (6 months). A total of 327 of these patients had been started on single PI regimens: hard-gel saquinavir (HG-SQV): 22%, indinavir (IDV): 32%, ritonavir (RTV): 23%, nelfinavir (NFV): 23%. Sixty patients had received boosted PI: SQV-RTV (1000–1250 mg/100 BID): 12%, IDV/RTV (800/200 mg or (800/100 mg BID): 63%, Lopinavir (LPV)/RTV

(400/100 BID): 25%. Long-term virological responses (12 months) were evaluable in 193 patients treated with single PIs (HG-SQV: 15%, IDV: 35%, RTV: 23%, NFV: 27%) and in 37 patients on boosted-PI-therapy (SQV-RTV: 8%, IDV/RTV: 68%, LPV/RTV: 25%).
Results: Patients receiving single PI-treatment with HG-SQV had less favorable treatment responses when compared to single IDV-, RTV- or NFV-treated patients, both in the short term analysis (25% vs. 75, 67, 70.6%, respectively; $P < 0.05$) and the long-term analysis (25.5% vs. 80.6, 71.1, 69.8%, respectively; $P < 0.05$). To avoid HG-SQV induced effects, patients treated with this PI were excluded from further analysis. Initial viral suppression below the limit of detection (<400 copies/mL) was achieved in 61.7% of patients on single PI therapy and in 83.3% of patients treated with boosted PIs ($P < 0.05$). Durable viral control (<400 copies/mL) over 12 months was found in 67% of patients from the single PI group and 91.8% of patients receiving boosted PI-treatment ($P < 0.05$).
Conclusion: Boosted protease inhibitor therapy with SQV/RTV, IDV/RTV and LPV/RTV seems to induce higher rates of virological suppression both in short- and long-term follow-up, compared to conventional single PI treatment.

O281 HIV quasispecies evolution and profile of cell-derived membrane proteins acquired by virions in the plasma and spinal fluid of AIDS patients

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Objective: Cell-derived membrane proteins (CMP) acquired by HIV provide information of the cellular source of circulating virus. CMP profile of HIV present in the plasma and in the spinal fluid of AIDS patients as well gp120 hypervariable region (V3 loop) quasispecies heterogeneity were established in order to analyze relationships with coreceptor usage and compartmentalization of viral replication in these body sites.
Methods: CMP profile was established by immunocapture of purified virions from plasma and spinal fluid with monoclonal antibodies to cell membrane markers including: CD26, CD45RO, CD36, CD58, N-CAM, VCAM-1, ELAM-1, CD44 and glutamate receptor. Viral quasispecies were analyzed by *V3 loop cloning and sequencing.*
Results: Seven patients were included in the study. Direct comparison between plasma and spinal fluid was possible on five paired specimens. Incorporation of CMP in viral envelope was lower for HIV from spinal fluid as compared to plasma virions. CD44 was the most represented CMP in virions from spinal fluid, while the monocyte marker CD36 was the most prominent CMP on plasma virions. V3 loop sequences in HIV from both body compartments were compatible with a predominant usage of CCR-5 as coreceptor. Virion heterogeneity in V3 loop was generally lower in spinal fluid. Viral quasispecies evolution in two patients indicated a distinct clusterization of clones derived from the two body fluids, while in the remaining patients spinal fluid and plasma V3 loop clones were not separated.
Conclusions: Our data indicate that in AIDS patients HIV present in the spinal fluid and plasma may evolve separately. Accordingly, CMP profile of virions derived from the two body compartments may be quite different, suggesting distinct cellular source of the HIV, although a similar pattern of coreceptor usage is suggested by the V3 loop aminoacid pattern. The comparative analysis of CMP profile and quasispecies heterogeneity may help in understanding the evolution of HIV during the natural history of the infection and may be useful in the study of its cellular reservoirs.

O282 Correlation between human immunodeficiency virus type 1 levels and cytokine profile into the cervicovaginal secretions of a female cohort

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Objectives: To evaluate the role of cytokines on human immunodeficiency virus type 1 (HIV-1) replication and shedding into the cervicovaginal secretions (CVS) and their relationship with clinical parameters of HIV-1 disease.
Methods: Sixty paired cervicovaginal and blood samples were collected from HIV-infected women followed at colposcopic unit of Department of Obstetrics Gynaecology, Pavia. Fifty-one out of 60 were in active antiretroviral treatment (ART). CVS specimens were analyzed for cytokines levels (interleukin-IL-1-β, tumor necrosis factor TNF-α, IL-6) by ELISA (Chemicon, Italy), for presence and load of HIV-RNA and HIV-DNA by RT-PCR, and for detection and type of human papillomavirus (HPV) by PCR-RFLP. Plasma HIV-RNA viral load was determined by RT-PCR.
Results: The prevalence of HIV-RNA viremia was 45%. HIV-DNA, cell-associated and cell-free HIV-RNA were detected in 76.7, 70 and 71.7% of CVS specimens, respectively. The median levels of TNF-α, IL-1-β and IL-6 in CVS were 27.8, 30, and 4.15 pg/mL, respectively. There was a direct correlation between the CVS concentration of IL-1-β, plasma HIV-RNA viral load, HIV-DNA and cell-associated HIV-RNA in CVS. Fifty-one women in active ART had significantly higher concentration of IL-6 and lower concentration of TNF-α in CVS than the nine untreated subjects.
Conclusion: These results suggest that local immune activation is associated with presence and load of HIV-1 in the CVS and local concentration of proinflammatory cytokines may influence the risk of HIV transmission.

O283 Influence of HIV infection in the evolution of Legionnaires' disease

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Objectives: Although Legionnaires' disease (LD) is rarely described in AIDS patients, the incidence of this infection is similar to that in those without HIV infection. Since 1983, 15 cases of AIDS with LD have been seen in HUGTiP. A previous descriptive analysis demonstrated that mortality and morbidity were high in this subset. Clinical features and complications of LD were compared between HIV and non-HIV patients.
Methods: Data related to HIV and LD were retrospectively collected. Fifteen HIV patients (group A) and 64 non-HIV patients (group B) with LD were included in the study. Patiens in group B had not received previous immunosuppressive therapy.
Results: No statistical differences were observed between the two groups in the origin (community, nosocomial), delay in treatment (>3 days), or antibiotic therapy (erythromycin, fluoroquinolones). Fine score >3 was not significant in either group ($P = 0.11$). Most patients in the two groups were males with a mean age of 37.8 (group A) and 57.4 (group B). Among intrinsic risk factors, liver disease ($P = 0.00019$) and smoking ($P = 0.01$) were significantly more frequent in HIV patients. Previous β-lactam treatment was also more frequent ($P = 0.03$) in HIV patients. Respiratory symptomatology was present in 12 (80%) versus 42 (65.6%) of groups A and B, respectively ($P = 0.22$) with dyspnea being of note in group A ($P = 0.01$). Extrarespiratory symptoms were present in seven (46.6%) versus 32 (50%) patients in groups A and B, respectively ($P = 0.95$). Hyponatremia ($P = 0.004$) and an increase in AST ($P = 0.01$) were significantly more frequent in group A. Radiologically, HIV patients showed more bilateral pulmonary infiltration ($P = 0.006$). Complications ($P = 0.04$) and specially respiratory failure ($P = 0.07$) were also more frequent in this group. Lastly, mortality was significantly greater ($P = 0.02$) in HIV patients.
Conclusions: Morbidity and mortality of LD are significantly higher in HIV versus non-HIV patients.

Rickettsial diseases 2002: an update (Symposium organized by EUWOG)

S298 Ehrlichiosis: trends in Europe

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Ehrlichiosis in humans and animals in Europe is caused by intracellular organisms of the families *Ehrlichia* and *Anaplasma*. As the order Rickettsiales currently is reorganized with new species combinations being used, the nomenclature is still unfamiliar. Ehrlichioses are named as granulocytic or monocytic depending on which cell lineage is predominantly infected. Human monocytic ehrlichiosis is caused by *Ehrlichia chaffeensis*. The principal tick vector for *E. chaffeensis* is *Amblyomma americanum*. Based on serology, sporadic cases of monocytic ehrlichiosis have been reported from a small number of European countries. The ehrlichiosis of considerable clinical importance in Europe is granulocytic ehrlichiosis caused by *Ehrlichia phagocytophila* (proposed new name; *Anaplasma phagocytophila*). The vector of *E. phagocytophila* in Europe is the common tick *Ixodes ricinus*. Clinical cases of human granulocytic ehrlichiosis have been described in several European countries, and serological evidence of human infection is being reported from countries all over Europe. Related seroprevalence rates in tick-exposed populations varies between 1 and 25%. The most common clinical manifestations of granulocytic ehrlichiosis are fever, headache, and myalgia. Additional nonspecific symptoms may include rigors, malaise, and arthralgia. However, several studies assert that the infection often is mild and self-resolving.

Resistant staphylococci (Joint ESCMID/ICAAC symposium)

S306 Alternative therapies for staphylococci and their problems

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Staphylococci remain important causes of morbidity and mortality, both in and out of the hospital setting. While modalities to prevent infections caused by these organisms, as well as to ameliorate the consequences of a systemic inflammatory response that may occur, are being pursued, the mainstay of management remains antimicrobial agents. The resistance of staphylococci to β-lactams and to alternatives, some of which have excellent bioavailability, is well known and helped spur studies of other therapies. Two such therapies have been approved for use in recent years in many parts of the world. The first of these, quinupristin/dalfopristin (Q/D), is a combination of streptogramins that together show enhanced, and even irreversible, binding to ribosomes; binding, and the bactericidal effect, is decreased in the presence of the cMLS-B (constitutive) phenotype, encoded by *erm* genes, which results in ribosomal methylation. Q/D is administered intravenously (long line preferred) and produces considerable myalgias, arthralgias and interactions with other drugs. Combinations of Q/D with other antimicrobials generally appear to be beneficial or indifferent, although results can vary in different assays. Linezolid, an oxazolidinone, is used either IV or PO, with excellent bioavailability. It is a generally bacteriostatic, protein synthesis inhibitor, but some killing may be observed in vitro and in animal modes. Reversible anemia and thrombocytopenia are seen, usually after prolonged exposure. Newer fluoroquinolones with enhanced Gm + activity include gatifloxacin, moxifloxacin and gemifloxacin; however, MICs are increased, often into the resistant range, with MRSA. Another new antibiotic with Gm + activity is telithromycin (a ketolide more potent than macrolides and a poor inducer of the iMLS phenotype), which is active against most MSSA but not MRSA (which usually display the cMLS phenotype). Agents in comparative clinical trials include oritavancin (LY333328) and daptomycin (both have completed phase III trials for complicated skin and soft tissue infections), tigecycline (GAR936, a glycylcycline), MBI226 (an antimicrobial peptide in phase III for prevention of bloodstream infections), and ABT733, a ketolide. A rapidly bactericidal antistaphylococcal agent that is bioavailable is still needed.

Viral respiratory infections (Joint ESCMID/ESCV symposium)

S307 Seasonal influence on the epidemiology of viral respiratory infections

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Respiratory infections are major causes of illness observed in all age groups and all year round. These infections are mainly caused by respiratory viruses (influenza A and B; respiratory syncytial virus; parainfluenza viruses I, II and III; adenovirus; enterovirus; rhinovirus; coronavirus). These viruses have different characteristics such as clinical presentations, seasonal patterns and age distribution. By combining diagnostic procedures (antigenic detection plus culture plus PCR plus serodiagnosis), viral etiology can be assessed in 70–80% of patients presenting with acute respiratory tract infections. This high rate of diagnosis allows a clear characterization of the epidemiology of these viruses in the different regions of the world. When comparable, the studies conducted in different countries are in agreement. Common cold is the most frequent upper respiratory tract disease. It is mainly due to rhinoviruses and coronaviruses, and occurs during early fall and spring. Influenza and RSV are responsible for the severe acute lower respiratory tract infections mainly observed during winter. The age distribution differs for these two viruses since RSV is observed in very young children (age below five) and in the elderly while influenza is mainly observed in older children (more than 5-year-old) and young adults. RSV is responsible for severe bronchiolitis leading to admission to the hospitalization, while para I and II, observed also during the winter period, can be responsible for mild bronchiolitis. Para III infections are mainly recorded during summer. Adenovirus has no obvious seasonal pattern, but seems to be responsible for small located epidemics (serotype 4 and 7) that are observed in young adults. This virus is also observed in very young upper respiratory tract infections that resemble influenza presentations. Even if the seasonal pattern and the epidemiology of these respiratory viruses is better understood, it remains a real challenge to assess for each individual case that the virus responsible for the clinical presentation observed is the one supposed to be circulating at that time of the year. Since, efficient techniques for the diagnosis of infections due to respiratory viruses are now available and efficient in numerous laboratories, this diagnosis should be carried out, particularly in the age of development of specific antiviral agents.

S310 A newly discovered human pneumovirus isolated from young children with respiratory tract disease

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From 28 young children in the Netherlands, a paramyxovirus was isolated that was identified as a tentative new member of the Metapneumovirus genus based on virological data, sequence homology and gene constellation. So far,

avian pneumovirus was the sole member of this recently assigned genus, hence the provisional name for the newly discovered virus: human metapneumovirus. The clinical symptoms of the children from whom the virus was isolated were reminiscent of those caused by human respiratory syncytial virus infection, ranging from upper respiratory tract disease to severe bronchiolitis and pneumonia. Serological studies showed that by the age of 5 years, virtually all children in The Netherlands have been exposed to human metapneumovirus and that the virus has been circulating in humans for at least half a century. We have generated sequences for all hMPV open reading frames (ORFs) and intergenic sequences as well as partial sequences of the genomic termini. The overall percentage amino acid sequence identity between APV and hMPV N, P, M, F, M2-1, M2-2 and L ORFs was 56–88%. Some nucleotide sequence identity was also found between the noncoding regions of the APV and hMPV genomes. Although no discernible amino acid sequence identity was found between two of the ORFs of hMPV and ORFs of other paramyxoviruses, the amino acid content, hydrophilicity profiles and location of these ORFs in the viral genome suggest that they represent SH and G proteins. The high percentage sequence identity between APV and hMPV, their similar genomic organization (3′-N-P-M-F-M2-SH-G-L-5′) as well as phylogenetic analyzes provide evidence for the proposed classification of hMPV as the first mammalian metapneumovirus.

Quinolone and macrolide resistance

O315 Topoisomerase mutations associated with in vitro selection of resistance to moxifloxacin in *Streptococcus pneumoniae*

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Moxifloxacin (MOX) is an 8-methoxyquinolone very active against *Streptococcus pneumoniae*. In a previous study [1], we showed, using isogenic transformants with different mutations in the QRDR of ParC or GyrA, that a similar low level of resistance was obtained for MOX, although it did not indicate if a preferential target existed. In this work, we demonstrated the order of acquisition and the mutations selected on MOX in two wild-type pneumococcal strains, R6 and 5714. Selection of first-step mutants was performed by plating 10^9 to 10^{10} cells on MH blood agar plates containing MOX or levofloxacin (LEV) incubated for 72 h, while the second selection step was done on MOX. Selection occurred only at 2 and 4× MIC and frequencies of selection of first- and second-step mutants did not differ significantly whatever was the fluoroquinolone (FQ) used, ranging from 10-7 to 10-9. Analysis of R6- and 5714-derived first-step mutants ($n = 19$) selected on MOX (2× MIC) revealed two main types of mutants. First, 10 derivatives showed only two-fold increased MICs of the FQs tested, but no mutation in the QRDR of GyrA, GyrB, ParC or ParE. Since no decrease in MICs were observed with reserpine, the resistance mechanism has still to be determined. The other eight mutants showed a single GyrA mutation at position 81, associated with two- to four-fold increased MICs of MOX and sparfloxacin and at most a two-fold increase in MICs of LEV, ciprofloxacin, and pefloxacin, consistent with the GyrA phenotype [1]. As expected, when LEV was used first as selecting agent (2× MIC), only mutants with the ParC phenotype [1] were selected showing four-fold increased MOX MICs. All the second-step mutants selected on MOX from first-step mutants (GyrA S81F, selected on MOX) had a second mutation either in ParC at position 79 or 83, or in ParE at position 435. Similarly, all the second-step mutants selected from ParC mutants (S79Y, selected on LEV) had a second mutation in GyrA at position 81 or 85. This work indicates GyrA as the primary target of MOX. Since a single mutation in GyrA or ParC similarly affects its MICs, MOX could belong to the FQs which target preferentially GyrA, although probably acting through both gyrase and topoisomerase IV. Since a two-step mutation process modifying GyrA and ParC or ParE is necessary to MOX resistance, any other FQ able to select first-step ParC mutants will favor the emergence of MOX resistance.

Reference

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O316 Patterns of mutations in target genes in septicaemia isolates of *Escherichia coli* and *Klebsiella pneumoniae* with resistance or reduced susceptibility to quninolones

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Objectives: To describe mutations in QRDR in *E. coli* and *K. pneumoniae*, of subunits of topoisomerase II and IV in septicemia isolates with reduced susceptibility to ciprofloxacin (CIP), and to correlate mutations to the degree of resistance.

Methods: DNA probes were used as primers to amplify and sequence QRDR of *gyrA*, *gyrB*, *parC* and *parE* in blood isolates of *E. coli* and *K. pneumoniae*. The strains were selected on the basis of reduced susceptibility to CIP as determined by the disc test and MIC determinations. The strains were also tested for susceptibility to nalidixic acid (NA).

Results: In *E. coli*, mutations in the *gyrA* gene were seen at codons 83 and 87, leading to amino acid substitutions Ser → Leu (TCG → TTG) and Asp → Asn (GAC → AAC), together with single or double mutations in *parC* leading to Ser80 → Ile (ACG → ATC),Glu84 → Gly (GAA → AAA) or Gly78 → Cys (GGC → TCG) in all CIP resistant strains (MIC > 8 mg/L). Mutations in *parE* were uncommon and no mutations were seen in *gyrB*. Strains with reduced susceptibility (MIC 0.125–1 mg/L) showed a single mutation in *gyrA* (Ser83 → Leu) and also in *parC* (Gly78 → Cys or Ser80 → Ile). No *parC* mutations were found in the absence of *gyrA* mutations. All strains were resistant to NA (MIC > 8 mg/L). In *K. pneumoniae*, only one resistant strain with resistance to CIP (MIC 8 mg/L) was found with a double mutation in *gyrA* in codon 83, leading to Thre → Phe (ACT → TCC) and in codon 87 to Asp → Gly (GAC → GGC), whereas strains with reduced susceptibility (MIC 0.25–2 mg/L) harbored a single mutation in *gyrA*, leading to Thre83 → Ser or Tyr (ACT → TCC or TAC). Resistance to NA (MIC 8 → 256 mg/L) was present in all strains. The same mutation in codon 83 with Thre → Ser was also observed in six CIP susceptible strains (MIC 0.016–0.064 mg/L). In two wild-type strains the MICs were 0.008–0.016 mg/L. These eight strains were susceptible to NA (MIC 2–4 mg/L).

Conclusion: *E. coli* and *Klebsiella* blood isolates with high-level CIP resistance harbor double mutations in *gyrA* and *E. coli* also in *parC*. Strains with single mutation in *gyrA* remained susceptible, but the MICs were increased. All the strains were resistant to NA. CIP-susceptible strains of *K. pneumoniae* with one mutation in *gyrA*, leading to Thre83 → Ser and with susceptibility to NA might be the first step towards reduced susceptibility. Additional mutations in *parC* and efflux mechanisms have to be tested in *K. pneumoniae*. NA resistance was predictive of reduced susceptibility to fluoroquinolones.

O317 Different contribution of efflux to fluoroquinolone (FQ) resistance in clinical isolates and laboratory-created mutants of *Streptococcus pneumoniae* (SPN)

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Objectives: The reserpine-sensitive efflux pump PmrA has recently been shown to confer FQ resistance in SPN. The aim of this study was to compare the contribution of efflux to ciprofloxacin (cipro)-resistant (MIC ≥ 4 μg/mL) clinical isolates (CLIN) and lab-created mutants (LAB) resistant to cipro, gatifloxacin (gati), gemifloxacin (gemi), levofloxacin (levo), and moxifloxacin (moxi).

Methods: A total of 34 cipro-resistant CLIN were collected as part of an ongoing national respiratory organism surveillance program and their MICs were determined by broth microdilution (NCCLS, M7-A4, 1997). A total of 39 single-step LAB were created from three susceptible SPN isolates at 1–16× MIC of cipro, gati, gemi, levo or moxi. The Quinolone Resistance Determining Regions (QRDRs) of GyrA and ParC were sequenced in all the CLIN and LAB. The role of efflux was evaluated via reserpine studies for all isolates.

Results: QRDR changes were observed in 14/34 (41%) and 32/34 (94%) of CLIN for GyrA and ParC, respectively. Ciprofloxacin-positive efflux (a four-fold or greater decrease in MIC in the presence of reserpine) was observed in 12/34 (35%) of the CLIN. Conversely, QRDR changes were observed in 6/39 (15%) of GyrA and 4/39 (10%) of ParC in the LAB and 100% of the ciprofloxacin-resistant mutants were efflux-positive. The 20% of gati, 44% of gemi, 67% of levo, and 22% of moxi-resistant mutants were able to efflux cipro but none were positive for efflux of the FQ with which they were selected.
Conclusions: The clinical isolates predominately showed QRDR changes (94% in ParC and 41% in GyrA), but only 35% were positive for efflux. Alternatively, efflux of cipro contributed to 100% of cipro-resistant laboratory mutants whereas only 15 and 10% had GyrA and ParC changes, respectively. Resistance in clinical isolates was primarily due to QRDR changes whereas resistance in laboratory mutants was predominately a result of efflux.

O318 Macrolide resistance (Macr) by ribosomal mutation detected in clinical isolates of *Streptococcus pneumoniae* isolated from PROTEKT 2000

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Objectives: PROTEKT is a global, longitudinal study of the antimicrobial susceptibility of bacterial pathogens associated with community-acquired lower respiratory tract infections. We have been screening clinical isolates from PROTEKT 2000 for the genetic determinants of Macr (total of 1047 isolates) and have discovered 17 isolates negative for known efflux or rRNA methylase mechanisms. The aim of this study was to determine the molecular basis of Macr in these strains.
Methods: Segments of the L4 and L22 riboprotein genes and the four copies of the 23S rRNA (domains V and II) gene were amplified, sequenced and mutations determined. RT-PCR and primer extension was performed at the region of the 23S mutations to determine the relative proportion of RNA having the mutation. SPN R6 and macrolide resistant strains negative for *erm(A)*, *erm(A)* subclass *erm(TR)*, *erm(B)*, *erm(C)* and *mef(A)* genes were tested.
Results: Three isolates carried an *A2058G* mutation in three alleles of the 23S rRNA gene (erythromycin A [ERY] MIC 64–128 mg/L). Five isolates had *A2059G* mutations in all four 23S rRNA copies (ERY MIC 16–128 mg/L). Three German isolates had *A2059G* mutations in 1, 2, and 3 of the 23S rRNA copies, respectively (for isolates 1, 2, 3: ERY MIC 1, 8, 64 mg/L; Clarythromycin [CLA] 1, 2, 16 mg/L, Azithromycin [AZI] 0.5, 128, 128 mg/L). One Canadian isolates had an *A2059G* mutation in two alleles (ERY MIC 32, CLA 8, AZI 128 mg/L). One isolate from the USA had a C2611G mutation in three 23S rRNA alleles (ERY MIC 128, CLA 32, AZI 8 mg/L). An L22 riboprotein G95D amino-acid substitution combined with *A2059G* mutation in all four copies was found in three Japanese isolates (ERY MIC 64–128 mg/L). L4 riboprotein gene mutations were not found in any isolates. The telithromycin MIC range for the 17 isolates was 0.015–0.25 mg/L.
Conclusions: This study shows that a small number of Macr *S. pneumoniae* have ribosomal alterations as the mechanisms for macrolide resistance. The isolates are global in distribution. The data suggest a dosage effect for resistance; as the number of alleles with the mutation increases so does the MIC. The data also suggest that some macrolides may be more affected than others by these mutations. Of particular interest is the large increase in MIC to azithromycin when the *A2059G* mutation is present in one compared to two copies. Telithromycin MIC values remain low for all 17 isolates suggesting that these mutations do not have a major effect on this new ketolide antimicrobial.

O319 Macrolide resistance (Macr) mechanisms in *Streptococcus pyogenes* (SPY) isolated in the PROTEKT 2000 study

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Background: PROTEKT is a global, longitudinal study of the antimicrobial susceptibility of bacterial pathogens associated with community-acquired lower respiratory tract infections. The objective of this study was to compare Macr mechanisms in SPY isolates from countries participating in PROTEKT 2000.
Methods: The presence of Macr genes (*erm(A)*, *erm(A)* subclass *erm(TR)*, *erm(B)*, *erm(C)* and *mef(A)*) were determined in 143 Macr isolates collected from 19 countries (total sample size 1485 isolates) using a novel rapid-cycle multiplex PCR assay with microwell-format gene specific probe detection [1].
Results: Overall, 46.3% of the isolates tested were *mef(A)*, 31.3% were *erm(B)*, 20.9% were *erm(A)* subclass *erm(TR)* and no isolates were negative for all the genetic markers tested. The *erm(A)* or *erm(C)* gene was not found in any isolate and none showed more than one Macr mechanism. The majority of the countries surveyed showed low levels (<10 isolates) of Macr (these being Argentina, Australia, Brazil, France, Germany, Hong Kong, Poland, Hungary, Sweden, Switzerland, Turkey and USA), and therefore, it was not possible to obtain meaningful data regarding distribution of resistance types from these sites. However, Italy, Japan, South Korea, Canada, Mexico, Portugal and Spain could be analyzed. For Italy, Portugal and South Korea at least 70% were *erm(B)*, but the reverse was true for Japan, Mexico and Spain where at least 70% were *mef(A)*. No *erm(B)* were found in Canadian isolates (58.3% *erm(A)* subclass *erm(TR)*, 41.7% *mef(A)*)
Conclusions: The distribution of Macr mechanisms was found to vary widely between countries and different geographical regions and further analysis is required to try to explain this phenomenon. The low number of Macr isolates ($N = 143$) is a reflection of the overall low level of macrolide resistance in SPY rather than the sample size (143/1485, 9.6%).

Reference

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O320 Activity of the ketolide antibiotic telithromycin is refractory to *erm* monomethylation of bacterial rRNA

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Objectives: Methylation of specific nucleotides in ribosomal RNA is one of the means by which bacteria achieve resistance to macrolide–lincosamide–streptogramin (MLS) antibiotics. The degree of resistance is determined by how effectively the rRNA is methylated. We have implemented a bacterial system in which the rRNA methylations are defined, and investigated what effect *erm* mono- and dimethylation of the rRNA has on the activity of representative MLS, including ketolide, antibiotics.
Methods: The methyltransferase gene, *rrmA*, was inactivated in *Escherichia coli* AS19, a hyperpermeable strain. This strain (AS19-*rrmA*-negative) was then transformed with plasmids containing either the monomethyltransferase *ermN*, or the dimethyltransferase *ermE*, or with a control plasmid without an *erm* gene. MICs were determined for the AS19-rrmA-negative strains by using the agar dilution method according to the NCCLS guidelines. For each strain, approximately 10 000 cells were applied to surface of Luria–Bertani agar plates containing serial two-fold dilutions of clindamycin, clarithromycin, erythromycin, lincomycin, pristinamycin IA, telithromycin (HMR 3647) or HMR 3004. The levels of methylation at *A2058* were measured by reverse transcriptase extension of a primer complementary to nucleotides 2061–2078 of the 23S RNA, in the presence of dTTP and ddCTP. Primer extension products were analyzed by polyacrylamide gel electrophoresis, and the degree of methylation was calculated by scanning the relative intensities of the unmethylated and dimethylated bands with a PhosphorImager.
Results: In the test system, >80% of the rRNA molecules are monomethylated by *ermN* (TlrD) or dimethylated by *ermE*. The *ermE* dimethylation confers high resistance to all MLS and ketolide drugs. The *ermN* monomethylation predictably confers high resistance to the lincosamides clindamycin and lincomycin, intermediate resistance to the macrolides clarithromycin and erythromycin, and low resistance to the streptogramin B pristinamycin IA. In contrast to the macrolides, monomethylation only mildly affects the antimicrobial activities of the ketolides telithromycin and HMR 3004, and these drugs remain 16–250× as potent as clarithromycin and erythromycin.
Conclusions: These differences in the macrolide and ketolide activities could explain the recent reports of variation in the MICs of telithromycin for streptococcal strains that have constitutive-*erm* MLS resistance, and which are highly resistant to erythromycin.

O321 Association of genes responsible for erythromycin and tetracycline resistance in *Streptococcus pyogenes* and their expression

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Objectives: The presence of more than one gene responsible for erythromycin resistance associated or not with tetracycline resistance determinants in the same isolate of *S. pyogenes*, was investigated in a sample of strains in order to (i) determine their genetic linkage (ii) evaluate their ability to be cotransferred and (iii) study the expression of these determinants.
Methods: In a previous PCR screening for erythromycin-resistant *S. pyogenes*, 13 strains with associated genes, *erm(TR)*, *mef(A)*, *erm(B)*, *tet(O)* were found. The genetic linkage of genes was determined by PFGE restricted with SmaI followed by hybridization with *erm(TR)*, *mef(A)*, *erm(B)* and *tet(O)* probes. Conjugation experiments were performed using erythromycin-resistant *S. pyogenes* as donor and *E. faecalis* JH2-2 as recipient. Studies of expression of associated genes were performed by RT-PCR.
Results: All *S. pyogenes* showed an MLSB phenotype. Among 11 tetracycline resistant strains no. 7 carried *erm(TR)*, *erm(B)*, and *tet(O)* genes while no. 4 carried *erm(TR)* and *tet(O)* genes. Only two tetracycline susceptible strains carried both *erm(TR)* and *mef(A)* genes. Southern blot analysis with the probes for each gene revealed that *erm(TR)* and *tet(O)* genes hybridized in the same SmaI fragment (242 kb), while the *erm(B)* probe hybridized in a 97-kb fragment, except for two strains in which *erm(TR)* and *tet(O)* hybridized in a 291-kb SmaI fragment. No association in the erythromycin-resistant tetracycline-susceptible strains was found between *erm(TR)* and *mef(A)* genes. Preliminary conjugation experiments showed that it was possible to transfer the erythromycin and tetracycline resistance to a recipient strain. RT-PCR experiments demonstrated the expression of all genes with the only exception of five *S. pyogenes*, carrying *erm(B)*, *erm(TR)* and *tet(O)* genes, in which *erm(B)* was not expressed.
Conclusion: Our study demonstrated that *erm(TR)*, in spite of its recent origin in *S. pyogenes*, is a quite diffuse gene in this species and it is always functional, even if associated with other erythromycin-resistance determinants. In our sample it is frequently associated and localized in the same fragment of the *tet(O)* gene, instead of *erm(B)* or *mef(A)*. The *erm(B)* gene was not expressed in five strains, demonstrating that the *erm(TR)* gene alone is responsible for the MLSB phenotype. Our results demonstrated that a single phenotype of resistance can be determined by different genes with different levels of expression and mobility.

O322 Efflux-mediated quinolone resistance in *Bacteroides fragilis* group strains

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Objectives: In the present study, the possible efflux of quinolones were investigated in 35 *Bacteroides fragilis* group isolates with moderate to high levels of resistance to quinolones (ciprofloxacin, levofloxacin, moxifloxacin and clinafloxacin) and compared to five ATCC strains by determining: (i) the impact of a known efflux inhibitor, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), on the intracellular ciprofloxacin accumulation, and (ii) the CCCP and/or reserpine-induced growth inhibition in the presence of moxifloxacin.
Methods: The accumulation of ciprofloxacin by 35 *B. fragilis* group strains, isolated from fecal samples or clinical specimens, was measured by a silicon oil based fluorescence method. These strains had previously been characterized according to mutations in their GyrA QRDR and antimicrobial susceptibilities. Strains were incubated in 10 μg/mL ciprofloxacin with and without the addition of CCCP. The cells were separated from the extracellular solution by centrifugation through a silicon oil barrier. The entire pellet was placed in glycine–HCl buffer to obtain cell-lysis. After centrifugation, the amount of ciprofloxacin in the supernatant was determined by spectrofluorometry. The possible presence of efflux was further examined using growth inhibition assay by studying the effect of reserpine and CCCP on the growth of the same strains in various concentrations of moxifloxacin. Samples were removed after 24 and 48 h in order to determine the amount of viable cells per ml by 10-fold dilution to 10–7, inoculation on blood agar plates, incubation and colony counting.
Results: The CCCP-mediated ciprofloxacin accumulation was expressed as ratio between the accumulation with and without CCCP. The ratio varied between 0.9 and 4.9. Eleven of the 35 isolates had a ratio of >2 indicating an increased efflux activity. Accordingly, all of these were highly quinolone resistant with MICs of 8–256 μg/mL. In most of these strains efflux inhibitors mediated an increased susceptibility to moxifloxacin.
Conclusion: In 11 of the 35 studied isolates, the presence of CCCP produced marked increases in the amount of intracellular ciprofloxacin. In the majority of these strains, inhibition of the efflux-pump led to growth suppression when cultured in the presence of moxifloxacin. These results indicate overexpression of efflux-pumps in quinolone resistant *B. fragilis* group isolates.

Time to re-evaluate antimicrobial agents in an era of increasing resistance (Symposium arranged by Roche)

S329 Is antimicrobial resistance also subject to globalization?

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In recent years, one of the more alarming aspects of clinical microbiology has been the dramatic increase in the incidence of antibacterial resistance among pathogens causing nosocomial as well as community-acquired infections. Resistance to antimicrobial drugs is known to vary profoundly depending on geographic location, probably depending also on a different use of antibiotics. With regard to the major respiratory tract pathogens and in particular to *S. pneumoniae*, the most common cause of CAP, macrolide resistance (erm/mef) prevails in β-lactam-refractory strains but may be high (>30–50%) independently and in Italy it reaches 40%, whereas fluoroquinolone resistance is rare but increasing. While the problem has attained pandemic proportions, the prevalence of penicillin resistance and multiple resistance is highly variable depending on site, e.g. the Netherlands percentage: ?; India: <5%; Italy: 5–15%; South Africa: 30–40%; Spain, France: 50%, Japan, Korea: 70–80%. In terms of clinical consequences, penicillin resistance (MIC 4 mg/L) may not represent a threat in nonmeningeal infections if only crude mortality rates are considered. On the contrary, both low-level macrolide resistance and fluoroquinolone resistance may lead to clinical failures in CAP. With regard to *Haemophilus influenzae*, a major cause of CAP in COPD patients, the prevailing mechanism of resistance is represented by β-lactamase synthesis for which considerable variations (from 10 to 40%) have been reported: in Italy, resistance to amoxicillin has progressed sharply (contrary to the situation prevailing in *S. pneumoniae*) from 5% in 1997 to 16% in 1999. The important differences in terms of prevalence of resistance observed when comparing Italy to other countries, and especially those belonging to the European Union, may be tentatively attributed to different habits in the usage of drugs, both in terms of antibiotic classes and of route of administration. In Italy, the preference of physicians and patients alike goes towards the adoption of parenteral antibiotics in the treatment of severe infections, with third generation cephalosporins displaying a long serum half life being the most frequent choice. The distinctive microbiological and pharmacokinetic advantages of these drugs allowing for a rapid bactericidal activity and sustained MBCs in tissues, the easier compliance obtained over most compounds administered by the oral route may all have contributed to abate the rates of resistance in *S. pneumoniae* and other RTI pathogens.

S331 Bacterial resistance: the clinical challenge

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Antibiotic resistance leads to treatment failure, prolonged morbidity and occasional mortality. The costs of antibiotic resistance are poorly defined but include prolonged hospitalization, increased laboratory investigations, alternative management approaches and a greater economic burden. The relationship between antibiotic use and resistance is complex. In many developed countries prescribing is professionally controlled but varies markedly in

indications, choice, dose and duration of therapy. Thus it is not surprising that prescribing strategies to date have had little impact on the increasing rates of resistance among hospital and community pathogens. Professional concern has stimulated action from governments, international agencies and, in Europe, the EU. Strategies for controlling resistance have emphasized the importance of antibiotic resistance surveillance, monitoring antibiotic use, education of professionals and the public, prudent prescribing, improved hygiene, and the development of new anti-infective drugs and vaccines. Prudent prescribing is essential and yet the majority of drug administration, especially within the community, remains empirical. The clinician can seek prescribing guidance from several sources, including government and professional societies, and, increasingly, management guidelines, which may be based on consensus after a critical analysis of the literature. Guidelines are now available in abundance but are often not accessible in an easily digestible form at the point of prescribing. CD-ROMs and web-based sources provide additional prescribing support. Prescribing information must take account of changing patterns of antibiotic resistance. Surveillance systems are now available in abundance at local, national and international levels, but the data are often selective or lack robustness and are rarely presented in a timely manner to aid the prescriber. Drug licensing authorities, e.g. the EMEA, are placing greater emphasis on the need to include regional susceptibility data in the SPCs of anti-infective drugs. However, such data are rarely helpful for individual prescribing events, especially when this remains empirical and without a microbiological diagnosis. In summary, the prescribing clinician is in a dilemma. He feels responsible for the problem of resistance and yet is rarely presented with robust tools to bring about its control. It is therefore essential that strategies to control resistance be integrated and tested scientifically to ensure that prescribing is better supported. The goal must be cost-effective prescribing whilst minimizing the risks of adding to the burden of antibiotic resistance.

Secretory immunity – a major adaptive defense system

K341 Mucosal immunity – a major adaptive defense system

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The mucosal surface area of an adult individual amounts to some 400 m^2. This extensive and generally quite vulnerable epithelial barrier is protected by numerous innate defense mechanisms that cooperate intimately with a local adaptive immune system. Its most prominent feature is an abundance of supepithelial plasma cells and B-cell blasts (collectively called immunocytes), constituting at least 80% of all antibody-forming cells of the body. These local immunocytes produce mainly dimeric IgA (dIgA) with incorporated J chain. Facing the large host of environmental challenges, specific mucosal immunity has developed two strategies: (i) immune exclusion performed by secretory antibodies (SIgA and SIgM), mainly to inhibit colonization of pathogenic microorganisms and penetration of harmful antigens; and (ii) immunosuppression to avoid local and peripheral hypersensitivity towards innocuous substances bombarding the mucosal surfaces. In the gut, the latter phenomenon is called oral tolerance; it apparently involves several down-regulatory mechanisms and explains why most individuals show no adverse immune reactions against persistent contact with food proteins and the normal microbial flora. Immune exclusion depends on an ingenious cooperation between the enormous mucosal B-cell system and a transmembrane epithelial glycoprotein called secretory component (SC), or the polymeric Ig receptor (pIgR). This is quantitatively the most important receptor of the immune system because it is responsible for external transport of large amounts of locally produced dIgA and pentameric IgM. The pIgR shows high and selective affinity for these two ligands because of their incorporated J chain. The epithelial receptor belongs to the Ig supergene family and can be up-regulated by various cytokines, particularly interferon-γ and interleukin-4 (IL-4) derived from activated T cells as well as IL-1 and TNF-α from macrophages. In this way, the secretory immune system can be modulated according to the intensity of local immune responses. During a variable period after birth, both immune exclusion and oral tolerance are poorly developed; appropriate generation of the underlying network of regulatory mechanisms depends both on the establishment of a normal microbial flora and on the introduction of dietary antigens (timing and dose). Breast-feeding is immunologically important during this vulnerable period, not only as a natural 'substitution therapy' for the lacking secretory antibodies but apparently also for its immune-modulating properties. The infant's secretory immune responses are initially dominated by SIgM, but after 1–2 months SIgA usually predominates, although there are large individual variations in the development of mucosal immunity. Mucosal defense provided by dIgA/SIgA is more favorable than that provided by pentameric IgM/SIgM because (a) dIgA more easily gains access to the basolaterally expressed epithelial pIgR and is therefore efficiently transported to the lumen; (b) SIgA is a particularly stable antibody class and is therefore well suited to perform immune exclusion; (c) dIgA generally functions in a noninflammatory manner and may be involved in epithelial clearance of soluble antigens from the mucosa as well as intraepithelial virus neutralization with no cytolytic side-effect; (d) dIgA avidly binds to Fc-α R on phagocytes and may normally dampen their proinflammatory potential but enhance phagocytosis; and (e) dIgA and especially SIgA (and SC on a solid phase) can efficiently activate eosinophils and thus enhance their proinflammatory potential in situations where immune exclusion fails, such as in parasitic infestations. The recent construction of pIgR/SC knockout mice has substantiated the importance of secretory immunity for mucosal homeostasis and defense against toxin-producing pathogens.

Role and mechanisms of toxins in pathogenesis

K342 Bacterial toxins: from infectious diseases to *cellular microbiology*

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Toxins have been the first virulence factors of pathogenic bacteria discovered. Diphtheria toxin (DT), shown in 1888 by Roux and Yersin at the Pasteur Institute to be the only protein of *Corynebacterium diphtheriae* required for the death caused by an infection with this bacteria, was the microbial toxin which opened a prodigious way of investigations. Currently, studies on bacterial toxins, together with those on virulence factors translocated directly from the microbes to the host cells, has given rise to the emergence of a new discipline which has been named 'Cellular Microbiology'. Toxins, like DT, may represent the critical and unique virulence factor of a microbe which renders a bacteria highly harmful for the infected patient. Like DT, tetanus, botulinum or cholera toxins are the virulence factors of *Clostridium tetani*, *Clostridium botulinum* or *Vibrio cholera*, respectively, which determine entirely the pathogenic power of these bacteria. Other toxins do work only in cooperation with additional virulence factors made by the infecting bacteria. For instance toxins from *Bacillus anthracis* are only harmful if the producing bacteria is able to make a capsule. Also the vacuolating cytotoxin VacA from *Helicobacter pylori* appears to be an additional virulence factor which becomes fully efficient for pathogenicity when acting in cooperation with proteins encoded by the pathogenicity island termed 'Cag'. Finally many pathogenic bacteria may also produce toxins but their precise roles in the virulence of the producing microbes are not evident or still unknown. For instance uropathogenic *E. coli* strains produce a toxin named cytotoxic necrotizing factor-1 (CNF-1) whose roles in the urinary infections are still under debate. On the molecular and cell biology side, bacterial toxins are sophisticated tools for the biologist which allow the precise dissection of cellular signaling cascades or complicated processes such as endocytosis and intracellular trafficking. Finally, it is worth to note that recent scientific breakthroughs have been made by studies and utilizations of bacterial toxins.

Infection in cancer patients (Joint symposium with EORTC/IATG)

S344 Etiology of bacterial infections in cancer patients in Europe: an ever changing scenario

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Bacterial infections are still the most common cause of morbidity and mortality in patients with cancer. The past two decades have witnessed dramatic changes related to the characteristics of these infections which include changes in the spectrum and in antimicrobial susceptibility of isolates. Microbiological data from the studies performed by International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC) provided comprehensive evidence on the patterns and trends of bacterial infections. Starting from mid-1980s there was a shift in infecting organisms with increasing predominance of Gram-positive bacteria. Coagulase-negative staphylococci emerged as the leading cause of infection being responsible at least one third of all bacteremias and streptococci mainly viridans type closely followed. Penicillin, third generation cephalosporin and macrolide resistance has become prevalent among streptococcal isolates. In 1994, IATCG/EORTC Trail XI revealed that 69% of single-pathogen bacteremias were due to a Gram-positive agent. However, recent evidence indicates that the trend is reversing again. A comparison of data from two IATCG trials in 1993 and 2000 found that there was a significant increase in overall incidence of Gram-negative bacteremia and also single agent Gram-negative bacteremia (6.5% vs. 12%, $P < 0.001$). Incidence of bacteremias due to *Klebsiella* spp. and *Enterobacter* spp. also increased. Fluoroquinolone prophylaxis was used less frequently during Trial XIV (33% vs. 52%, $P < 0.0001$). This has led fewer isolation of *Escherichia coli* resistant to quinolones (20% vs. 38%). Increasing overall infection rate for Gram-negatives has also been reported from different centers in Europe and elsewhere. An alarming increase in the incidence of type1 beta-lactamase producing *Enterobacter* spp. and unusual but more resistant pathogens such as *Acinetobacter* spp. and *Stenotrophomonas maltophilia* may be challenging. It is imperative more than ever for physicians to be aware of changing trends in infecting microorganisms and their antimicrobial susceptibility patterns in cancer patients.

Prion disease: frontiers 2001

S348 Epidemiology of BSE in Italy and Europe

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Objectives: Bovine Spongiform Encephalopathy (BSE) is a fatal disease of cattle. Strong evidence indicates that BSE agent is responsible of the variant of Creutzfeldt–Jakob disease in human beings. Passive surveillance was the only way to detect BSE until rapid test became available. The public health concern along with pitfalls of the passive surveillance of BSE led to the implementation of a EU-wide active surveillance based on rapid tests. This paper describes the epidemiological situation regarding BSE after the set up of the active surveillance.
Methods: Since January 2001, all cattle for human consumption and a random sample of dead-on-farm cattle have been tested for BSE. From July 2001 the test has been extended to all dead-on-farm and emergency slaughtered cattle over 24 months.
Results and conclusions: The introduction of such a system allowed the identification of the disease in indigenous cows in Italy and in another countries as Germany, Spain, Austria, Finland. As of November, there have been about 1500 cases throughout Europe. In Italy, as of 4 December 2001 confirmed cases totaled 42 from 409 060 tests performed up to this date. Three of the animals had been imported from another country (2 from Germany, 1 from Switzerland). On the basis of the estimated size of the bovine population older than 24 months the raw incidence excluded the nonautochthonous cases is 12.5 cases per million animals. The geographic distribution of outbreaks partly reflects distribution of the national bovine population. Collectively, the northern Italian regions where most cases of BSE were identified (Aosta Valley, Piedmont, Lombardy, Veneto, Friuli Venezia Giulia, the province of Bolzano, Emilia-Romagna) represent 65.7% of the entire Italian bovine population. In the five regions where the observed cases are concentrated (Lombardy, Emilia-Romagna, Piedmont, Friuli–Venezia–Giulia with 36 of 42 cases), the incidence varies from 31.2 to 7.5 cases per million animals. The distribution of cases by breed shows analogies with what has been observed in other countries with a predominance of high milk production races. The incidence is: Frisian (24 observed cases; 16.6 per million animals over 24 months), Brown Swiss (11; 27.5 per million), Italian Simmenthal (4; 43.0 per million) and Herens (1; 62.9 per million). The lower incidence in Frisian breed is not easily explained. Collectively, 36 of the 41 identified cases were born after the meat and bone meal has been banned in feeding stuffs for ruminants. Preliminary results of the data analysis indicate that in all the outbreaks, during the period of presumed exposure, commercial animal feed or raw material had been purchased.

Advances in invasive fungal infection

S352 Trends in cryptococcosis in Europe

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Cryptococcosis (CR) is a life-threatening fungal infection, usually presenting as meningo-encephalitis. CR may occur in apparently healthy hosts but it mainly affects patients with significant underlying predisposing factors, particularly advanced HIV disease. Data on its incidence are still limited as CR is a nonreportable disease and few prospective epidemiological studies have been carried out. The CR trend has been monitored in France since 1985 by the National Reference Centre which provided evidence of the unchanged occurrence of cases in HIV-negative patients over the years, the progressive increase in number of cases associated with the AIDS epidemic and the impact of the highly active antiretroviral therapy (HAART) in the 1996–1998 when it reduced the annual incidence by >50%. The levelling trend observed after 1998, reported also in other studies, was thought to be related to the diminished effect of HAART because of side-effects, poor patient compliance with therapy and development of resistant HIV strains. To investigate the epidemiology of CR in Europe, a prospective survey was started by the European Confederation of Medical Mycology (ECMM) in July 1997. A standard questionnaire was used for each case to report basic information concerning the patient, underlying disease, clinical presentation and diagnostic and treatment approach. As of December 1999, 655 cases were notified and 565 evaluated. Most of the cases were reported by 8 of the 17 participating countries. CR was associated with HIV infection in 435 patients (77%, range 59–94% according to country) and was AIDS-defining in 57.5% (range 24–80%). CR was clearly under-reported, also where an active reporting network was present. The annual incidence could be estimated in Lombardia (Italy) where it was 0.85/100 AIDS population. The number of cases diagnosed on the basis of CSF-positive cultures (410/531, 77%) suggested late diagnoses. Treatment was started with amphotericin B in

50% of cases, combined with flucytosine in half of them, and fluconazole in 22% of cases. Various combination therapies were used for the remaining patients. The determination of the sero-genotype of the isolate provided new insights into the European distribution of the infecting strains. Continued monitoring of the CR trend is essential to evaluate the effectiveness of HAART, to provide more data on the unusual manifestations of the "immune recovery syndrome" associated with the use of HAART, and to verify the possibility of safe discontinuation of suppressive therapy after sterilization of cultures and rise of CD4 cell count have been achieved. The aim of ECMM is also to increase the number of reporting centers extending the network throughout Europe, to develop diagnostic skills, to raise awareness of cryptococcal infection also in non HIV-infected at risk patients, in whom CR is often diagnosed too late or overlooked, and finally to start drawing the European distribution map of *Cryptococcus neoformans* sero-genotypes.

Pneumococcus – ever increasing resistance (Joint symposium with ISC/FESCI)

S359 Macrolide resistance: from pumps to ribosomes

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Resistance to macrolides and lincosamides is increasingly reported in clinical isolates of Gram-positive bacteria, including pneumococci. Three ways have been used by bacteria to resist macrolides—target site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, efflux of the antibiotic and drug inactivation. In pneumococci, drug inactivation has still not been reported and the impact of the two other mechanisms in terms of incidence is unequal according to the countries. Modification of the ribosomal target confers cross resistance to macrolides and related antibiotics which have overlapping binding sites, the lincosamides, whereas efflux affects only some of these molecules and confers a lower level of resistance. Target modification is mostly related to the synthesis of ribosomal methylases encoded by erm(B) or erm(TR) (subset of erm(A) class) genes. The erm(B) genes are usually borne by conjugative transposons and the spread of macrolide resistance in pneumococci might result both from dissemination of clonal strains, horizontal transfer of the conjugative element and possibly from DNA exchange between strains by transformation followed by recombination. Efflux is due to the synthesis of pumps belonging to the MFS family and encoded by large transposable elements. Recently, laboratory strains and clinical isolates of pneumococci harboring ribosomal mutations have been reported. In survey studies, these strains represent generally <2% of erythromycin-resistant pneumococci. Various mutations of ribosomal structures, including 23S rRNA, L44 and L22 proteins have been characterized. Every mutation is characterized by a specific phenotype. Huge geographic differences, from 3 to 74%, are observed in the macrolide resistance frequencies according to countries. In general, higher is the rate of penicillin resistance, higher is the rate of macrolide resistance. Epidemiologic surveys have shown that the major mechanism of resistance of pneumococci to macrolides in Europe is target modification due to the presence of erm(B) genes, while in other countries, including USA, the efflux mechanism appears prevalent. The reasons for these epidemiological differences are unknown.

Update on parasitic infections

S367 Alveolar and cystic Echinococcosis: new trends

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Infection with *E. multilocularis* encounters either constitutional resistance, late resistance or full susceptibility to alveolar echinococcosis (AE). Dissection of humoral, cell- and cytokine-mediated immune responses in susceptible and resistant humans showed differences partially dependent on host MHC class II polymorphism. A murine AE model (C57BL/6) was used to document susceptibility to disease upon a marked nitric oxide-mediated immunosuppression phenomenon, including high inflammatory cytokine transcripts for IL-1, TNF-α. Concomitantly, genetic iNOS-deficiency resulted in a relative resistance to AE. Furthermore, the encapsulating parasitic laminated layer—a carbohydrate-rich envelope of the parasite—was identified to function as the key factor within the parasite survival strategy. This occurred by modulating the host immune response upon the T-cell independent nature the major carbohydrate antigen Em2(G11), a mucin-type highly glycosylated protein. Similar observations about the key role of carbohydrate molecules have been achieved with *E. granulosus* (cystic echinococcosis, CE). Thus, the major *N*-glycans from the *E. granulosus* LL were found to be noncharged structures having complex-type antennae and core fucosylation. Clinically, new tools are presently investigated to assess the viability status of treated and nontreated parasites in human AE and CE patients. Thus, the viability in FNABs can be tested upon 14-3-3-gene expression on the protein level or by RT-PCR. Thus, nonviable parasites are increasingly identified not only in successfully treated AE patients, but also in persons exposed to infection with a subsequent abortive asymptomatic course of AE. Spontaneous dying-out of hydatid cysts has also been described in CE patients and associated to immunological, predominantly Th1-linked phenomena, whereas Th2 cell activation appear to associate with susceptibility to disease. Taken together, there is an accumulation of facts that contribute to our understanding of how the parasite can develop in certain patients suffering from AE and how, in other infected individuals, the parasite dies out without causing disease.

Pertussis: the hidden epidemic

S371 The hidden high endemicity of infections with *Bordetella pertussis* in highly vaccinated populations

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Symptomatic infections with *B. pertussis* in elder children, adolescents and adults often may be subclinical and in cases in which a physician is consulted often pertussis is not considered in the differential diagnosis. Moreover, in countries in which pertussis-serology is not available or, when available, reliable criteria for positivity (for recent or actual infection) of values in a single serum are absent, laboratory-confirmation of pertussis in individuals >15 years is rarely achieved because most cases consult a physician at the (late) stage of disease in which culture for *B. pertussis* and pertussis-PCR have little or no solving power and in which the dynamic phase of the immunorespons already has taken place. In the Netherlands we used the seroprevalence of IgG antibodies to pertussis toxin (IgG-PT) in the population ($N = 7800$) to calculate the incidence of infection with *B. pertussis* in that population in the year before blood sampling (1995–1996). This could be done because we have established the rate of decline of IgG-PT after symptomatic natural infection in multiple follow-up sera (during 2–10 years) of 57 patients with pertussis which resulted in a formula for the functional relationship between time since infection and IgG-PT concentration. Applying this formula to the seroprevalence data of the population of 3–79 years (individuals <3 years were excluded because of possible interference of maternal IgG-PT or IgG-PT induced by vaccination) we estimated an incidence of infection with *B. pertussis* in the year before blood-sampling of 3900/100 000 (3.9%). The age-specific incidence per year was lowest for 3–4-year-olds (1.2%) and increased with age to 3.3% (5–9 years), 3.4% (10–14), 4.0% (15–19) and, maximally, 6.0% (20–24). Among individuals 25–60 years of age the incidence varied

between 3.5 and 5% per year. Among individuals of 60 years and older the incidence varied between 2 and 3% per year. In contrast, in the Netherlands the incidence of pertussis according to notifications in the study period 1994–1996 was highest among 3–9 years olds (75/100 000/year, i.e. 0.075%) and lowest among individuals >15 years (< 4/100 000/year, i.e. <0.004%)! The occurrence of *B. pertussis* infections in elder children, adolescents and adults is severely underestimated. These data are of importance not only for daily practice but also for determining optimal strategies for booster vaccination at later ages to diminish morbidity and, perhaps more importantly, to diminish transmission of *B. pertussis* to unimmunized infants.

Nosocomial infections of MRSA

O373 Improvement of perioperative antibiotic prophylaxis in prolonged cardiac surgery by automated alerts in the operating room

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Objective: The efficacy of perioperative antibiotic prophylaxis diminishes during the long procedures. This observation has prompted recommendations to re-dose the antibiotic during procedures of prolonged duration. Despite these recommendations, intraoperative re-dosing is often omitted. We aimed at assessing the impact of an automated intraoperative alert to re-dose the prophylactic antibiotics in prolonged cardiac operations.
Methods: We conducted a randomized, controlled, evaluator-blinded trial in a university-affiliated hospital. We included patients undergoing cardiac surgery that lasted more than 4 h after the preoperative administration of cefazolin, unless they were receiving therapeutic antibiotics at the time of surgery. Patients were randomized to an audible and visual reminder on the operating-room computer console at 225 min after the administration of preoperative antibiotic (reminder group, $n = 137$), or control ($n = 136$). After another 30 min, the circulating nurse was required to indicate whether a follow-up dose of antibiotic had been administered.
Results: Intraoperative re-dosing was significantly more frequent in the reminder group (93/137, 68%) than in the control group (55/136, 40%) (adjusted OR: 3.31; 95% CI 1.97–5.56; $P < 0.001$). The impact of the reminder was even larger when compared with the 6 months preceding the study period (129 re-doses/480, 27%, $P < 0.001$), suggesting some spill-lover effect on the control group. Re-dosing was formally declined in 18 of the 44 patients in the reminder group without re-dosing. The rate of surgical site infection in the reminder group (5/137, 4%) was similar to the one observed in the control group (8/136, 6%, $P = 0.4$), but significantly lower than in the pre-study period (48/480, 10%; $P = 0.02$).
Conclusion: The use of an automated reminder system in the operating room improved compliance with guidelines on perioperative antibiotic prophylaxis.

O374 Modeling therapeutic decision-making for antimicrobial therapy: an application to Gram-positive infections using vancomycin

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Objectives: To examine variations in therapeutic antimicrobial decision-making in hospitalized patients (pts) with well-defined infectious syndromes.
Methods: The study population consisted of adult inpatients admitted to either of two tertiary care hospitals between January 1999 and December 2000, who (1) had one of the following diagnoses: (a) nosocomial primary or catheter-related blood-stream infection owing to Gram-positive organisms (noso-BSI); (b) cellulitis/soft-tissue infection (skin inf); (c) osteomyelitis (osteo); and (2) received at least one dose of intravenous (i.v.) vancomycin (vanco). The primary outcome measure in the analysis presented here was the length of stay (LOS).
Results: The cohort from hospital A consisted of 260 pts and from hospital B 411 pts (671 total). A total of 333 pts had noso-BSI, 229 had skin inf and 107 pts had osteo. The most common organisms associated with noso-BSI were: *Staphylococcus* coagulase-negative (51%), *S. aureus* (25%), and *Enterococcus* (22%). In hospitals A and B, vancomycin was part of the initial antibiotic regimen in 40 and 43% of subjects with noso-BSI, 48 and 66% of subjects with cellulitis, and 75 and 65% of subjects with osteomyelitis, respectively. Overall, only 10% of subjects received vanco as their single i.v. antibiotic; pts received an average of four different antibiotics. The median duration of i.v. antibiotics was 13 days and i.v. vancomycin 6 days. The percent of pts with noso-BSI, skin-inf, and osteo receiving at least 5 days of vanco was 53, 21, 25%, respectively; the corresponding figures for receipt of at least 10 days of vanco were 34, 10, and 11%. The percent of pts switched from an i.v. to a completely oral antimicrobial regimen prior to discharge was low (8%). Pts with noso-BSI had the highest mean LOS (noso-BSI: 36 days; skin inf: 14 days; osteo: 14 days). For pts with skin inf, subjects started on vanco initially had shorter length of stay than subjects not started on vanco initially (hospital A: 10 vs. 22 days; hospital B: 10 vs. 21 days).
Conclusion: Vanco prescribing practices in pts with Gram-positive noso-BSI, skin inf, and osteo were comparable across these two hospitals. Lengths of stay were prolonged, particularly in pts with noso-BSI, and may have been affected by the initial antibiotic selection. Oral step-down regimens were inconsistently used. An analysis of the clinical factors that drive frequent modifications of antimicrobial therapy is in progress.

O375 Cost-effectiveness of an antiseptic-impregnated central venous catheter in intensive care patients

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Objectives: A case–control study to estimate the economic outcome associated with the use of an antiseptic-impregnated Arrow G+uard Blue™ versus standard catheter in the ICU unit.
Methods: Data of intensive care patients ($n = 138$) with central venous catheter were prospectively recorded over a 15-month period. Altogether 30 (21.7%) patients developed catheter-related bacteremia, of which 20 (14.5%) met the criteria for inclusion in the study. Pair-matched controls without catheter-related infection were assigned and the marginal costs were calculated. The cost of medication, blood products, examinations and medical equipment was calculated on the basis of consumption, whereas all remaining costs were calculated from data provided by the hospital administration.
Results: Marginal costs averaged € 85.52/day per case. Based on the direct comparison of the mean values, prolongation of the length of stay in the hospital was estimated at 10.8 days or, on the basis of transitional probabilities, at 2.8 days. The higher purchase cost of the antiseptic impregnated catheter of € 21/catheter, as against € 18/catheter for a normal, uncoated double-lumen central venous catheter (although other, more expensive models, can cost up to € 59.3), is offset by the additional cost ensuing through catheter-associated infection of € 82.5/day × 2.8 days = € 231. With an infection rate of 0.28–7.5% for conventional catheters and 0–6.25% for antiseptic-impregnated catheters, on average, the difference per 1000 catheters is € 2434.8 in favor of the antiseptic-impregnated catheter (conventional catheters: € 9098.1 antiseptic-impregnated catheters: € 6663.3).
Conclusion: The use of antiseptic-impregnated catheters appears to be cost-effective for intensive care patients in a university-hospital setting.

O376 Success rate of conservative treatment of totally implantable device-related bacteremia

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Introduction: Totally implantable venous-access device (TIVAD) represents a convenient approach for patients requiring long-term intravenous therapy but infection is an important complication. Its optimal treatment remains poorly defined.

Objectives: To review the outcome of TIVAD-related bacteremia in patients treated without the removal of the catheter and to determine the predictive factors of favorable outcome.
Methods: All patients with TIVAD-related bacteremia occurring from 1996 to 2000 in our 858-bed academic hospital were retrospectively reviewed.
Results: A total of 67 patients developed 92 episodes of infection. Isolated pathogens were Gram-positive cocci in 78.4% (coagulase-negative Staphylococci (CNS) 55.9%, *Staphylococcus aureus* (14.4%), Gram-negative bacilli (9.0%) and fungi (6.3%)). Early decision for catheter removal was taken in 32 episodes (35%), associated with the following etiologic agents or conditions: fungi (5), *S. aureus* (9), *Pseudomonas* (1), sepsis (14), local signs (10), or failure of a previous conservative treatment (8). Among the 57 evaluable episodes (62%) treated with antibiotics given for 10–14 days trough the infected TIVAD left in place (conservative treatment), we observed 32 successes (56%), 16 failures (28%) and 9 relapses (16%). Only one of these 57 patients (1.8%) died from TIVAD infection. The following conditions were associated with an increased risk of failure: local signs of infection (RR: 1.94, CI: 1.10–3.42; $P=0.029$), sepsis (RR: 2.27, CI: 1.09–4.74, $P=0.097$) and organism other than CNS (RR: 2.70; CI: 1.11–6.59; $P=0.021$). The rate of success for conservative treatment of CNS infections in the absence of local signs of infection or septic signs achieved 74% (25/34 episodes). The following conditions did not influence the risk of failure of conservative treatment: delay between insertion and infection, site of insertion, indication (parenteral nutrition vs. chemotherapy), neutropenia, polymicrobial versus monomicrobial infection.
Conclusion: In our study, treatment was conservative in 62% of TIVAD-related bacteremia. The cure rate in these episodes was 56%, but increased up to 74% for the subset of patients with CNS infections without local signs or sepsis. Because of limited vascular access in these patients, we favor the conservative treatment in the latter indication.

O377 Establishment of the HARMONY IUMS MRSA Global Database: a progress report

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On behalf of the HARMONY Typing Project participants and the IUMS Staphylococal Sub Committee

Objectives: In a DGXII-funded HARMONY project to establish an EU MRSA database.
Methods: Exchange scientists have performed mecRIA (*S. Salmenlinna*/W Witte) and binary typing (*W. Vanleeuwen*/A van Belkum) and ribotyping (A Lynch/V Fussing). The Bath (Wellcome Centre) has performed MLST (M Enright). This has informed the underlying genetic background of the various MRSA EU clones. Internet data transfer protocols (with the ENEMTI project) are nearing completion. FAFLP is also completed. Most importantly, the relevant aspects of PFGE (the universal typing method currently adopted by typing laboratories) that need to be standardized were identified. The group had analyzed MRSA in a blinded fashion with their own PFGE protocols and with an agreed protocol that standardized the run parameters. The group had been reluctant to standardize other parameters as they had each developed these over many years and it was felt to so do might hinder rather than help standardization.
Results: HARMONY, established as the International Sub-Committee of Staphylococci's global database. PFGE results: There were clonal trends, but there were many differences between the centers. The group met and felt that, in view of the results, many parameters (e.g. volume of agarose, position and type of standards) should now be standardized. The previous standard protocol took too long. S. Murchan visited A Deplano/M Struelens and others at the Belgian Centre to agree the finer technical details and a new pulsing protocol that would produce optimal separation of the bands in <24 h. This new protocol resulted in almost perfect concordance between the two centers and five others have now submitted near-perfect results. To our knowledge, this is the first time this has been achieved. We put our success down to meticulous attention to detail and joint ways of working (ésprit de corps). There will be a brief overview of the nomenclature for MRSA (there are currently 36 strain types in the database)and the clonality of spread within the EU (there are four internationally spreading clones which have even spread during the project over the last 2 years).
Conclusions: This database bodes well for the EU. Plans are underway to interact it with DG SANCO's HELICS and EARSS networks. The lessons can be extended to other pathogens such as enterococci.

O378 Methicillin-resistant *Staphylococcus aureus* (MRSA) clones identified at four health institutions in Italy

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Objectives: To evaluate the prevalence and epidemiology of MRSA clones collected at four health institutions in Italy.
Methods: A total of 204 MRSA were recovered during two collection periods, 1997 and 1998, from four hospitals located in three Italian cities: Naples (63 strains), Milan (48 strains), Rome (93 strains). All strains were characterized according to the antimicrobial susceptibility patterns and genotypic profiles defined by PFGE and hybridization with the *mecA* and Tn554 probes. Susceptibility patterns were performed against 12 antimicrobials: ampicillin (Amp), chloramphenicol (C), ciprofloxacin (Cip), clindamycin (Da), erythromycin (E), gentamicin (Gen), oxacillin (Oxa), rifampin (Rif), tetracycline (Te), trimethoprim-sulfamethoxazole (Sxt), teicoplanin (Teic), and vancomycin (Va). MICs were evaluated for quinupristin–dalfopristin (synercid) at the concentrations of 0.5, 1, 2, 4 mg/L.
Results: The 204 MRSA were clustered into four main clonal types: two of them already documented as widely disseminated in Europe – the Iberian MRSA (38 isolates, 18.6%) and the Brazilian MRSA (25 isolates, 12.2%); two new clones were detected in this sample – the 'Italian' clone comprising 90 isolates (44.1%) present in he four health institutions and the 'Rome' clone with 32 strains (15.6%) identified in Rome only. The remaining 19 MRSA were included into 11 minor clonal types. By ribotyping, all strains had a similar profile with the exception of isolates belonging to the Italian clone. Each clonal type could be associated with an antibiotype: 84% of the Iberian MRSA isolates were susceptible only to C and Sxt; 72% of the Brazilian MRSA were susceptible to C; 69% of the Italian clone strains were susceptible to C, Rif, Te, and Sxt, and 78% of the Rome-clone isolates were susceptible to C, Da, E and Sxt. In addition all the strains were susceptible to teicoplanin, and vancomycin. MICs to synercid of most strains were <0.5 mg/L (susceptible) except for 73 MRSA isolates, 62 out of which belonged to the Italian clone that shared an MIC > 0.5 mg/L.
Conclusions: The present study showed that the MRSA clones responsible for infection particularly at high-risk areas of Italian hospitals includes not only geographically spreading clones but also regional or local clones. This data will be useful for further epidemiological surveys and for the reinforcement of infection control measures to prevent their dissemination inside the hospital and at the community.

O379 Prevalence of methicillin-resistant *Staphylococcus aureus* and small colony variants *S. aureus* in Belgian cystic fibrosis patients

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Objectives: To study the prevalence of methicillin resistance and SCV phenotype among Belgian CF population colonized with *S. aureus*.
Methods: *S. aureus* strains were collected from six Belgian CF clinics and one re-validation Centre during a 6-month period. One or more *S. aureus* strain per patient were characterized by phenotypic methods (coagulase, latex test and Oxacillin Screen Agar) and a triplex PCR amplifying *nuc*, *mecA* and 16S rRNA genes. MRSA was defined by the presence of *mecA* gene.
Results: A total of 179 strains from 162 patients were analyzed. The *mecA* gene was present in 21 strains from 20 patients (12%). A total of seven patients were carrying both MRSA and MSSA. MRSA prevalence ranged from 0% in two centers to 19–24% in three centers. Median age of the patients was significantly higher (23 years vs. 15.5 years) in the three centers with higher prevalence of MRSA ($P<0.001$). Twenty-four percent (5/21) of the MRSA strains failed to grow on Oxacillin Screen Agar plate; 3/5 of these showed the SCV phenotype. 22 patients presented a SCV phenotype of *S. aureus* (14%). 12 patients carried both normal and SCV strains. Ten patients (6%) harbored the SCV phenotype only. SCV carriage ranged from 0% in three centers to 24–30% in two centers. A number of 21/22 SCV strains were found in one laboratory testing primary cultures from two centers.
Conclusions: (1) The overall prevalence of MRSA in Belgian CF centers was low (12% of *S. aureus* colonized patients). (2) MRSA prevalence by Centre was very heterogeneous. (3) Older CF patients were more likely to be

colonized with MRSA ($P < 0.001$). (4) 35% of MRSA carriers also carried MSSA. (5) The prevalence of SCV phenotype was much lower than described in a previous study and was mostly detected in one laboratory. (6) MSSA and MRSA prevalence could be underestimated due to use of laboratory methods that may be inadequate for detection of *S. aureus* SCV phenotype.

O380 Dissemination of epidemic strains in Central Europe and changes of resistance patterns in MRSA

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Objective: To follow emergence and spread of epidemic MRSA by means of molecular typing and computer-assisted comparison of typing patterns.
Methodology: Molecular typing by *Sma*-I-macrorestriction patterns and PCR detected genomic polymorphism, PCR demonstration of resistance determinants; computerized pattern analysis and comparison by use of the HARMONY MRSA collection.
Results: From 1991 to 1994, three clonal groups of MRSA with epidemic interhospital spread were predominant in Germany: the Northern German Epidemic MRSA (corresponding to the Iberic clone widely spread in several European countries), the Southern German MRSA (corresponding to epidemic strains from Belgium, Slovenia, Bulgaria, and UK [EMRSA]), and the Hannover area MRSA. These groups exhibited a broad pattern of multiresistance including aac6′-aph2″-based aminoglycosid resistance. After 1995, the prevalence of these clonal groups declined from 20 to 30% for each to about 3–1%, whereas that of newly emerging groups with less broad multiresistance as A: the Berlin epidemic strain with *mecA*, *grlA* (also in Sweden and in Belgium); B: the Barnim epidemic strain with *mecA*, *ermC*, *grlA* (identical to EMRSA15 from UK, also occurring in Sweden); and C: the Rhine–Hesse epidemic strain with *mecA*, *ermC* (also in Belgium and Finland) has increased (A: 1994–11%, 2000–26,7%; B: until 1996–0, 2000–19,8%; C: until 1998–0, 2000–6%). Besides an outbreak of GISA belonging to North German Epidemic MRSA, one of the other epidemic MRSA had exhibited this phenotype or acquired resistance to quinupristin–dalfopristin and to linezolid.
Conclusion: Increasing spread of newly emerging epidemic MRSA with less broad resistance patterns has led to decrease in MRSA of resistance to gentamicin (now 4.1%), to oxytetracycline (now 9.2%), to rifampicin (now 4.1%), and to trimethoprim (now 9.7%). Epidemic MRSA prevalent in Central Europe obviously have also been disseminated in other European countries.

β-lactamases and carbapenemase

O381 Comparative study of the development of resistance to imipenem and meropenem in *Acinetobacter baumannii* clinical isolates

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Objectives: To compare the development of resistance to imipenem (IMP) and meropenem (MEP) in *Acinetobacter baumannii* (Ab) clinical isolates, studying the mutation frequency for both antibiotics, and the stability of resistance in the mutants obtained.
Methods: Six strains of Ab were selected from 156 clinical isolates previously characterized by rep-PCR and having different resistance patterns to β-lactams. The MICs of IMP and MEP were studied by agar dilution following the 1998 NCCLS recommendations. The mutation frequency was studied by culturing each strain in Mueller–Hinton agar plates (MHA) with IMP and MEP concentrations eight-, four- and two-fold greater than their respective MICs. The colonies present in the plates with the higher concentration were subcultured in plates containing twice the MIC of MEP or IMP depending on the antibiotic used. The mutation frequency was calculated by dividing the number of mutants growing at concentrations eight-fold the original MIC or higher into the initial inoculum. A mutant was considered to be stable when there were no changes in the MIC after five subcultures in MHA.
Results: Sixteen mutants selected with MEP (M-MEP) were obtained in all six strains, but seven mutants selected with IMP (M-IMP) were obtained in only two strains. The M-MEP showed MICs of MEP that were 2–32-fold the original MIC, and remained susceptible to IMP. In the M-IMP, the MICs increased one- or two-fold, and all strains remained susceptible to IMP. The mutation frequency was higher for MEP than for IMP, from 4×10^{-10} to 2.8×10^{-4}. A total of 86.9% of the mutants were stable in their resistance to carbapenems.
Conclusions:
1 MEP-selected mutants more often than IMP, and a stable, high-level resistance to MEP was obtained in most of them.
2 Selection of resistance to MEP does not appear to affect resistance to IMP. Therefore, the mechanism of resistance involved could be different.

O382 Insertion sequence ISEcp1 as a genetic tool for expression and integration of the clavulanic acid inhibited extended-spectrum β-lactamases of the CTX-M type

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Objectives: The Ambler class A clavulanic acid inhibited extended-spectrum β-lactamase (ESBL) *CTX-M-18* has been characterized from a *Klebsiella pneumoniae* isolate from Vietnam. The genetic environment of this gene was characterized since several *CTX-M*-type genes are associated with insertion sequence ISEcp1. The ISEcp1 as a vector for *blaCTX-M* gene expression and mobility has been also assessed. The natural reservoir of ISEcp1 has been searched among constituent of bacterial flora in humans since the progenitors of several plasmid-mediated *CTX-M*-type β-lactamases are Enterobacteriaceae such as *Kluyvera* sp.
Methods: Shotgun cloning of *Bam*HI- or *Sau*3AI restricted plasmid DNA of the p *K. pneumoniae* isolate was performed into pBK-CMV followed by selection in *Escherichia coli* DH10B. DNA sequencing was performed with inserts of several recombinant plasmids. Additional plasmid constructs were performed consisting in subclonings (i) in the low copy vector pACYC184 to study the β-lactamase expression with or without the 3′-end of the insertion sequence ISEcp1, (ii) of parts of the sequence located upstream and downstream of blaCTX-M-18. A transposition assay using the conjugate plasmid pOX38 as recipient in *E. coli* was used to analyze ISEcp1 transposition. A series of bacterial species that are component of the enteric flora in humans were screened for ISEcp1. Hybridization were performed by the Southern technique with an internal probe for ISEcp1 and genomic DNAs of strains of enterobacterial species, *Pseudomonas* sp., *Aeromonas* sp., *Clostridium* sp., *Enterococcus* sp., *Staphylococcus* sp., *Bifidobacterium* sp., *Campylobacter* sp. . . .
Results: Sequencing of the surrounding sequences of *blaCTX-M-18* identified in the immediate upstream and downstream DNA region, ISEcp1 and another insertion sequence, IS903B, respectively. Deletion experiments and primer extension analysis identified −35 and −10 promoter sequences in the 3′-end of ISEcp1 for driving expression of *blaCTX-M-18*. ISEcp1 was active for transposition of the *blaCTX-M-18* gene without IS903B. None of the studied bacterial species of the human fecal flora was found to be the natural reservoir of ISEcp1. ISEcp1-like sequences were identified in one *Shigella sonnei* strain and one *Clostridium perfringens* strain without β-lactamase gene.
Conclusion: ISEcp1 has a still unknown natural reservoir and is a driving force for expression and mobilization of the *ESBL* genes of the *CTX-M*-type.

O383 Comparison of the kinetics parameters of the ceftazidime-hydrolyzing CTX-M-15 β-lactamase to those of its progenitor CTX-M-3, and analysis of surrounding DNA sequences of these β-lactamase genes

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Objectives: The extended-spectrum β-lactamase CTX-M-15 had been identified previously from New Delhi enterobacterial isolates. It differed from CTX-M-3 (identified from enterobacterial isolates from Poland) by an aspartate to glycine substitution in position ABL238. Biochemical properties

of CTX-M-15 and of CTX-M-3 against β-lactams were compared. Additionally, the DNA sequences surrounding these β-lactamase genes were compared.

Methods: From whole-cell DNAs of clinical enterobacterial isolates, the *blaCTX-M-15* and *blaCTX-M-3* genes were amplified by PCR and cloned into the same pCRScript cloning vector in *Escherichia coli* DH10B. Purified enzymes were obtained from β-lactamase extracts of cultures of *E. coli* DH10B-harboring recombinant plasmids that were purified by ion-exchange chromatography, and isoelectric focusing analysis and kinetic parameters were determined. Using PCR experiments and primers located in the β-lactamase genes and in insertion sequence ISEcp1 identified upstream of *blaCTX-M-15*, the 5′-upstream-located DNA sequences of *blaCTX-M-15* and *blaCTX-M-3* were compared.

Results: The specific activities of the purified enzymes CTX-M-3 and CTX-M-15 β-lactamases were 22.5 and 61.2 mM min^{-1} mg of protein, respectively, when 100 mM cefotaxime was used as the substrate. Both enzymes, with an identical *pI* value of 8.4, conferred a same level of resistance to β-lactams except to ceftazidime. The catalytic activity of CTX-M-15 was weaker than that of CTX-M-3 against penicillins (although identical for piperacillin whereas it was higher against cephalosporins (identical for cefotaxime) including ceftazidime. Finally, an insertion sequence ISEcp1 with putative −35 and −10 promoter sequences driving β-lactamase expression was found upstream of both *blaCTX-M*-type genes. However, an additional 79-bp sequence was found immediately upstream of *blaCTX-M-3* only.

Conclusion: This report further underlines that the clavulanic acid inhibited CTX-M-type extended-spectrum enzymes that usually hydrolyze cefotaxime and not ceftazidime may indeed hydrolyze ceftazidime through a single amino acid substitution. Thus, ceftazidime-containing treatment for curing infections due to CTX-M-type enterobacterial producers should be contra-indicated. Finally, this work gives additional evidence for ISEcp1-mediated spread and/or expression of *blaCTX-M*-type enzymes, as evidenced in distantly related enterobacterial isolates such as those from India and Poland.

O384 OXA-40, a novel oxacillinase with carbapenemase activity from an *Acinetobacter baumanni* clinical strain

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Objectives: A clinical strain of *Acinetobacter baumannii* (AbZZ) was isolated in the hospital Bicêtre (Le-Kremlin-Bicêtre, France) from a broncheal lavage of a Portugese patient who had been directly transferred from Portugal to France. The aim of this work was to analyze the β-lactamase content of AbZZ strain that was resistant to all β-lactams including carbapenems.

Methods: Shotgun cloning of *Hin*dIII fragments of genomic DNA of AbZZ was followed by selection onto amoxicillin (50 mg/mL)-containing trypticase soy agar of recombinant *Escherichia coli* clones and β-lactamase expression in *E. coli*. Purified β-lactamase was performed from *E. coli* DH10B cultures harboring one of the recombinant plasmids with ion-exchange chromatography followed by determination of kinetic parameters by spectrophotometry analysis. MICs were determined by an agar dilution method according to the NCCLS guidelines. The molecular biology techniques included those for plasmid extractions and analyzes, I-Ceu-I digests for determination of chromosomal location of β-lactamase gene associated with hybridization by the Southern technique with probes for the identified β-lactamase gene and for 16S RNA, and determination of integron-location of the gene using sequencing and PCR amplifications experiments with primers located in the 5′- and 3′-conserved regions for class 1 integron.

Results: Shotgun cloning identified a novel oxacillinase gene *blaOXA-40*. OXA-40 differed from OXA-24/−25/−26 by one or two amino acid substitutions. These oxacillinases had been identified mostly from the same geographic region in southern Europe, i.e. Spain, and conferred a decreased susceptibility to carbapenems. Detailed kinetic parameters (K_{cat}, K_m) of the purified OXA-40 enzyme from cultures of *E. coli* DH10B harboring a recombinant plasmid pOXA-40 indicated that this clavulanic-acid resistant enzyme has a significant carbapenemase activity. Plasmid analysis, I-Ceu-I digestion following by hybridization with 16S RNA and *blaOXA-40* internal probes and sequence analysis of the neighboring regions of *blaOXA-40* indicated that *blaOXA-40* was chromosomally located and, on the contrary to most of the oxacillinase genes, integron-free.

Conclusion: This report provides further evidence that multiple-antibiotic resistant Ab strains may carry carbapenemase genes that are undetectable clinically.

O385 The active insertion sequence IS1999 enhances expression of the integron-located *blaVEB-1* encoding an extended spectrum β-lactamase in *Pseudomonas aeruginosa*

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The integron-borne *blaVEB-1* gene encodes an extended spectrum β-lactamase that was initially found in an *Escherichia coli* isolate from Vietnam and subsequently in two *Pseudomonas aeruginosa* isolates from Thailand. In the latter two strains, *blaVEB-1* was associated with either one or two insertion sequences (IS1999 and IS2000) that were inserted upstream of *blaVEB-1*. Epidemiological surveys have identified *blaVEB-1* in up to 48 and 94% of unrelated ceftazidime-resistant enterobacterial and *P. aeruginosa* isolates from Thailand, respectively.

Objectives: The aim of this work was (i) to study the distribution of IS1999 and IS2000 in *blaVEB-1* positive enterobacterial and *P. aeruginosa* clinical isolates from Thailand (ii) to analyze their contribution in β-lactamase expression and to determine transposition ability of IS1999.

Methods: PCR analysis and dot blot experiments were used to study distribution of ISs. To determine whether IS1999 may influence *blaVEB-1* expression, several recombinant plasmids containing *blaVEB-1* alone, associated with IS1999 or/and IS2000, were introduced into *E. coli* and *P. aeruginosa* reference strains. Specific β-lactamase activities and MICs of β-lactam antibiotics were determined for these strains. Primer extension experiments were used to characterize functional promoters.

Results: IS1999 was present in 90% of the *blaVEB-1*-containing *P. aeruginosa* strains and among these latter strains 7% possessed an additional IS2000 inserted within the IS1999 sequence. In Enterobacteriaceae, out of 18 *blaVEB-1* positive isolates, none of them contained either IS1999 or IS2000 except one *Klebsiella pneumoniae* strain that has a *blaVEB-1* gene associated with IS1999. In *P. aeruginosa*, the β-lactamase expression was enhanced in the construct with IS1999 upstream of *blaVEB-1* leading to a 4–8 fold increase of MIC values for β-lactams. Primer extension identified two active promoters located in the left inverted repeat of IS1999 in *P. aeruginosa* while only one of them was functional in *E. coli*. Additionally, a transposition assay in *E. coli* characterized IS1999 transposition and revealed that the 5′-upstream located sequence of *blaVEB-1* is not a hot spot for IS1999 transposition.

Conclusion: The *blaVEB-1* expression is enhanced in *P. aeruginosa* by an upstream insertion of IS1999. This may explain the high prevalence of the association between IS1999 and *blaVEB-1* in these ceftazidime-resistant *P. aeruginosa* isolates.

O386 Detection of extended spectrum β-lactamase production amongst *Acinetobacter* species

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Objectives: To investigate extended spectrum β-lactamase (ESBL) frequency amongst *Acinetobacter* species with two different methods.

Methods: Eight-one non-duplicate *Acinetobacter* strains isolated from clinical specimens were enrolled in the study. Double disk synergy (DDS) test and OXOID combination disks [ceftazidime (CD_{02}, 30/10 μg) and cefotaxime (CD_{03}, 30/10 μg) with clavulanic acid] were used to detect ESBL frequency. Thirty microgram cefotaxime, ceftazidime, cefepime, aztreonam and ceftriaxone (OXOID) disks were placed 25 mm (center-to-center) from a 20/10-μg amoxicillin–clavulanate disk for the conventional DDS test. A clear extension of the edge of the inhibition zone of any of the antibiotics towards the disk containing clavulanate was interpreted as positive for ESBL production. For the strains having very small zone sizes around the disks, the test was repeated decreasing the distance between the disks. For the OXOID combination disks, the zone sizes of ceftazidime and cefotaxime disks were measured and compared with the zone sizes measured with the combination disks. Five millimeter or above enhancement of the zone sizes with the combination disks were detected as having ESBLs.

Results: A number of 79 (97.5%) of the strains were identified as *A. baumanni*, two (2.5%) as *A. junii*. With the conventional DDS test 29 (35.8%) of the strains were detected as producing ESBLs, with the OXOID combination disks the number was 28 (34.6%). While testing the strains with DDS test, the differences between the disks were lowered even to 15 mm in the strains

having very small zone sizes. There were additional strains detected as producing ESBLs at that distance.
Conclusions: To visualize the ESBL production, DDS test may have to be run several times to get the best distance between the disks for detecting enhancement. OXOID combination disks seem sensitive and easy for routine clinical application in the detection of ESBLs.

O387 Decreased production of AmpC β-lactamases by ciprofloxacin, salicylate and 2,4-dinitrophenol in *Citrobacter freundii* strains

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Objectives: Quinolone-resistance in *C. freundii* strains has been associated with a decreased production of chromosomal AmpC β-lactamases[1] and with the expression of *marA* in *Escherichia coli*. Likewise, hypersusceptibility of the *Pseudomonas aeruginosa nfxB* mutants to β-lactams is owing to reduced expression of the AmpC β-lactamase. The objective of the present study was to compare the effect of subinhibitory concentrations of ciprofloxacin (CPX) and two inducers of *marRAB* expression, salicylate (SL) and 2,4-dinitrophenol (DNT), on chromosomal AmpC β-lactamase expression in two *C. freundii* strains.
Methods: Two chromosomal AmpC β-lactamase overproducing *C. freundii* strains (DM1 and DM2) were used. Their β-lactamase crude extracts (β-CE) were obtained after growing in presence of CPX (0, 0.03, 0.06 and 0.12 mg/L), SL (0, 3, 5 and 10 mM), DNT (0, 0.5, 1 and 2 mM), with and without 10 mg/L cefoxitin (FOX) as an inducer of AmpC β-lactamase production. Their specific activities were determined using 0.1 mM cephaloridine (CR) as substrate. The effect of CPX (0.12 mg/L), SL (10 mM) and DNT (2 mM) in the outer membrane proteins (OMP) was analyzed by electrophoresis in urea–SDS–PAGE.
Results: The specific activity (SA) of non-induced β-CE from the DM1 and DM2 strains on 0.1 mM CR decreased progressively with the increment of CPX, SL or DNT concentrations in the culture medium. SA decreased: 24–93% with CPX, 63–90% with SL and 22–81% with DNT in the DM1 strain β-CE and 33–84% with CPX, 77–99% with SL and 46–95% with DNT in the DM2 strain β-CE. β-CE obtained in the presence of CPX, SL or DNT and FOX hydrolyzed CR 1.5–12 times faster than non-induced β-CE. Only the exposure to SL or DNT resulted in changes in OMP expression (a 44-kDa protein did not express). The diminution of SA in β-CE from the DM1 and DM2 strains by the exposure to CPX, SL, or DNT was concomitant with decreased FOX (2–4 times), cefotaxime (2–16 times), ceftazidime (2–8 times), ceftriaxone (2–64 times) and imipenem (2–4 times) MICs.
Conclusions: The exposure of the DM1 and DM2 strains to subinhibitory concentrations of CPX, SL or DNT resulted in a decreased production of AmpC β-lactamases and an increased susceptibility to cephalosporins and imipenem. Only the presence of SL and DNT repressed the expression of OMP in the DM1 and DM2 strains.

Reference

1 Tavío-Pérez MM, Amicosante G, Franceschini, N *et al. Microb Drug Resist* 1999; 5: 235–40.

O388 Effect of ceftizoxime and piperacillin/tazobactam dosing on efficacy and mutation frequency of *Bacteroides fragilis* and *Enterobacter cloacae* in mixed infection murine abscesses

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Objectives: Emergence of resistance may occur when microorganisms are exposed to sustained low dosing of β-lactam antibiotics. Little or no data are available for BF. Using a murine abscess model (Antimicrob. Agents Chemother., 45, 1394–1401), we studied the effect of dosing increases on efficacy and frequency of resistance to CZX and PT in mixed infections of BF and EC.
Methods: Mice received a daily dosing of 6–1536 mg/kg (q2) CZX or 24–6144 mg/kg (q2) PT starting 30 min before inoculation with 7 log 10 cfu BF ATCC 23745 and EC 22491 (cefoxitin-resistant) clinical isolate (MICs 1, 0.25 (CZX) and 1, 4 (PT) mg/L, respectively). After 24 h, abscesses were homogenized and 20 µL of 10-fold dilutions plated onto agar plates containing 0, 1×, 2×, 4×, 8× or 16 × MICs of CZX or PT. MFs were calculated from total bacterial counts and mutant stability assessed by multiple subculture on antibiotic-free media.
Results: The respective ED_{50} of BF/EC were 555/422 mg/kg/day for CZX and 84/528 mg/kg/day for PT. CZX-resistant BF and EC mutants were isolated from untreated controls at a MF of 0.001–0.000001 on up to 16 × MIC plates and PT-resistant EC at a MF of 0.00001–0.000001 on up to 4 × MIC plates. No PT-resistant BF were isolated on plates containing >1 × MIC PT. With CZX dosing 12–384 mg/kg/day, the MF of EC increased to 0.1–0.01. Isolated mutants with a >256-fold MIC increase produced high levels of a stably depressed cephalosporinase. The MFs of CZX-resistant BF were unchanged with increasing CZX dosing. No CZX-resistant mutants were isolated at >768 mg/kg/day. Increasing PT dosing had little effect on the MFs of PT-resistant EC with no mutants isolated at >384 mg/kg/day.
Conclusions: Sustained exposure of BF/EC mixed infections to low dosing CZX could select stably derepressed CZX-resistant EC mutants in early abscess development. PT efficacy is similar (EC) or superior (BF) to CZX but PT is more efficient in the suppression of EC and BF mutants.

Non-molecular diagnostic methods I

P389 Usefulness of selective broth media for the detection of group B streptococci (GBS) in pregnant women

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Objective: To confirm the usefulness of selective broth media for detecting group B streptococci (GBS) in pregnant women in order to include its use in our procedures.
Methods: From March to July 2001, all samples from pregnant women were included in a prospective study. Vaginal and anorectal swabs were inoculated onto Columbia with colistin and nalidixic acid (CNA) and Todd Hewitt broth supplemented with 5% blood, gentamicin and nalidixic acid (SBM). All culture media were incubated at 35 °C in 5% CO_2. After 24 h of incubation SBM broths were subcultured onto CNA plates and all negative cultures were incubated for additional 24 h.
Results: A total of 694 samples from 370 patients were evaluated (368 vaginal and 326 anorectal). GBS was found in 113 (16%) samples from 64 (17.2%) women. Of the 113 positive specimens GBS was recovered from 100 (49 vaginal and 51 anorectal) samples in CNA (88.4%). SBM recovered GBS in 13 additional samples from 12 women. Evaluating paired samples of these women, in six (9.3%) was the diagnostic method. Of 113 positive samples there were 17 that were isolated in direct CNA plates but were not detected in SMB subcultures. Of these 17 samples 12 (70%) were anorectal samples.
Conclusions: SBM broth improves the recovery of GBS in pregnant women. Nearly 10% of our patients were misdiagnosed without the use of this medium. These data support the routine use of a selective broth media for the detection of GBS in pregnant women.

P390 Growth of *Burkholderia cepacia* complex and other organisms on *B. cepacia* selective agar (BCSA)

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Objective: To evaluate the growth on BCSA of isolates belonging to the *Burkholderia cepacia* complex and organisms other than *Burkholderia cepacia* complex.
Methods: A total of 127 *B. cepacia* complex isolates were included in the study: two from cystic fibrosis (CF) patients, 119 from non-CF patients, and six from

hospital environment. The following organisms other than *B. cepacia* complex were tested: 23 *S. maltophilia*, 12 *A. baumannii*, 10 *P. aeruginosa*, one *P. putida*; 10 *S. marcescens*, nine *E. coli*, nine *K. pneumoniae*, eight *E. cloacae*; 22 *Staphylococcus* spp., and 14 *Enterococcus* spp. The identification of these 245 strains was performed using conventional biochemical analysis and/or commercial systems. The testing of the efficacy of BCSA was conducted in two phases. In phase 1 polymyxin and gentamicin were included in BCA-base. In phase 2 vancomycin was added to this formulation. BCSA plates were inoculated with standardized bacterial suspensions and were incubated at 32 °C up to 72 h.
Results: A total of 226 of 245 strains grew on BCSA-base. For 19 *Staphylococcus* spp, this base had inhibitory effect. All *B. cepacia* complex isolates, six of 23 *S. maltophilia*, and seven of 14 *Enterococcus* spp. grew on BCSA phase 1. None of the tested organisms of family Enterobacteriaceae, *Pseudomonas* spp., *A. baumannii*, and *Staphylococcus* spp. showed growth on BCSA with polymyxin and gentamicin. The addition of vancomycin to B1CSA phase 1 inhibited two of seven enterococci but had no effect on the growth of the nonfermentative Gram-negative isolates.
Conclusions: BCSA demonstrates high sensitivity for isolation of the *B. cepacia* complex organisms. This medium inhibits most of the non-*B. cepacia* complex strains but it is not enough selective for *S. maltophilia*. This organism is particularly problematic species since, it has similar to the *B. cepacia* complex biochemical properties and can be found in specimens of CF patients. Our data show that BCSA is effective medium for identification of putative *B. cepacia* complex organisms and should be included in primary culture protocols.

P391 Evaluation of two different selective solid media for detection of group B streptococci (GBS) in pregnant women

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Murcia, E

Objective: To evaluate Granada agar plate and Columbia with colistin and nalidixic acid agar (CNA) for the detection of GBS in pregnant women.
Methods: From March to July 2001, 694 (368 vaginal and 326 anorectal) samples from 370 pregnant women were processed in our laboratory for the detection of GBS. All samples were inoculated onto GM and CNA plates. After 24 h of incubation in 5% CO_2 at 37 °C all culture negative plates were reincubated for additional 24 h.
Results: GBS was isolated from 115 of 694 samples (16.5%) from 68 (18.4%) patients. Fifty-five (15%) of 368 vaginal swabs and 60 (18.4%) of anorectal swabs were positive. GBS was recovered from 109 specimens in GM and from 100 specimens in CNA giving sensitivities of 94.7 and 86%, respectively. GBS grew only in Granada agar in 15 cases (six vaginal and nine anorectal samples) and in CNA agar in six cases (three vaginal and three anorectal samples). The 15 samples only positive in GM were from 13 women, evaluating these cases in 10 of them GBS was isolated in CNA of paired sample (anorectal or vaginal). Only in three (4.4%) patients GBS was detected exclusively in GM. GBS was detected in two (3%) patients only in CNA media.
Conclusions: There were no significant differences between both culture media. GM because of characteristics colored colonies is an easier and faster method for detecting GBS in pregnant women.

P392 Staphychrom 2, a new specific staphylocoagulase test using a chromogenic substrate

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Objectives: Tested with 297 *Staphylococcus* strains, Staphychrom 1 (International Microbio, France), a 2-h fluorogenic test using human prothrombin and protease inhibitors for the detection of staphylocoagulase has been shown to be more specific, and as sensitive as the reference tube coagulase test (TCT), and more specific and more sensitive than the latex agglutination test (LAT) Pastorex Staph Plus (Bio-Rad). Staphychrom 2, a new version of Staphychrom 1, uses a chromogenic substrate and a simplified methodology. This study compared Staphychrom 2 to (i) Staphychrom 1 (ii) a reference TCT and (iii) the LAT Slidex Staph Plus (bioMérieux, France) for the rapid identification of *Staphylococcus aureus*.
Methods: A total of 113 clinical isolates including 92 *S. aureus* (78 methicillin sensitive and 14 methicillin resistant), and 21 non-*S. aureus*, were tested. Moreover, 15 collection strains: four *S. aureus* (two of which with a negative reference TCT) and 11 non-*S. aureus*, selected for their ability to give false-positive results either with the TCT or a LAT, were tested.
Results: With the clinical isolates, Staphychrom 2, Staphychrom 1 and the TCT yielded a positive-coagulase test with 91 of 92 *S. aureus* strains (sensitivity 98.9%) then a negative coagulase-test with the 21 of 21 non-*S. aureus* and one *S. aureus* strains (specificity 100%). The LAT yielded a negative-agglutination with 18 of 21 non-*S. aureus* and three *S. aureus* strains (one methicillin sensitive and two methicillin resistant) and a positive-agglutination with three non-*S. aureus* strains (sensitivity 96.7% and specificity 85.7%). With the collection strains, Staphychrom 2 and Staphychrom 1 yielded a positive-coagulase test with two of four *S. aureus* and a negative-coagulase test with the 11 non-*S. aureus* and the two *S. aureus* known to be negative with the reference TCT. The TCT yielded a positive test with two of four *S. aureus* and eight of 11 non-*S. aureus* strains (three *S. schleiferi* subsp coagulans and four *S. intermedius* and one *S. hyicus*). The LAT yielded a positive-agglutination test with three of four *S. aureus* and seven non-*S. aureus* strains (two *S. schleiferi* subsp coagulans, three *S. intermedius*, one *S. hyicus* and one *S. delphini*).
Conclusions: Staphychrom 2 yields the same results as Staphychrom 1. This test is as sensitive and more specific than the TCT, and more sensitive and more specific than the LAT Slidex Staph Plus. This rapid, accurate and easy-to-use coagulase test seems to be a good substitute for the conventional TCT.

P393 Evaluation of a new commercial selective medium for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis

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Objectives: *Burkholderia cepacia* complex, is an important pathogen, causing respiratory-tract infections in patients with cystic fibrosis. Because several organisms misidentified as *B. cepacia* grew on commercially available agars intended to be selective for *B. cepacia*, we believed a medium that was more inhibitory yet that was enriched to support the grow of *B. cepacia* was required. The aim of our study is to verify a new selective agar medium *B. cepacia* Selective Agar (BCSA) in an effort to improve the speed and accuracy of the isolation of the *B. cepacia* complex.
Methods: In two Italian clinical microbiology laboratories (Istituti Clinici di Perfezionamento of Milan and G. Gaslini Institute of Genova) was carried out a prospective comparative evaluation (from 7 July to 10 September 2001.) of two commercial prepared media in the routine setup procedures for respiratory specimens from CF patients. The participating laboratories were allowed to incorporate the additional selective agars into their existing CF respiratory protocols as best suited the individual laboratory's procedures. We compared BCSA (manufactured by BioMerieux Italia S.p.A) to Oxidation-Fermentation Agar Polymyxin Bacitracin Lactose agar (OFPBL) (from BBL, Cockeysville). Bacterial strains were identified at species level by conventional biochemical method, the *B. cepacia* complex status was assesed by genus and species-specific polymerase chain reaction assays (Agodi JCM 2001, Bevivino JCM in press).
Results: A total of 333 respiratory specimens from CF patients were processed. The 211 of 333 (63.4%) cultures had no growth of any organisms on any selective agar for *B. cepacia* complex; 25 of 333 (7.5%) cultures grew *B. cepacia* complex on both media; 43 of 333 (13%) grew other than *B. cepacia* complex on BCSA agar and 122 of 333 (36.6%) on OFPBL agar. Other organisms that grew on BCSA and OFPBL agar included: 74 *Staphylococcus aureus* (one on both media, 73 on OFPBL only); 37 moulds (13 on both media, 24 on OFPBL only); 15 *Achromobacter xylosoxidans* (11 on both media, fouron OFPBL only); 57 yeasts (two on both media, 55 on OFPBL only); nine *Pseudomonas aeruginosa* (eight on both media, one on OFPBL only).
Conclusions: We conclude that (i) BCSA and OFPBL was similarly in their ability to support the growth of *B. cepacia* complex. (ii) BCSA is superior to OFPBL in suppressing growth of organisms other than *B. cepacia* complex.

P394 Evaluation of two different paediatric blood culture bottles of BacT/Alert system for the detection of *Neisseria meningitidis*

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Objectives: To evaluate the performance of the new BacT/Alert pediatric aerobic bottle with activated carbon (FAN) in comparison with the old BacT/Alert pediatric aerobic bottle (STD), to support the growth of *Neisseria meningitidis* (*N. meningitidis*).
Methods: We studied 30 frozen strains, sensitive and moderately sensitive to penicillin and belonging to serogroups B and C, isolated from patients with sepsis and/or meningitis. We prepared two dilutions of each strain of $1:10^5$ and $1:10^6$ UFC/mL. Of each dilution one bottle STD (vented) and two bottles FAN (one vented and one nonvented) were inoculated with 0.4 mL of inoculum and 1 mL of human blood. Bottles were vented prior to loading into the BacT/Alert system and were incubated for 5 days or until the instrument signaled that they were positive. Before inoculating bottles we made a quantitative inoculum control.
Results: There were no significant differences in the capacity of recovering strains of *N. meningitidis* between both types of bottles, with average inocula between 174 and 1497 UFC/mL. There were no significant differences in detection time (in hours) related to serogroup or penicillin sensitivity. There were statistically significant difference in detection time between STD bottles and FAN bottles in favor of STD. There were statistically significant differences in detection time between FAN vented and FAN nonvented, in favor of FAN vented.
Conclusions: The FAN bottles were comparable with STD bottles in their ability to recover *N. meningitidis*, although they needed more incubation time. According to our results, we suggest venting the bottles before incubation when sepsis and/or meningitis are suspected, in order to obtain the results early.

P395 An assessment of the cost effectiveness of a chromogenic agar (ABC medium) for the selective isolation of salmonellae from routine stool samples

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Objectives: To investigate whether a novel chromogenic agar (α-β-chromogenic (ABC)) medium, Lab M, Bury, England) was more efficient in terms of workload (Welcan units) and cost than commonly used selective enteric media.
Methods: ABC medium exploits the fact that salmonellae may be distinguished from other members of the Enterobacteriacae by the presence of α-galactosidase (α-gal) activity in the absence of β-galactosidase (β-gal) activity. Salmonellae produce a characteristic green colony (α-gal positive, β-gal negative) whereas non-*Salmonella* strains are either clear (α-gal and β-gal negative) or black (β-gal positive). The study was performed in two district general hospitals, A and B. All stool samples were subcultured from selenite broth onto ABC medium and either desoxycholate lactose agar (DCLS) or xylose lactose desoxycholate (XLD) agars. Hospital A routinely used DCLS and XLD agars whilst hospital B used XLD only. Costings and Welcan units were calculated for each medium.
Results: A total of 866 stool samples were processed from which 14 salmonellae (1.6%) were isolated. The 620 samples were cultured onto XLD and 246 onto DCLS. XLD/DCLS media produced a greater number of suspect colonies (227) that required further investigation compared to ABC medium (31 suspect colonies). In hospital A, one salmonella was detected by ABC medium only. Overall, the sensitivity and specificity was superior for ABC medium ($100 \pm 45.2\%$, respectively) than for XLD/DCLS media ($92.3 \pm 5\%$, respectively). Comparative specificities for ABC medium were significantly different at the two hospital sites (hospital A 100% and hospital B 50%). This was probably due to hospital A's previous experience with this medium. The difference in Welcan units between XLD/DCLS and ABC was 2197 units. Using half-plates for ABC medium, consumable costs were comparable with XLD/DCLS media.
Conclusions: Selective chromogenic agar has much improved specificity for salmonella identification and will have a significant impact in reducing laboratory workload and costs.

P396 Synthesis and evaluation of a new chromogenic substrate for detection of β-ribosidase

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Objectives: To synthesize a chromogenic substrate which allowed visualization of β-ribosidase activity in agar media and to evaluate the diagnostic potential of this substrate for the differentiation of pathogenic bacteria.
Methods: 3′,4′-dihydroxyflavone is a molecule which chelates iron to form a black insoluble precipitate. This molecule was chemically derivatized to form a nonchelating β-riboside and this substrate was incorporated with iron into an agar-based culture medium. A total of 158 bacterial strains were inoculated onto this medium and incubated overnight. When the riboside substrate was hydrolyzed by bacterial enzyme activity the 3′, 4′-dihydroxyflavone was released and reacted with iron to form black bacterial colonies.
Results: Among the Gram-negative bacteria tested β-ribosidase activity was widespread but not universal. Most strains of Enterobacteriaceae were positive for this activity but *Yersinia enterocolitica* and *Vibrio cholerae* were negative. β-ribosidase activity was also highly useful for the differentiation of *Shigella* spp. (positive) and *Proteus* spp. (negative). Among Gram-positive bacteria there were also useful findings. For example, *Corynebacterium diphtheriae* was a strong producer of β-ribosidase whereas most streptococci were negative. This could be of diagnostic importance for the isolation of *C. diphtheriae* from throat swabs.
Conclusions: This is the first microbiology report of the diagnostic utility of a β-ribosidase substrate. The substrate allowed for the clear recognition of bacteria producing β-ribosidase due to the formation of black colonies. We conclude that this substrate could be applied in new chromogenic media for the rapid detection of bacterial pathogens.

P397 Early detection of *Listeria monocytogenes* with selective CHROMagar *Listeria* medium

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Background: Listeriosis is a major public health problem in developed countries, where expansion of the agro-food industry has resulted in the possibility of a wide variety of food becoming contaminated with *Listeria monocytogenes*, which may cause large food-borne outbreaks. Rapid detection of the bacterium in food products is thus essential for industrial food producers. Various selective chromogenic media differentiating *L. monocytogenes* from the other species of Listeria on the basis of metabolic reactions (color of the colonies, opaque halo around colonies) are now available.
Methods: We compared the time required for the specific detection of *L. monocytogenes* with the most recent CHROMagar™ Listeria medium (CHROMagar™) with that on ALOA® medium (AES Laboratoires). We tested 194 strains of *Listeria* sp. (152 *L. monocytogenes*, 25 *L. ivanovii*, eight *L. innocua*, nine other *Listeria* sp.). The interpretation criteria were: the color of the colonies, the presence or absence of an opaque halo, the diameter of the halo measured after various times of incubation at 37 °C (18, 24 and 48 h). Statistical analysis was performed with EpiInfo6.
Results: Halos were observed for *L. monocytogenes* and *L. ivanovii* strains only, on both media. At 18 h, the halo was significantly larger with CHROMagar™ Listeria medium than with ALOA® medium in 75% of cases. At 24 h, the halo was still significantly larger with CHROMagar™ Listeria medium. At 48 h, there was no significant difference in halo size between the two media.
Conclusions: The use of chromogenic media can significantly reduce the time required to detect of *L. monocytogenes* in food products from that required for detection with the reference method (ISO 11290–1). Our results suggest that the opaque halo appears earlier on some chromogenic media than on others.

P398 Evaluation of *Burkholderia cepacia* selective agar (BCSA) on supporting growth of *B. cepacia* complex bacteria from clinical and environmental sources in Italy

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Background: *Burkholderia cepacia* (BC) complex is an opportunistic pathogen present in the environment that cause pulmonary infections in cystic fibrosis (CF) patients (pts). There are now nine established species or genomovars (gvr) in the BC complex. The most frequent gvr recovered in CF pts is BC gvr III, but a careful and continuous microbiological monitoring in airways specimens for recovery BC complex belonging species, is important to completely understand the epidemiology of these pathogens.
Objective: We evaluated a new selective medium, *B. cepacia* Selective Agar (BCSA) (BioMerieux Italy), with others commercial prepared selective media in their ability to support the growth of BC complex strains from clinical (CF) and environmental sources.
Methods: We studied a collection of 68 strains isolated from sputa of 53 pts from different Italian regions attending the CF center of Genova and 75 strains isolated from the maize rhizosphere in three Italian regions. Strains were assigned to the BC complex by polyphasic analysis employing (i) conventional tests: API 20 E, API NE Systems (BioMerieux), OF basal medium (Difco) for six sugars, oxidase test, growth at 42 °C, pigment and hemolysis production; (ii) genus and species-specific polymerase chain reaction assays (Bevivino JCM in press). BCSA plates were compared with OFPBL and Cepacia Medium (CM) (both from BBL, Cockeysville). Growth study was carried out by a subculture of strains on blood agar for 24–48 h and aliquots of a bacterial suspension (1×10^3 to 1×10^4 cfu/mL) placed on BCSA, OFPBL and CM. Recovery efficiency for strains was determined after incubation at 30 and 35 °C for 72 h.
Results: BC complex status of studied strains was: 2% gvr I, 68% gvr III, 3.5% *B. stabilis*, 19.6% *B. ambifaria*, 5.6% *B. pyrrocinia*. Conventional tests were able to recognize *B. stabilis* but failed to discriminate BC gvr I to BC gvr III and to *B. ambifaria*. The 100% of studied strains grown on BCSA and OFPBL and 97% on CM (three CF strains of BC gvr IIIA and one CF gvr III failed to growth on CM). All strains displayed good growth on BCSA and OFPBL after 48 h at 35 °C but on 24 h incubated BCSA plates the colony growth was yet detectable. CM needed an increased incubation time for 72 h to improve the optimum growth rate.
Conclusions: Our results show that BCSA is superior to OFPBL and CM for rapidity and quality of growth of Italian BC complex strains of CF and environmental origins.

P399 Blood cultures: time required for recovery of bacteria and yeasts with BacT/Alert system

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Objectives: Prompt detection of bacteraemia and fungaemia impacts positively on patient management and continues to be one of the most important responsabilities of clinical microbiologist. The aim of this study is to evaluate the time of grow of bacteria and yeasts in blood cultures of the automated BacT/Alert system (Biomérieux).
Methods: Blood cultures, FAN aerobic and anaerobic, obtained from hospitalized patients between 19 June 1998 and 15 December 2001 were studied. Bottles were incubated for a standard 7-day protocol or until the instrument signaled that they were positive. In this study, the time of growth is defined by number of days between the collection and the detection of growth by the technician (cultures are examined daily, 7/7 days, at 08.00 and 15.00 h) and matches with the days required to report the positive result and Gram stain to the clinician.
Results: A total 87 785 bottles for blood cultures were performed. Positive cultures were found in 10 851 cases (12.4%). The microorganisms isolated were 11 921. The time required for recovery of microorganisms was as follows: 0 days (i.e. the same day of the collection, 0.6%), 1 day (61.1%), 2 days (28.1%), 3 days (5.6%), 4 days (1.8%), 5 days (1.1%), 6 days (0.9%), 7 days (0.7%).

	Detection of growth (days)								
Micro-organisms	0	1	2	3	4	5	6	7	Total
Enterobacteriaceae	43	2656	297	78	21	13	5	5	3118
Aerobic Gram-neg. bocilli (others)	1	273	87	28	3	4	1		402
Aerobic Gram-pos. bocilli	3	90	68	17	5	2	6	2	193
Aerobic Gram-neg. cocci		4	3	1					8
Aerobic Gram-pos. cocci	23	4076	2642	474	142	55	28	27	7467
Propionibacterium spp.			5	3	3	35	57	55	158
Yeast		96	116	21	7	1	2		243
Total (*n*)	72	7287	3344	673	215	127	112	91	11921
Total (%)	0.6	61.1	28.1	5.65	1.6	1.07	0.94	0.76	

Conclusion: Based on this study, the clinician can expect the results of positive blood cultures and Gram stain the day after in 61.7% and 2 days after in 89.8% of the bacteriemic patient. The identification and susceptibility of direct inoculation from positive culture can provide an interim final results within 3 days nearly in 90%.

P400 Evaluation of Agar blood–neomycin–nalidixic acid and Agar Columbia–colistin–nalidixic acid in the isolation of group B *Streptococcus*, in pregnant women

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Objective: Group B *Streptococcus* (GBS) is a major cause of neonatal morbidity and mortality worldwide. We study 442 cervicovaginal specimens of pregnant women with 25–26 weeks gestation, in Trujillo City, Peru from main Hospitals and Centers of Health, to determine the presence of (GBS) and to compare the quality of isolation of media.
Methods: We evaluated 442 cervicovaginal specimens. A swab was cultured in a selective isolation Agar blood–neomycin–nalidixic acid (ASNA) and Agar Columbia–colistina–nalidixic acid (ACCA), the identification of GBS was carried out by means of the catalasa tests, Bacitracin, CAMP test and serology for Agglutination with antisera Lancefield DIFCO.
Results: We compare ASNA and ACCA, determining the effectiveness of each one, and then to be able to use it like routine media. We determined that 15.84% are carriers of GBS. Observing that in ASNA GBS grows more quickly than in ACCA. Also in ASNA grow less contaminants than in ACCA.
Conclusion: We concluded that the medium more appropriate for the isolation of SGB in pregnancy women is the ASNA medium in comparison with the ACCA.

P401 Evaluation of the performance of bioMérieux campylosel agar

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Campylobacter cause diarrhea with abdominal pains and fever. *Campylobacter jejuni* is the species most frequently isolated by bacteriology laboratories. Campylosel agar is a selective medium, enabling isolation of these pathogenic intestinal bacteria from polycontaminated specimens (liquid stools). The growth of contaminant bacteria can, in the event of low or insufficient inhibition, lead to incorrect results due to the relatively slow growth of *Campylobacter* strains.
Purpose of study: The purpose of this study was to compare the performance of the bioMérieux preplated medium, in terms of nutrient capacity and selectivity, with the main competitor media available: Oxoid and Becton Dickinson.
Materials and methods: 25 *C. jejuni* strains and other *Campylobacter* species were tested. They were obtained both from internal strain and ATCC collections. For selectivity, 25 interfering strains were used, including Enterobacteriaceae, nonfermenting Gram-negative bacilli, Gram-positive bacteria, yeasts and anaerobes. For each strain, a two MCF sterile physiological saline solution suspension was produced. Inoculation was performed for

the three isolation media to be compared using a 10-μL calibrated loop. Columbia agar with 5% sheep blood inoculated in parallel was used as a nonselective reference. The plates were incubated under microaerophilic conditions for 72 h at 37 °C. A growth intensity and colony size reading was performed simultaneously for all the media after 24, 48 and 72 h of incubation.
Results: The *C. jejuni* strains studied were used to classify the culture media in order of nutrient capacity. The Oxoid medium was found to have the richest growth after 48 h, followed by those of Becton Dickinson and bioMérieux. However, overall, for all the strains tested, only the bioMérieux medium enabled visible colony growth after just 24 h of incubation. In the same way, the media were classified in order of selectivity according to the growth of interfering strains. The bioMérieux medium proved to be the most selective, followed by those of Becton Dickinson and Oxoid.
Conclusions: The bioMérieux Campylosel agar has a nutrient capacity similar to those of its main competitors with a better performance in terms of selectivity. This culture medium is a reliable product, well adapted to the needs of *Campylobacter*, which is difficult to isolate due to the generally very abundant accompanying flora.

P402 CPS ID3, an advanced chromogenic medium for diagnosis of urinary tract infections

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Objective: CPS ID was the first Chromogenic Medium designed for easy diagnosis of urinary tract infections, enabling the enumeration of microorganisms, the identification of *Escherichia coli* and the preliminary identification of enterococci, Proteeae, and the Klebsiella–Enterobacter–Serratia group (KES). CPS ID2 included a new chromogenic substrate for easier identification of *E. coli*, especially in multimicrobial cultures. CPS ID3 is an evolution of this medium incorporating an innovative chromogenic substrate for the detection of deaminase in bacteria of the tribe Proteeae (patent pending).
Methods: This new medium was compared to CPS ID2 using a collection of 350 microorganisms commonly encountered in urine. A preliminary comparison was also made using urine specimens.
Results: All the microorganisms grow on CPS ID3, and the colonies of Gram-positive cocci and yeasts are larger. The sensitivity of *E. coli* identification is slightly better on CPS ID3 and the specificity is 100% on both media, as this identification is based on the highly specific glucuronidase activity. The sensitivity of Proteeae detection is higher on the new medium; deep brown colonies are produced without the addition of any reagent and there is no diffusion in the agar. On CPS ID3, colonies of *Citrobacter* are similar to those of KES. The detection of enterococci is 100% sensitive on both media, but the specificity is higher on CPS ID3 with better discrimination between enterococci and group B streptococci. These results are confirmed with urine specimens: the growth of staphylococci is better and the detection of Proteeae is easier than with CPS ID2.
Conclusion: CPS ID3 enables easy detection and enumeration of Proteeae even in mixed cultures. The growth of Gram-positive cocci and yeasts has been improved and other parameters have been optimized. CPS ID3 is the chromogenic medium perfectly adapted to the diagnosis of urinary infections, even with the most complex specimens.

P403 New Oxoid Dryspot tests for detecting important non-O157 *E. coli* serotypes isolated from different culture media

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Objectives: To evaluate the new range of Dryspot Serocheck tests with non-O157 *E. coli* and other Gram-negative lactose fermenting organisms from different culture media.
Methods: Oxoid Ltd. has developed a range of latex agglutination kits for the individual identification of somatic antigen from six of the most common verotoxigenic non-O157 *E. coli* serotypes (O26, O91, O103, O111, O128 and O145). A total of 112 strains of *Escherichia coli* and other lactose-fermenting Gram-negative bacteria were grown overnight (18–24 h) at 37 °C on MacConkey, Enterohaemolysin and Nutrient agars (all manufactured by Oxoid Ltd) and tested with each Serocheck kit.
Results: All serotypes (O26, O91, O103, O111, O128, O145) of *E. coli* tested were positive with the appropriate *E. coli* Serocheck kit, regardless of agar type used. With the exception of a single bacterium (*Edwardsiella tarda*) that agglutinated weakly with all *E. coli* Serocheck test latex reagents, there were no false positive reactions with other serotypes of *E. coli* or other Gram-negative bacteria regardless of agar type used. *Edwardsiella tarda* can be differentiated from *E. coli* by its production of hydrogen sulphide.
Conclusions: Excellent sensitivity (100%) and specificity (99%) was demonstrated for each of the Serocheck kits with strains grown on the three different agar types. Dryspot *E. coli* Serocheck kits can be stored for two years at room temperature. The tests provide fast and simple screening procedures for non-O157 serotypes implicated in human disease.

P404 The influence of the presence of albumin in the growth and/or motility of Gram-positive and -negative bacteria

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Objectives: Little attention has been given to the role of albumin play in determining the growth and motility of bacteria. The data presents the growth and motility of motile and nonmotile bacteria in laboratory cultures. Firstly, the effect of type of bacteria on their growth for 6 days. Secondly, the effect of different degrees of temperature on the growth of motile and nonmotile bacteria. Finally, the effect of albumin at different concentrations on the growth and motility of motile and nonmotile bacteria at different degrees of temperature and at different environmental conditions.
Methods: The organisms used for the determination the effect of albumin were: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883) and *Staphylococcus aureus* (ATCC 25923). Growth of microorganisms in the presence of albumin was determined by culturing in duplicate in order to incubate at 37 °C and 43 °C under aerobic condition. The radius of bacterial spread from the central inoculum was measured (in mm) daily for up to 6 days of incubation. Motility of bacteria was observed under a phase contrast microscope, using a flat capillary tubes of path 0.2 mm, called microslides under aerobic and anaerobic conditions.
Results and conclusions: Culture media with the different levels of albumin which inoculated with one strain of bacteria: *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 13883) and *S. aureus* (ATCC 25923). Samples were incubated at either 37 °C or 43 °C and viable counts were made at 24, 48, 72, 96 and 144 h. At 37 °C, *P. aeruginosa* and *S. aureus* grew well in full strength at the presence of high concentration of albumin, whereas, *E. coli* showed little growth. The failure of *K. pneumonia* to grow can be attributed to high osmolarity of the albumin. No increase in growth of all used organisms at 43 °C, except for *P. aeruginosa* which reflects the tolerance of this organism to unusual growth conditions. The results of the study show that the bacteria survive and may highly multiply even at high concentration of albumin and at both used temperature.

Antibiotic usage

P405 Assessment of antibiotic therapy after 72 h: impact of a questionnaire on the use of antibiotics

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Objective: Antibiotic therapy (AB) can be reassessed after 48–72 h, based on clinical evolution, and microbiological and laboratory results. Such assessment may lead to a more appropriate use of antibiotics. A questionnaire was developed for this purpose and evaluated in a randomized study.
Methods: Patients hospitalized in the surgical and medical wards of a university hospital were screened for inclusion by daily chart review. The eligible patients were those receiving an i.v. antibiotic for 72–96 h. They were randomly allocated to either no intervention ($n = 125$) or mailing of a questionnaire to the resident in charge ($n = 126$). The questionnaire asked three questions about continuation of AB and, in case of continuation, indication and possible adjustment. The primary outcome was time until modification of AB (interruption, adjustment, switch to oral therapy). The results in the two groups were compared by Cox-proportional hazard ratio modeling. Comparison was also made with 151 patients treated during the 2 months preceding the study. A sample of 50 (20%) patients was randomly selected to evaluate the appropriateness of AB within the 48 h following inclusion (clinical evaluation sample).
Results: There was a nonsignificant trend towards shorter time to modification of AB in the intervention group (adjusted hazard ratio 1.2; 95% CI 0.93–1.56). Time to modification was significantly shorter in the intervention group than during the pre-study period (adjusted hazard ratio 1.15; 95% CI 1.02–1.31). According to this latter result, the questionnaire would infer 15% less days until modification of AB in the intervention group. In the clinical evaluation sample, the management of AB within 48 h following the inclusion was considered inappropriate in 44% of patients in each group. However, no withdrawal of antibiotics was considered inappropriate, suggesting that the questionnaire did not induce hazardous decisions.
Conclusion: The present study failed to demonstrate a significant reduction in time until modification of AB by use of a questionnaire aimed at assessing antibiotic treatment after 72–96 h. However, this may be an underestimate of the impact of the intervention by a contamination effect to the control group, as the study was randomized at the patient level. Although some benefit may arise from this simple and easily automatable approach, multiple strategies are probably needed to improve antibiotics use.

P406 First evaluation of the impact of an antibiotic management team on the antibiotic consumption in a general hospital

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Objective: To evaluate the impact of an antibiotic management team (AMT) on AB consumption in a general hospital (H).
Methods: Three hospitals (HA, HB, HC) were united in a 755-beds general hospital in 1999. After providing a single AB formulary, an AMT composed of an infectious diseases specialist (IDS), a pharmacist, a microbiologist, an internal medicine specialist and a computer-analyst was created in December 2000. AB controlled were "Broad spectrum AB" (piperacillin ± tazobactam, ceftazidim, cefepim, ceftriaxon, aztreonam and carbapenem), glycopeptides and quinolones. Period I (PI) (1 Janruary to 30 September 2000) and period II (PII) (1 Janruary to 30 September 2001) were compared. In HA, an AB policy existed from 1996 co-ordinated by the microbiologist and the pharmacist. On PII, the IDS and microbiologist made advised consultations in all services with educational programs on AB use twice a week. In HB and HC, no AB policy existed before AMT intervention (PI). In HB, during PII, the AMT initiated the same AB policy as in HA. In HC, controlled AB prescription was not notified. The IDS made advised consultations without direct control on AB prescription. Data of AB consumption were expressed in Defined Daily Dose per 1000 hospitalization days (DDD/1000 J).
Results: The H global AB consumption was 261.11 DDD/1000 J in period I and 201.32 in period II (−23%). HA decreased from 296.38 in PI to 230.32 in PII (−22%), HB from 269.01 in PI to 202.79 in PII (−24.6%) and HC from 193.42 in PI to 145.54 in PII (−24.7%). The H Glycopeptides consumption was 10.44 DDD/1000 J in PI and 8.43 in PII (−19.2%) with decrease of 27.3 and 39.6%, respectively, for HA and HB, and increase of 25% for HC. The H 'Broad spectrum AB' consumption was 34.98 DDD/1000 J in PI and 23 in PII (−34.25%) with decrease of 26.3 and 50.20%, respectively, for HA and HB, and increase of 22% for HC. The H Parenteral Quinolones consumption was 5.05 DDD/1000 J in PI and 4.29 in PII (−15%) with decrease of 14.6, 12.1 and 32% for, respectively, HA, HB and HC. The H oral Ciprofloxacin consumption was 18.17 DDD/1000 J in PI and 14.5 in PII (−20%) with increase of 41% for HA and decrease of 27.5 and 32%, respectively, for HB and HC.
Conclusions: The AMT succeeded in reducing H global AB use through advised consultations, educational programs and particularly daily notification of controlled AB prescription. The results of the AB consumption decrease on patient clinical outcome, hospital epidemiology and cost should be evaluated further.

P407 An infectious diseases (ID) day-hospital (DH) service in a tertiary care center in the third millennium: a 12-month survey of patients according to the spectrum of diagnoses, length of stay, and diagnosis-related group (DRG) parameters

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Objective: To perform a survey of patients hospitalized at the day-hospital (DH) of an infectious diseases (ID) department of a teaching hospital in the year 2000.
Methods: All DH hospitalizations were analyzed according to the discharge diagnosis, mean number of daily admissions, and DRG parameters, in order to assess the performance of our service, and plan future needs and resource allocation.
Results: In 1-year period, 304 patients were hospitalized in 302 working days using the four available DH beds: the overall 2478 days of admission led to a mean 8.15 daily admission rate, responsible for a 205% occupancy of DH beds. As noticed since 1998, liver diseases accounted for the majority of main diagnoses (135 cases: 44.4%), followed by HIV disease (107 patients: 35.2%), neoplasms (eight patients), fever of unknown origin and viral diseases (seven each), CNS infections (6), respiratory tract, genito-urinary-tract, and bone/joint diseases (four patients each), and skin/soft tissue infection (3) were the remaining diagnoses, together with miscellaneous ID (19 patients). Although an overall 8.15 daily DH admissions per patient was calculated, a significant difference was found between HIV-infected patients (10.5 ± 3.2) and patients with liver disease (5.4 ± 1.9; $P < 0.001$). These figure substantially overlap the trimmed mean length of admissions of other ID DH of Emilia–Romagna region for the same DRG groups, when excluding a slightly more prolonged stay for uncomplicated HIV and liver disease, balanced by a shorter length of hospitalization for AIDS and complicated hepatic disorders. An overall mean 1.03 DRG weight was calculated for our DH patients, but a significantly greater index was recognized for HIV-related admissions (1.47 ± 0.39; $P < 0.001$).
Discussion: HIV-related DH admissions largely overcame those owing to other illnesses until 1997 (with a maximum rate of 90.7% in the year 1996), but as a result of the changed natural history of HIV disease, the majority of DH hospitalizations now regards non-HIV-infected patients (53.4 to 64.1%, 1998–2001). Concurrently, with shortage of inpatients beds, a notably reduced mean time of DH stay, and the increased need of DH hospitalization for other diseases, significant issues are emerging about the changing referral to our DH, modified epidemiology and delivery of care of ID, and eventual needs for patients isolation, when potentially transmissible diseases are of concern. A continued monitoring of DH admissions and their features is strongly advisable to adjust standards of care to the moving target represented by ID patients in the third millennium, and to optimize patients care in the next future.

P408 A protocol for medical audit and peer review in infectious diseases – experiences with the method

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On behalf of the Medical Audit Group of the Swedish Society of Infectious Diseases

Objectives: Since 1993, the Swedish Society of Infectious Diseases has developed indicators of medical quality in the diagnoses endocarditis, pneumonia, bacterial meningitis, HIV, malaria, the follow-up of nosocomial infections and the use of antibiotics. A national quality register for treatment of endocarditis has been established. In collaboration with the Swedish Medical Association and The Swedish Society of Medicine we have developed protocols for medical audit and peer review of departments of infectious diseases in Sweden.
Methods: The medical audit group has developed two protocols; one describing structure and process quality data of the department reviewed, and one describing the medical quality using the indicators for each diagnosis and for nosocomial infection assessment and antibiotic use. The department carries out the self-assessment of the medical audit. Two experienced consultants perform the peer review during 3 days, which includes audit of protocols and medical records, interviews of hospital management staff and colleagues in other departments.
Results: Eleven audits using the peer-review method have been performed. The chosen indicators of medical quality have been accepted and successively integrated in the infectious diseases departments in Sweden. The register of endocarditis is the largest of its kind including more than 1400 episodes. The experience is that the self-performed medical audit is very interesting and not too time-consuming. The peer review has been very appreciated among the colleagues.
Conclusions: The peer-review method has given further insights into the medical quality of the reviewed departments not detectable just by medical indicators of quality. Audit of medical records is essential for peer review. Some deficits of medical quality can only be detected by study of records and interviews of external persons. Colleagues find the method very well suited for quality assurance in clinical practice. (Protocols available at http://www.infektion.net)

P409 Experience of a specialized infectious diseases home-care service from 1995 to 2001

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Objective: In 1995, a specialized infectious diseases (ID) home-care service was created in our institution in order to improve the quality of life of patients with prolonged parenteral antimicrobial therapy requirements and to reduce their hospital stay. Clinically stable patients with infections that required i.v. antimicrobial therapy were considered for inclusion in the service. We review the experience of this service between 1995 and 2001.
Methods: Analysis of the number of included patients per year, number of patients with HIV infection, ID Diagnosis and administered antimicrobial treatment.
Results: The number of patients included each year during the period 1995 through 2001 were: 54, 57, 86, 232, 213, 321 and 280, respectively. In 1995, 50 (92.5%) were HIV patients; in 1996, 53 (93%); in 1997, 65 (75.6%); in 1998, 95 (41%); in 1999, 64 (30%); in 2000, 90 (28%) and in 2001, 68 (20%). The main ID and antimicrobial treatment per year are shown in Tables 1 and 2. In the case of endocarditis, parenteral outpatient therapy reduced 504 days per year of hospital stay since 1998.

Table 1

Diagnosis	Number of patients included per year						
	1995	1996	1997	1998	1999	2000	2001
Citomegalovirus	27	39	27	26	15	6	6
Other HIV-related	13	3	9	28	25	44	24
Fungal infection	3	9	9	8	2	2	7
Bacterial endocarditis	5	3	6	26	22	20	29
Osteomielitis	2	1	1	14	15	27	31
Celulitis	0	0	1	15	8	33	27
Pneumonia	0	0	2	47	49	89	57
Urinary-tract infection	0	0	6	41	26	78	64

Table 2

Treatment	Number of daily antimicrobials given per year						
	1995	1996	1997	1998	1999	2000	2001
Antiviral	285	723	892	442	130	86	46
Antibiotic	53	179	420	1002	1270	1736	1285
Antifungal	3	17	164	225	158	93	188
GSF	210	498	253	48	14	25	10

Conclusions: The number of patients included in our specialized ID home-care service have been increasing in the last years. The percentage of HIV-patients included with respect to non-HIV-patients have decreased since 1996, probably, in relation to the introduction of highly active antiretroviral therapy and a better control of opportunistic infections. Urinary-tract infection, pneumonia, osteomielitis and endocarditis are actually the more frequent infections treated in this service, with reduction of the hospital stay.

P410 The evaluation of rational antibiotic use in a teaching hospital in Turkey

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Objective: To evaluate rational antibiotic use in relation to diagnosis and bacteriological findings in an 1100-bed teaching hospital in Turkey.
Methods: An antibiotic restriction policy for ceftazidime, cefepime, imipenem, meropenem, ticarcillin–clavulanate, piperacillin–tazobactam, vancomycin, teicoplanin and intravenous formulations of quinolones was initiated in Ankara Numune Hospital in January 1999. In July 2001, a point prevalence study of the hospitalized patients who received antibiotics was performed. Two infectious diseases specialists assessed the prescribed antibiotics according to published guidelines.
Results: Of the 713 patients hospitalized, 281 (39.4%) patients received 377 antibiotics. Fifty-eight (20.6%) patients received antibiotics for the treatment of hospital-acquired infections. Among 30 different antibiotics the most frequently prescribed were first generation cephalosporins (19.9%), ampicillin-sulbactam (19.1%), aminoglycosides (11.7%), quinolones (11.1%) and carbapenems (7.2%). Most of the antibiotics (75.9%) were given in parenteral form. The dosage of 35 (8.9%) antibiotics was out of standards. Eighty-six antibiotics were administered to 75 (26.9%) patients who had no evidence of an infection or indication of prophylaxis. Unnecessary antibiotic use was 30% in surgical wards, 14% in intensive care units and 8% in medical wards. Ampicillin-sulbactam and first generation cephalosporins were the most frequently unnecessarily used antibiotics (35 and 31%, respectively). Bacteriological samples had been taken before the antibiotic prescription in 114 (40.6%) patients. Antibiotic prescriptions were switched to another antibiotic in 43 (15.3%) patients; the main reason was susceptibility test result (44.62%). Antibiotic use was appropriate in 67%. Rational antibiotic use was highest when the results of bacteriological samples were positive.
Conclusions: Irrational antibiotic use was high for ampicillin-sulbactam and first generation cephalosporins. Antibiotic use was inappropriate particularly in surgical wards. Additional interventions such as postgraduate training programs and elaboration of local guidelines could be beneficial.

P411 Glycopeptide overuse and the impact of a restriction policy

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Glycopeptide (G) overuse is considered as a risk factor for the emergence of glycopeptide resistant enterococci (GRE). The isolation of such strains in our

hospital in late 1999 and during 2000 correlated well with the increasing G consumption during the last years. As a result, a restriction policy has been implemented since April 2001. According to this policy every G prescription has to be authorized by an infectious diseases physician. ICU was not included (a different policy exists there). The main target was to effectively reduce the inappropriate use of G in cases where another anti-staphylococcal agent could be used. We present the results of an interim evaluation of our policy (for the period 1 April to 30 September 2001). A significant decrease in G consumption was recorded in our hospital during this six-month period, in comparison with the same period of year 2000. With ICU data not included, vancomycin (V) use was reduced from 20.6 to 15.25 DDDs/1000 patient-days (−26%) and that of teicoplanin (T) from 17 to 8.6 DDDs/1000 patient-days (−50%). In contrast an impressive rise in cloxacillin and fusidic acid use was observed (+800 and +400%, respectively). An increase was also recorded for clindamycin (from 23.8 to 31.3 DDDs/1000 patient-days, + 31.5%). When ICU data were included, the reduction for V use was still 26%, but only 3.5% for T, because of increased consumption of this agent in the ICU. In the same period, the mean length of stay in our hospital showed a slight decrease (from 4.87 to 4.6 days) and so did the mortality from all causes(from 2.52 to 2.47%) (excluding ICU in both measurements). While the increase in cloxacillin and fusidic acid use is most welcomed, this is not the case for clindamycin since its overuse is also a risk factor for GRE emergence.

Conclusions: It is encouraging that, at least in the short-term, our policy seems to effectively decrease G consumption without adversely affecting length of stay or mortality. The impact on GRE problem in our hospital is being evaluated and will be presented in a future publication. Lastly, a restriction policy is probably needed for the ICU as well.

P412 Distribution, trends and seasonal behavior of antimicrobials used in five Spanish sanitary districts

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Objective: To describe the outpatient antibiotic use in five sanitary districts in Comunidad Valenciana (Spain), its trends, relative distribution and seasonal behavior.

Methods: The study was performed in five sanitary districts (2nd, 4th, 9th, 19th and 20th – 1 300 000 inhabitants) in the Comunidad Valenciana (Spain). Monthly DDD per 1000 inhabitants-day (DDH) of all antimicrobial used in community were obtained from prescriptions financed by the National Health System. From January 1996 to August 2000 in districts 2nd, 4th, 9th and 19th, and from January 1994 to August 2000 in district 20th, time series were performed using such data. In order to asses trends and seasonal behavior, ARIMA models were adjusted. Multiplicative seasonal factors where estimated using Season SPSS procedure.

Results: We observed a seasonal distribution with the highest overall use of antibiotics during winter months (e.g. in terms of DDH: January 1996: 29.4; January 1997: 33.3; January 1998: 30.4, January 1999: 32.9; January 2000: 26.9). Figures for summer months are nearly half of those of winter months. Highest January seasonal factors correspond to macrolides (1.62), followed by penicillins with extended spectrum (1.61), third-generation cephalosporins (1.49), second-generation cephalosporins (1.43) and combinations of penicillins (incl_ β-lactamase inhibitors) (1.39). Trends of each antimicrobial group are presented.

Conclusions: Upper respiratory-tract infections are the most frequent community infections in winter months. This fact can explain seasonal pattern in antimicrobial use, despite the frequently unknown infection aetiology. Intervention studies, guidelines and educational programs to reduce the prescription of antibiotics for respiratory-tract infections are necessary. Time Series Analysis techniques are useful tools in order to improve knowledge about elements that can help to control of bacterial resistance problems.

Acknowledgments: This project is supported by Grant no. 00/0002 of Fondo de Investigación Sanitaria, Ministerio de Sanidad, Spain.

P413 Antimicrobials use control at a teaching hospital, Surabaya, Indonesia

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Inappropriate use of antimicrobials (AM) is the key driver of AM resistance. Therefore, since 1984 Dr Soetomo Hospital has conducted a comprehensive program to promote rational use of AM. Starting with AM use reviews, it was found that there were huge problems in using AM. For example, the results of review on using AM for surgical prophylaxis in surgery wards in 1986 showed that irrational use according to the indication: 76.5% for clean and 27.5% for clean contaminated operations. Indications were correct, but inappropriate according to (a) timing: 57.2% for clean and 26.3% for clean contaminated (b) route of administration: 42.8% for clean and 10.6% for clean contaminated (c) duration: 100% for both categories. As results of these efforts, a guideline for AM usage was developed in 1990. The guideline was not only a list of AM, but also a policy on regulation and an educational tool. However, it was obvious that regulations and education alone were not effective in changing behavior. Regulations and educational measured should be supported by managerial steps. Unfortunately the roles of microbiological laboratory were not developed yet. Continual AM utilization studies provided feedback, among others were: peer reviews, focused group discussions, retraining, providing reliable literatures, etc. which in turn would bring awareness regarding the need for rational management and use of AM. The AM utilization studies and feedbacks also stimulated the need to revise the guideline periodically. Therefore, the second edition guideline was published in 1992. Although the results of the review was promising, i.e. the number of rational use of AM had been increasing steadily, 20–80%, it also showed that the lack of microbiological information was the key factor in preventing the progress. Since the development of the guideline was not fully supported by systematic and standardized microbiological surveillance, this caused de-motivation in the adherence towards the guideline. Now with the support from The Royal Netherlands Academy of Arts and Sciences, Dr Soetomo Hospital together with Dr Kariadi Hospital, Leiden UMC, Erasmus UMC, and Nijmegen UMC are conducting study on Antimicrobial Resistance in Indonesia: prevalence and prevention (AMRIN Study). It is expected that the results will enhance the rational use of AM since microbiological laboratory is involved.

P414 Antibiotic prescribing patterns in outpatients with bacterial infections. A questionnaire study of general practitioners in Denmark, Norway and Sweden

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Objectives: To investigate the antibiotic prescription patterns for common bacterial infections among general practitioners (GP's) in Denmark, Norway and Sweden, and to compare these patterns with the official guidelines for the respective countries.

Methods: A questionnaire concerning how to handle eight cases of common bacterial infections (urinary-tract infection (UTI), sinusitis, otitis media, skin infection, acute and relapsing tonsillitis, acute exacerbation of chronic bronchitis (AECB) and pneumonia) was sent to 1000 GPs in each country. From the questionnaires returned, answers were recorded according to country, age and sex of the GP. Antibiotic treatment guidelines from the three countries were collected and compared to the answers recorded.

Results: The questionnaires were answered and returned from 46.8% (D), 34.2% (N) and 38.8% (S), respectively, of the GPs. The male/female ratio was 2.5 (D), 2.7 (N) and 1.5 (S), respectively, and the age median between 50 and 60 years in D and S, 40–50 in N, reflecting the sex and age distribution among GP's in the three countries. There were major differences in treatment of UTI, AECB, relapsing tonsillitis and skin infections while treatment regimens were quite similar for the other infections. For otitis media, GPs in D and N awaited symptoms before antibiotic treatment in contrast to Swedish GPs, who were more prone to treat at once. The treatment modalities reflected the guidelines for the three countries.

Conclusion: The questionnaire study was a relatively easy method to unravel the attitudes among GPs in the three countries, and the answers concurred with the antibiotic treatment guidelines for the individual countries. Changes in antibiotic treatment among GPs in the three countries could probably be accomplished by changing the treatment guidelines, which seem to be followed closely.

P415 The time and costs required for the intravenous administration of antibiotics: an activity-based costing approach

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Objectives: To assess the true costs of intravenous antibiotics administration by an activity-based costing approach.
Methods: Core of this study was a time and motion analysis conducted in a large peripheral hospital in the Netherlands. A total of 100 routine acts of preparing and administering intravenous antibiotics were observed and timed by two research nurses. An extensive inventory and work flow chart was made of the whole process of intravenous antibiotics administration. This preliminary analysis was used firstly to develop appropriate data transcription forms and secondly to be able to determine personnel costs and additional costs attributable to i.v. antibiotic therapy, such as overhead costs. The average costs per administration were calculated for each of the different administration methods used in the hospital.
Results: Four different methods of intravenous drug administration were used in the study hospital: administration by volumetric pump, by syringe pump, 'unaided' infusion bag administration, and direct intravenous injection. Average times per procedure, including preparation and administration of the drug, were 4:49 (SD 2:37), 4:56 (2:03), 5:51 (3:33) and 9:23 (minutes:seconds). When these times were related to personnel salaries, and the costs of materials used were added (excluding drug costs), this resulted in average costs of resp. EUR 5.65, 7.28, 5.36, 3.83 per administration. The insertion of an intravenous catheter, necessary for a first time administration to patients without an intravenous site, was measured separately, and was found to be 10:15 (6:31) on average, associated with a cost of EUR 9.17. A few typical examples are given of how these data can be used to calculate the total personnel and material costs due to the preparation and administration of intravenous antibiotics for a full treatment of a particular patient. In addition, an attempt was made to estimate the overhead, storage and other miscellaneous costs attributable to intravenous antibiotics therapy. Although a reasonably accurate estimate of those costs proved difficult, it seemed unlikely that such 'hidden' costs will add significantly to personnel and material costs.
Conclusion: The true costs of intravenous antibiotics administration consist of personnel time, material costs and overhead in addition to the costs of the drugs themselves. In our analysis, the nursing time required and materials used were identified as the main cost drivers.

P416 Comparison of hospital resource use between linezolid and teicoplanin for Gram-positive bacterial infections including skin and soft tissue infections: results from European Cohort of a multicentre trial

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Background: Linezolid (LZD) is a new synthetic oxazolidinone antibacterial agent, which is active against multiresistant Gram-positive organisms. It is available in both i.v. and oral forms with 100% bioavailability. It is as effective as standard therapies including flucloxacillin and vancomycin for a range of infections. In a randomised multicentre trial, LZD was compared to Teicoplanin (TEI), a commonly used glycopeptide agent in Europe, for clinical efficacy and hospital resource utilization. The latter data are presented here.
Objective: Compare length of hospital (LOS) and i.v. days of study medications and discharge rate (DR) between Linezolid and Teicoplanin for the treatment of Gram-positive infections with special reference to skin and soft tissue infections.
Methods: From a randomised multicentre trial, 227 hospitalized patients from the UK, Germany, Sweden, Italy and Spain caused by Gram-positive bacteria, including complicated skin/soft tissue infection (SSTI) subset, were treated with Linezolid (i.v. followed by oral) or Teicoplanin (i.v. followed by optional intramuscular (i.m.)) for a minimum of 6 days and up to 28 days. This was followed by a further 21 days follow-up observation. The analytic sample consists of 210 patients after excluding 17 deaths not related to treatment. Kaplan–Meier survival analysis and *t*-test were used to estimate mean and median LOS and i.v. days, respectively. The hospital discharge rates are also reported. Discharge dates, were confirmed for 88% of the sample (185/210) while the remaining LOS data were censored.
Results: Results for the total intent-to-treat (ITT) and the subgroup of Skin/Soft Tissue Infection (SSTI) are shown below.

			LOS		% Patients discharged by			
Study sample and medication	*N*	i.v. Day mean (days)	KM mean (SE)	KM median (95% CI)	1st week	2nd week	3rd week	4th week
ALL (LZD)	108	5.8	15 (1)	9 (7–11)	35	61	75	82
ALL (TEI)	102	9.3	17 (2)	9 (7–11)	29	58	69	75
P value		0.000	0.5	0.5	0.37	0.63	0.3	0.16
SSTI (LZD)	65	5.3	14 (2)	8 (6–10)	42	65	75	82
SSTI (TEI)	53	8.9	16 (2)	8 (7–9)	36	60	74	79
P value		0.0002	0.82	0.82	0.53	0.64	0.82	0.75

Conclusion: Linezolid patients tend to have shorter LOS, higher DR and significantly shorter i.v. days.

P417 A 10-year study of the consumption of quinolones, trimethoprim and mecillinam in relation to the development of antimicrobial resistance in a large number of bacterial species

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Objective: The correlation between the use of antibiotics and resistance is not always evident. In Kronoberg county, Sweden, we have more than 10 years of quantitative data on antimicrobial susceptibility and on outpatient antibiotic consumption. This study attempts to relate resistance to consumption during this period.
Methods: In a stable population of 176 000 during 1990–2001, all susceptibility tests were performed according to the Swedish Reference Group of Antibiotics. All inhibition zone diameters were measured and yearly zone diameter distributions were analyzed. The mean and SD of the wild-type populations were calculated. Resistance was measured using epidemiological breakpoints, and defined as the proportion of isolates outside −3 SD. In Sweden sales of drugs are registered centrally which allows the analysis of yearly drug consumption by outpatients. Data were analyzed as defined daily doses per 1000 inhabitants and year (DDD). Three classes of antibiotics were studied, each representing different trends in consumption.
Results: The consumption of trimethoprim was stable over 10 years (0.54–0.66 DDD/1000 IY). Resistance increased in *E. faecalis* (8–28%) and decreased in *S. agalactiae* (10–2%). In Staphylococci, *E. coli*, *Klebisella* and *Proteus mirabilis* the resistance levels were stable between 5 and 15%. The consumption of mecillinam more than doubled (0.54–1.22 DDD/1000 IY). Resistance levels were stable in *E. coli* (9–10%), *Klebsiella pneumoniae* (10–9%), *Klebisella oxytoca* (16–16%), and decreased in *Proteus mirabilis* (15–6%). The use of fluoroquinolones increased from 0.55 DDD/1000 IY to a maximum 1.47 DDD/1000 IY in 1994 after which a gradual decrease to 0.98 DDD/1000 IY was recorded in 2000. Increasing resistance levels were recorded in *Campylobacter* (7–42%) and *Pseudomonas aeruginosa* (14–22%). In *E. coli*, with more than 3000 isolates analyzed per year, quinolone resistance has been slowly increasing to a level of 5% over the last 6 years.
Conclusion: The relation between consumption of and resistance to antimicrobial agents is not always immediately evident. In the present study, we were able to analyze high quality data over 10 years for large number of isolates in a stable catchment population for three antibiotics showing different consumption trends. There was no clearcut simple correlation between consumption and resistance development.

P418 European surveillance of antimicrobial consumption (ESAC): availability and accessibility of antibiotic utilization data in European countries

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Objectives: ESAC (granted by DG/SANCO of the EC for the period November 2001–October 2003) is an international network of national surveillance systems, aiming to collect comparable and reliable antibiotic use data in all European countries. In order to obtain insight in the starting position of all participating countries, a preparatory questionnaire was distributed among all national representatives.
Methods: The questionnaire was sent to 26 national representatives, all returned the completed forms. Sales status of antibiotics, reimbursement system, availability of consumption data and details about the structure of existing databases for community care and hospitals were questioned.
Results: In 21 out of 26 countries, antibiotics are prescription only, OTC is still possible in the remaining 5. In 15 countries, an identical reimbursement system is used for all antibiotics with a percentage of reimbursement ranging from 0 to 100%. In most countries, government (Ministery of Health, Medicines Agency) as well as other organisations (Health Insurance Company, Pharmacists, Marketing Research Company) are capable of data collection. For ambulatory care, in most countries ($n = 18$) census data are available, per county ($n = 16$) and on a quarterly ($n = 8$) or monthly ($n = 6$) base. For hospitals, it seems that the availability of data is more limited. Anatomical Therapeutic Chemical (ATC) classification of antibiotics is used in all countries except 2 and volume can be expressed in defined daily dose (DDD) in most of them. An increasing number of countries have antibiotic consumption data available in recent years ranging from 16 in 1997 to 21 in 2000 for ambulatory care and from 10 in 1997 to 16 in 2000 for hospitals. Data of 2001 will be available between March and October of 2002.
Conclusion: It is the intention of the ESAC project to create a dynamic and up-to-date databank covering all aspects of antibiotic use. At the end of the pilot period, databases of continuing surveillance of antibiotic use, containing retrospective annual data for the period 1997–2001 and detailed prospective data for 2002, will be accessible for scientists and health authorities in order to link utilization data to resistance patterns and to assess the impact of intervention strategies at the community and hospital level.

P419 Consumption of 26 parenteral antimicrobial agents in 50 Northern France hospitals. Data from the ARECLIN Hospital Pharmacists Study Group

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Objectives: Little data is available on hospital antibiotic (AB) consumption in France. ARECLIN is a network of infectious diseases and infection control professionals in North France hospitals. It covers approximately 7% of the French population, i.e. 4 million individuals A project to record AB consumption was initiated in 1998.
Methods: Annual data were collected retrospectively since 1995 and prospectively after 1998. Simultaneously, ARECLIN promoted the use of automatic stop orders for hospital AB. Data collected included consumption of 23 AB (amikacin, aztreonam, cefepim, cefotaxime, cefpirome, ceftazidime, ceftriaxone, ciprofloxacine, fosfomycine, fusidic acid, levofloxacin, imipenem, isepamicin, ofloxacine, pefloxacine, piperacillin, quinupristin/dalfopristin, sulbactam, tazocillin, teicoplanin, ticarcillin, ticarcillin-clavulanic acid, vancomycine) and three antifungals (amphotericin B lipid formulations and fluconazole), total hospitals AB spending and activity data. AB consumption was compared using WHO defined daily doses per 100 hospitalization days (DDD/HD).
Results: Participation in the study varied by hospital size: 100% for hospitals with >400 acute care beds ($n = 9$), 80% for 200–400 beds ($n = 12$) and 27% for <200 beds ($n = 128$), $P < 10–7$. The non-participating hospitals had a mean bed number of 68 compared to 241 for participating hospitals. Thus, 50 hospitals (40 publics, 10 private) were enrolled in 2000. AB consumption was higher in hospitals with >400 beds (16.3 ± 3.5 DDD/HD) than in 2–400 beds hospitals (10.2 ± 4 DDD/HD) or <200 beds hospitals (10.5 ± 13 DDD/HD). A difference in per-admission AB cost was also noted: 23.9 ± 7 € (>400 beds), 14.6 ± 5.1 € (2–400 beds), 15.2 ± 15 € (<200 beds). Over 6 years, overall evolution of AB consumption was marked by a non significant increase (from 14.4 DDD/HD in 1995 to 17.2 DDD/HD in 2000, $P = 0.88$). The only significant difference observed in AB class consumption was a dramatic decrease of aminoglycoside consumption (2.4 DDD/HD in 1995 to 1.5 DDD/HD in 2000, $P < 0.002$). Conversely, the mean AB cost per admission decreased from 33.5 ± 23 to 16.8 ± 12 € ($P < 0.02$).
Conclusions: This study provides interesting information on the wide variation of AB consumption among hospitals in the same geographical setting as well as among hospitals with similar size and activity. It will help us target hospital specific AB control strategies.

P420 Use of antibiotics and development of resistance at a university hospital

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Objective: The de-escalation approach in treatment of severe infections is generally accepted at our university hospital. Several antibiotic agents are used in parallel for this purpose. The study was done to assess the impact of this approach on resistance development in nosocomial pathogens.
Methods: Data were collected on antibiotic use since 1997. MICs were established for all pathogens isolated from relevant specimens of infected patients of the university hospital.
Results: The following overall use of selected antibiotics was made:

	mg/patient days			
Agent	1997	1998	1999	2000
Imipenem	19	29	35	29
PIP/TAZ	177	257	214	181
Ceftazidime	45	45	51	51
Gentamicin	19	19	15	8
Ciprofloxacin	4	4	7	5

During the time period studied, no changes in resistance patterns, i.e. for *Pseudomonas aeruginosa* were observed (MIC distributions will be shown).
Conclusions: Employing several antibiotic agents for de-escalation therapy in parallel seems to be at least as effective in reducing selective pressure as scheduled changes of antibiotic classes.

P421 Antibiotic consumption in hospitals of Clalit Health Services in Israel, 1995–1999

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Objectives: This study was performed with the intent to obtain information about the pattern of antibiotic consumption in eight Clalit Health Services general hospitals. An antibiotic control policy under the supervision of infectious diseases physicians exists in all the Clalit Health Services hospitals, except one. The most expensive antibiotics, with broad-spectrum activity and a major impact on bacterial resistance, are restricted (vancomycin, ureidopenicillins, ceftazidime, carbapenems, amikacin, quinolones).
Methods: The data presented are based on the antibiotics supplied to all the hospitals during the years 1995–1999, which represent the actual hospital antibiotic consumption. The ATC/DDD 2000 methodology was used. The data are expressed as Defined Daily Dose (DDDs) per 100 bed days and is statistically analyzed for each year, for each hospital and, in addition, according to hospital type (tertiary or community).
Results: The total DDDs for each hospital were around 120 without significant variation over time. The DDDs of restricted antibiotics varied from 6.6 to 7.9 during the study period. Use of broad-spectrum penicillins — J01CA was the highest (57 DDDs) followed by cephalosporins — J01DA (27.5 DDDs). The patterns of DDDs differed during the 5 years, like coamoxiclav, which increased from 14 to 27 ($P < 0.01$) and amoxicillin, which decreased from 24 to 16 DDDs ($P < 0.001$). The patterns of consumption differed between tertiary and community hospitals. Penicillin G was

highly used in community hospitals ($P < 0.001$) and amikacin, carbapenems, vancomycin and tazocin were highly used in tertiary hospitals ($P < 0.001$).
Conclusions: The positive impact of infection control units is reflected by the low DDDs of restricted antibiotics. However, more than 120 DDDs/year in each hospital reflect high consumption of antibiotics in general and control measures should be directed to decrease the consumption of nonrestricted antibiotics, as well.
Keywords: antibiotics, consumption, DDDs

P422 Prevalence of antibiotic use in nosocomial infections

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Background: To know the prevalence of antibiotic utilization is important for correct appreciation of antibiotic use and for estimation of medical expenses.
Objectives: To obtain information on antibiotic utilization in nosocomial infections (NI) in a county hospital.
Methods: The study was carried out in the County Hospital Constanta, Romania (1875 beds), in 1997, in the departments with a high epidemiologic risk of transmission of NI: normal and pathologic obstetrics, newly born, pediatrics, general surgery, intensive care unit, hemodialysis and plastic surgery. The study is based on cross-sectional surveys. The prevalence of NI was calculated for patients with one or more NI, and the use of antibiotics for each episode was taken into consideration.
Results: A total of 26 NI were detected in 502 surveyed patients in the studied departments. This represent a prevalence of NI of about 5.17%, and in a patients with one or more NI, of about 4.78% (SD = 15.07). The prevalence of antibiotics use was 46.2% with highest values in intensive care unit (90.47), plastic surgery (89.5%), pediatrics (70.49%) and general surgery (54%). The most frequent antibiotics used in NI treatment were: Penicillin G (38.46%), Gentamycine (69.23%), Ampicillin (30.76%), Norfloxacine (11.53%), Cephalosporines of third generation (19.23%), and Metronidazol (15.38%). There were used next associations of antibiotics: Ampicillin + Gentamycine + Metronidazol in four cases, Penicillin G + Gentamycine in 14 cases. Monotherapy was performed with Cephalosporines of third generation in five cases, with Fluorquinolons in three cases and with Imipenem in one case. The supplementary expenditures on NI were estimated.
Conclusions: We found an antibiotic abuse in surgery departments, pediatrics and intensive care unit. Optimization of antibiotics use require a good collaboration between clinician, pathologist, pharmacist and pharmaceutical companies for the best policy in antibiotic use. The results obtained in this study show the need for another national multicenter study, based on the same methodology.

P423 Perioperative antibiotic prophylaxis (AP) in five Italian hospitals

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Objectives: To evaluate the AP for prevention surgical site infections (SSI) in five Italian hospitals. To assay the degree of knowledge and use of AP. To study the effectiveness of guidelines (GD) for rational use of antibiotics.
Methods: A retrospective study was conducted to determine AP use according to surgical procedures (SP) in maxillo-facial, thoracic, urologic, gynecological, orthopedic and general wards. The review included the classification of the SP and the types of antibiotics used, route, timing and additional doses. In general ward the SP were divided by category and each one including a set of similar procedures.
Results: The review of 162/306 (52.9%) patients (pts) undergoing SP revealed a SSI rate of 1.6%. AP was received by 150/162 (92.6%) pts. AP was not indicated in 6.2% of cases and in 1.2% was not performed. Cephalosporins were administrated in 117/150 (78.0%) pts and penicillines in 15/150 (10.0%). Cephazolin was somministrated in 39.4% of cases, particularly in all SP of thoracic, maxillo and urologic wards. Ceftriaxone was used in 26.0% of cases and in orthopedic surgeries was administrated in 22/59 (44.1%) cases. 56/59 (94.9%) of the orthopedic SP were clean (prosthesis: 26/56, 46.4%); teicoplanin (30.8%) and cephazolin (26.9%) were used. All gynecological SP were clean-contaminated and cephazolin was used in 16/25 (64%) of pts. In general ward AP varied between each categories of SP (34/56 clean-contaminated, 13/56 contaminated and 1/56 dirty). Cephazolin was used in 28.6% of pts; ceftriaxone and cefepime in 17.8% of cases, respectively. The 5.3% of pts received metronidazole + gentamicin for colorectal SP. AP was incorrect for timing in 37.5% of cases and for dosage in 34.4%; AP became therapy in 75% of cases.
Conclusion: Application of GD for correct AP was started in orthopedic and maxillo-facial wards. A major goals are to standardize the correct use of AP by international GD in all wards and to control the incidence of SSI with a surveillance system.

P424 Laboratory physicians: an alternative resource for implementing organization-wide quality systems

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Objective: To raise awareness of in-house expertise for the implementation of organisation-wide quality systems.
Comment: Healthcare institutions are introducing quality improvement programs that have been in use in industry for over 30 years. These programs cost money, much of it spent on external consultants and middle management hired to implement them. Pathology physicians in diagnostic laboratories are well versed in quality issues and for many years have been subjected to accreditation which addresses administration, personnel, safety, the physical environment, test procedures, quality assurance and quality improvement. Laboratories have existing safety committees with skills re. vaccination, control of and exposure to dangerous chemical and biological agents, their safe disposal and systems for the reporting and recording of injuries. These skills can be put to use across organisations implementing standards such as ISO 9000 (Quality systems), ISO 14000 (Environmental Management) and OHSAS 18000 (Occupational Health and Safety Assessment Series) as well as ISO 17000 (Laboratory Systems). In addition, under pressure from the threat of 'league tables' and other forms of comparison, organisations are implementing Quality Improvement Programs similar to those used in laboratories. Aspects of care can be tracked mathematically with statistical process controls in routine use in chemistry and serology laboratories, e.g. run charts and control charts. These methods can and should be applied to processes to identify abnormal or unexpected outcomes, e.g. unscheduled returns to the operating theatre, implant infection rates, caesarian section rates. There is an opportunity for Pathology physicians to apply their expertise and share their experience in areas beyond the laboratory.
Conclusion: Healthcare Institutions can make use of in-house Pathology expertise that can be harnessed to address Quality Control and Improvement throughout the Institution.

P425 Public health and public pharmacy

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The community pharmacy roles in the public health are:the epidemic diseases control, the nonepidemic diseases control, the use of the psychodisleptic drugs control, the prevention of illness, the sanitary education of the public, the protection of mother and child, the family planning and the reproduction health, the stomatological prevention, the education for healthy nourishment, the protection of the environment, the prevention of alcoholism, the control of the medicine abuse and self-medication (automedication). Within the present work, we determine the importance of the self-medication with antibiotics and the role of the pharmacist in the automedication control. We use the method of the specific questionnaire and the study of the pharmaceutical services with in the community pharmacy, during a period of 60 days. The list of the compensated medicines from our country has 2092 products, containing 260 active ingredients. The antibiotics represent 380 products (18%) based upon 23 active ingredients (8.8%), the list containing also 154 antimicrobial products (7.3%), meaning that the antibiotic products have an important role as therapeutic agents. Between the most prescribed antibiotics upon the compensated receipts during the 2000 years, we find amoxicillin (2.7%), ampicillin (2.5%) and cephalexin (1.5%). This means the antibiotic products are frequently prescribed in our country, because of the specific morbidity. A research of the most prescribed medicines upon noncompensated receipts during the 2000 years, gave the following results for the

antibiotics: amoxicillinum (1.4%), ampicillinum (1.4%), nystatinum (1.13%), erytromycinum (0.95%), phenoxymethtlmethycillini (0.8%), gentamicynum (0.7%), doxyciclinum (0.7%). The conclusion of our study: the pharmacist is able and has the prestige to control the antibiotic abuse and the self-medication, explaining the consequences to the patients. All these represent a part of the pharmaceutical services offered by the pharmacist in the community pharmacy.

P426 Outpatient parenteral antibiotic therapy (OPAT): the Italian registry

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Objectives: In the early eighties, in the US the advantages (reduced costs, no hospitalization trauma in children, no immobilization syndrome in elderly, reduction of nosocomial and acquired infections by multiresistant organisms) of OPAT were identified and suitable therapeutic programs were established. Following the US experience, other countries set up their own OPAT programs which vary considerably from country to country because of different ways in which infections diseases are managed in different parts of the world and because of different reimbursement systems. In order to understand the ways of managing OPAT and its results, a national OPAT registry was set up in 1999 in Italy belonging to a wider International OPAT database, which collects data also from USA, Canada, Spain, Uruguay and Argentina.
Methods: Up to now 400 patients and as many antibiotic courses have been included in the national registry by eight different centers. The analysis of data permits to get information about the criteria of patient's selection, treatment (route of administration, site of care, choice of antibiotic, dosage and duration), outcomes and possible side-effects.
Results: Italian results offer a quite peculiar picture of OPAT in this country when comparing data with those of other countries. In contrast with other countries where soft tissue infections and osteomyelitis are the most frequent diagnoses for including patients in OPAT programs, in Italy pneumonia and bronchitis are the top two amenable infections. Ceftriaxone, Teicoplanin and Amikacin are absolutely the top three antibiotics selected for OPAT in Italy which confirm that a single daily dose regimen represents a great advantage in terms of compliance. Finally a large percentage of antibiotic courses (50%) are carried out by using the i.m. administration route which is very unusual in other countries.
Conclusions: OPAT registry is still ongoing and it will give us more detailed information in the future about the management of infections in the outpatient setting, but it already permits to define an actual picture of OPAT in our country and/or to compare and correlate data and information from different countries.

Resistance in Gram-negative bacilli

P427 Infections due to β-lactamase producers among critically ill hospitalized patients and their susceptibility to cefoperazone-sulbactam

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The problem of infections owing to the β-lactamase-producing organisms is increasing globally at an alarming rate. Most laboratories in developing countries do not employ tests for detection of β-lactamase owing to the high cost of such testing. In this study, significant pathogens like *Enterococcus fecalis*, coagulase-negative *Staphylococcus* and *Staphylococcus aureus* were tested for β-lactamase production by nitrocefin disc test (BBL, Difco) and the multidrug-resistant Gram-negative organisms with suspicious antibiogram were tested for ESBL production by a simple disc approximation test using cefotaxime and cefotaxime-clavulanic acid discs. A total of 49 *Enterococcus fecalis*, 60 coagulase-negative *Staphylococcus* and 48 *S. aureus* strains were tested by nitrocefin test, and 71 *E. coli*, 120 *Klebsiella* spp., 21 *Citrobacter* spp., 7 *Enterobacter* spp., 32 *Proteus* spp., 85 *P. aeruginosa* and 93 nonfermenter Gram-negative bacilli were tested for ESBL production. These were obtained from the critically ill hospitalized patients from sourses, like urine (17%), respiratory (20%), wound (20%), blood (27%) and other sterile body sites (16%). Of the 157 Gram-positive isolates tested, 76 were β-lactamase producers of which 32 (42%) were susceptible to cefoperazone-sulbactam. Of the 429 Gram-negative isolates, 283 multidrug-resistant isolates were tested for ESBL production. A total of 217 isolates were found to be ESBL producers of which 158 (72%) were susceptible to cefoperazone-sulbactam. Cefoperazone-sulbactam can be used emperically for treatment of infections in critically ill in-patients in hospitals with a high prevalence of infections owing to the ESBL-producing Gram-negative organisms.

P428 Epidemiology of *Enterobacter aerogenes* in Belgium: preliminary results of a multicenter survey

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Objectives: A national prospective study was conducted in 2000–2001 to characterize the epidemiology of *E. aerogenes* clinical isolates from hospitalized patients in Belgian hospitals.
Methods: Proportion rates and incidence data for *E. aerogenes* clinical isolates recovered from routine cultures during the second semester 2000 were evaluated using a questionnaire. *E. aerogenes* strains were defined as multiresistant (MREA) if they were resistant to at least one of the third generation cephalosporins. Antimicrobial susceptibility (MICs of 10 antimicrobials determined by agar dilution) and ESBL production (detected using the double-disk synergy test and Oxoid combination disks) were determined on strains collected from hospitalized patients (five consecutive nonduplicate MREA strains for each center) from May 2000 to May 2001.
Results: Forty per cent ($n = 50$) of the centers answered the questionnaire. The regional distribution showed 21 participants from Wallonia, 22 of Flanders and 7 from the Brussels region. MREA represented 51% (range 0–98) of the strains and mean incidence of MREA among hospitalized patients was evaluated at 5/1000 (range 0–17) admissions; 78% (range 0–100) of the MREA strains were nosocomially acquired. 65 centers sent 313 strains isolated from patients (mean age 72 years) hospitalized mostly in medical (35%), long-time care (20%), intensive care (18%) and surgical (15%) units. Strains originated mainly from the urinary (44%) and respiratory (32%) tracts, from wounds swabs (13%) and blood cultures (5%). More than 85% of the strains were resistant to ceftazidime and ciprofloxacin, less than 15% to aminoglycosides, less than 8% to cefepime and imipenem. No meropenem-resistant strains were recovered. ESBL production was detected by the two methods in 65% of the strains, all being resistant to both ceftazidime and ciprofloxacin.
Conclusions: This study strongly confirms the widespread dissemination of ESBL-producing MREA strains among patients admitted to a large number of Belgian hospitals and their distribution in almost all types of wards.

P429 Molecular typing of extended spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates in a neonatal intensive-care unit in Greece

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Objectives: The aim of this study was to determine clonal relations among extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (EPKP) isolated from neonates.
Methods: Isolation and species characterization were performed according to standard procedures. Susceptibility to antibiotics was determined by a disk diffusion method and evaluated using NCCLS guidelines. Genomic DNA fingerprints were generated by *Xba*I restriction and pulsed field gel electrophoresis (PFGE).
Results: During the period 1997–1998, 21 infections and 23 colonizations with EPKP were recorded in the NICU of a children's hospital in Athens. Seventeen of the infected and 12 of the colonized neonates had been referred

from other hospitals. The remaining infections and colonizations occurred during hospitalization in our hospital. PFGE typing showed that the latter cases were owing to an outbreak strain that persisted in the unit, whereas the repeated introduction of EPKP-carriers was mostly owing to the clonal outbreaks in two maternity hospitals.
Conclusions: Extended-spectrum β-lactamase-producing *K. pneumoniae* (EPKP) strains are frequently implicated in outbreaks in neonatal intensive-care units. Past studies have documented the high prevalence of EPKP in various Greek hospitals. The data presented here indicate that these microorganisms have also been spread in pediatric and maternity hospitals in the Athens area.

P430 First detection of CTX-M-3-like β-lactamase-producing *E. coli* in Europe

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Objectives: Surveillance of the epidemiology of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in Bulgaria and screening for uncommon enzymes.
Methods: Enterobacteriaceae isolated from clinical specimens between 1997 and 2001 were screened for the production of ESBL by their MICs for oxyiminocephalosporins and aztreonam together with combinations including sulbactam. Strains supposed to produce ESBLs were further analyzed to identify their enzymes.
Results: An *E. coli* strain from urine of a female patient demonstrated MIC of >512 and 256 mg/L for cefotaxime and ceftazidime, indicating the production of a cefotaximase. The *pI* of the enzyme was slightly above that of CTX-M-3. The gene encoding this enzyme was transferrable and could be amplified with CTX-M group specific primers. Sequencing of the amplificate and deduction of the amino acid sequence demonstrated identity with CTX-M-3 except for one amino acid substitution at position 240 from aspartate to glycine. Therefore the enzyme was identified as CTX-M-3 like β-lactamase previously descibed by Karim, A et al. (FEMS Microbiol Lett 201 (2001) 237–241).
Conclusion: Bulgaria is the first place in Europe where CTX-M-3 like β-lactamase was detected after it had been isolated from patients in Japan and in India in 2000. Continuing surveillance can show whether this β-lactamase gene may spread among patients in Bulgaria.

P431 National and regional susceptibility of Gram-negative organisms to extended-spectrum cephalosporin and fluoroquinolone antibiotics: results of the Antimicrobial Resistance Management (ARM) Program

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Objectives: The ongoing ARM program was developed to document susceptibility patterns, including for Gram-negative organisms, in both in- and out-patient isolates. Since 1987, more than 10 million isolates have been collected on 19 organisms and 46 antibiotics from 103 US hospitals in the North-east, North-central, South-east, South-central, and South-west.
Methods: Antibiograms and sensitivity reports of isolates for *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Serratia marcescens* were reviewed for susceptibility to extended-spectrum cephalosporin (cefuroxime, cefoxitin, cefotetan, cefotaxime, ceftazidime, ceftriaxone, and cefepime) and fluoroquinolone (ciprofloxacin, levofloxacin, ofloxacin, and trovafloxacin) antibiotics.
Results: The total number of isolates and percentage of isolates susceptible to the antibiotics were determined for each organism. Nationally, *E. coli* isolates had greater susceptibility to ceftriaxone ($n = 318\,802$) than fluoroquinolones ($n = 571\,551$). This was also seen for *K. pneumoniae*, except in South-west, where isolate susceptibility to ciprofloxacin (98.5%) differed from ceftriaxone (98.0%). Isolate susceptibility of ciprofloxacin and levofloxacin to *P. mirabilis* was suppressed, specifically for ciprofloxacin in South-central (70.6%); cross-resistance is suggested by similar increases and decreases in susceptibility to these agents within regions. Isolate susceptibility differed nationally between ceftriaxone (99.4%, $n = 58\,422$) and ceftazidime (96.5%, $n = 29\,344$); especially in the North-east (98.9% vs. 86.8%). For *P. aeruginosa*, national susceptibility to ciprofloxacin (73.6%, $n = 141\,034$) and levofloxacin (67.3%, $n = 41\,673$) was similar, except in South-west, where levofloxacin was more susceptible (64.3% vs. 77.0%); nationally, greater susceptibility was seen for ceftazidime (88.1%; $n = 159\,066$) versus cefepime (78.4%, $n = 10\,052$), a difference present within each region. For *S. marcescens*, 94.4% of isolates nationally were susceptible to ceftriaxone ($n = 16\,960$), compared with 87.6% to ceftazidime ($n = 9799$), also seen in North-central (94.8% vs. 89.2%) and South-east (93.7% vs. 85.0%); a difference was noted between ceftriaxone and cefotaxime in North-east (97.3% vs. 92.3%) and North-central (94.8% vs. 90.6%).
Conclusions: A greater percentage of Gram-negative organisms remain susceptible to extended-spectrum cephalosporin than to fluoroquinolone antibiotics, a difference validated nationally and regionally within the US.

P432 Epidemiology of ciprofloxacin resistance and its relationship to ESBL-producing *Klebsiella pneumoniae*

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Objectives: To examine the epidemiology of ciprofloxacin resistant ESBL-producing *Klebsiella pneumoniae* strains.
Methods: Sixty-nine unique patient isolates of *K. pneumoniae* isolated from a variety of clinical specimens submitted to the clinical bacteriology laboratories of the Royal Infirmary of Edinburgh and associated GP practices were identified and susceptibility testing performed with the Vitek system. Strains that flagged as extended spectrum β-lactamase (ESBL) positive by the Vitek system were subjected to isoelectric focusing.
Results: The results suggested that all 69 isolates harboured at least one ESBL. Of the 69 isolates, 32 (46.6%) were found to be resistant to ciprofloxacin, 11 (16%) were intermediate and 26 (37.6%) were sensitive. To investigate the epidemiological relationship between the ciprofloxacin resistant/ESBL positive strains, pulse-field gel electrophoresis (PFGE) was performed. Rapid-dest® software was used to calculate the genetic distance by the Nei distance method. PFGE analysis indicated that the clinical isolates belonged to four distinct genotype cluster groups (A, B, C and D), each group or cluster was homogenous or compact with respect to certain characteristics. Group A consisted of 25 isolates, Group B three isolates and Groups C and D, two isolates each. PCR was used to amplify the *gyrA* gene from genomic DNA of the ciprofloxacin resistant isolates. The amplified product was sent for analysis by automated DNA sequencing and the resulting DNA sequences compared with the *gyrA* gene of *K. pneumoniae*.
Conclusions: These results indicate that the spread of fluoroquinolone resistance is largely as a result of the dissemination of a single clonal strain. The sequencing results demonstrated that alteration of the GyrA subunit DNA gyrase at amino acid 83 and/or amino acid 87 played a central role in conferring high-level fluoroquinolone resistance in *K. pneumoniae* possessing ESBLs.

P433 Extended spectrum β-lactamases-producing *Klebsiella pneumoniae* in a Spanish hospital (1993–1999)

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From January 1993 to December 1999, 487 extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) were isolated from clinical samples of 223 patients admitted in our hospital (isolates from 25 patients were not available for further studies).
Objectives: To characterize the clonal relationship between these strains and to study the ESBL.
Methods: Antibiotic resistance pattern analysis, pulsed-field gel electrophoresis (PFGE) and isoelectric focusing of β-lactamases were performed.
Results: Eight antibiotypes, 28 PFGE clones and 8 ESBL-production patterns of p*I*s were found during the study period. A dominant epidemic clone by PFGE was found from January 1993 to June 1995 in clinical samples from 132 patients. These isolates showed five ESBL-production patterns of p*I*s (N): (a) 7.6 (41); (b) 8.2 (68); (c) 7.6 + 8.2 (20); (d) 7.6 + 5.4 (2); and (e) 8.2 + 5.4 (1). All ESBL production patterns were transferred by conjugation to *Escherichia coli* J-53–2. This epidemic clone was only found in four isolates between December 1997 and January 1998. The remaining 62 isolates were classified in 38 different clones by PFGE, of them, 91.9% produced an ESBL of p*I* 7.6. Among these isolates six clones were found in clinical samples from more than one patient.

Conclusions: The ESBL-Kp isolated in our hospital during the study period were mainly owing to the spread of an epidemic clone until June 1995. After this date, small outbreaks caused by other ESBL-Kp clones were detected. The ESBL phenotype in Kp is mainly owing to the enzymes belonging to the SHV family.

P434 Drug resistance and molecular epidemiology of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolated from an intensive-care unit in a clinical hospital in Szczecin, Poland

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Endemic and epidemic nosocomial infections caused by extended-spectrum β-lactamase-producing (ESBL+) *Klebsiella pneumoniae* (K. pn.) represent a persistent problem in intensive-care units (ICU). Epidemic strains of ESBL + K. pn. have been associated with increased morbidity and mortality in hospitalized patients.
Objectives: The aims of the present study were to analyze occurence and antibiotic resistance ESBL+ strains of K. pn. and to delineate the clonal diversity and transmission patterns of these strains by genome macrorestriction analysis.
Materials and methods: During the period from 1999 to 2001, 70 strains of K. pn. ESBL+ were isolated from various materials from patients hospitalized in ICU in Clinical Hospital No. 2 in Szczecin. Susceptibility to antimicrobial agents was tested by the disc diffusion method (according to NCCLS standards) and the double-disc test for detection ESBL expression. Genome macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) was obtained by using *XbaI*. Patterns were visually compared into clonal groups and clonal variants.
Results: 55% of ESBL+ strains of K. pn. was susceptible to piperacillin/tazobactam, but only 7% to amoxicillin/clavulanic acid; 12 strains were intermediate susceptible to cefotaxime and 5 to ceftazidime. They were distinguished by different susceptibility to fluoroquinolones (62%) and to aminoglycosides (40%). All strains were susceptible to imipenem. Chromosomal DNA digested with *XbaI* produced an average of 20 fragments ranging in size from less than 50 kb to approximately 700 kb. TypA was dominant-35 strains identical; clonal variants: typA1-20 strains closely related, typ A2–7 strains less related; 8 strains genetically unrelated.
Conclusion: High percent of K. pn. ESBL + isolates were observed. Most strains belong to the same clone. Time of isolations suggest that the patients hospitalized for long periods in ICU have been acted as a reservoir for cross infections. An increase in the number of ESBL + K. pn. isolates in ICU is ownd mainly to dissemination of resistant plasmids or mutations in existing plasmid mediated β-lactamases under the selective pressure produced by the overuse of the third-generation cephalosporin.

P435 Molecular identification of *Escherichia coli* and *Klebsiella* with suspected extended spectrum and AmpC β-lactamases

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Objectives: Resistance to cephalosporins is mediated not only by the frequently reported extended-spectrum β-lactamases (ESBLs) but also by plasmid-mediated and hyperproduced chromosomal AmpC β-lactamases (AmpCs). Little is known about the contributions of AmpCs to extended-spectrum β-lactam resistance in Germany. Therefore, we investigated the frequency of ESBL- and AmpC-mediated resistance in a group of *E. coli* and *Klebsiella* strains.
Methods: Between February 2000 and January 2001, 24 *E. coli*, 12 *K. pneumoniae* and 3 *K. oxytoca* strains from five Berlin hospitals were submitted because of reduced susceptibility to third generation cephalosporins. For these strains, minimum inhibitory concentrations (MICs) of seven β-lactams were determined. Based on these MICs, the strains were divided into those with a suspected ESBL and those with a suspected AmpC. The β-lactamases were then identified at the molecular level by PCR and subsequent sequencing of the β-lactamase genes.
Results: Of these 39 strains, 55% derived their resistance from the production of AmpCs, 40% showed ESBL production, 2.5% hyperproduced a TEM-1 enzyme and 2.5% produced an inhibitor-resistant β-lactamase (IRBL). At the species level, 74% of the *E. coli* and only 27% of the *Klebsiella* derived their resistance from the production of AmpCs. Of the *E. coli* AmpC producers, 76% hyperproduced the chromosomal AmpC are because of a combination of promoter mutations and 24% possessed plasmid-mediated AmpCs.
Conclusions: There is a high percentage of extended-spectrum β-lactam resistance which is due not to ESBLs but to AmpCs. This percentage varies with the species. Therefore, it is imperative to identify the species and these enzymes so that an appropriate therapy can be devised. Plasmid-mediated AmpCs are of epidemiological importance because the mobility could lead to a rise in resistance.

P436 Trend of vancomycin (VAN)-resistant enterococci, and third generation cephalosporin, imipenem (IMP)- and fluoroquinolone (FQN)-resistant Gram-negative bacilli (GNB) in Korea

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Objectives: Increasing trends of VAN-resistant *E. faecium*, and IMP- or FQN-resistant GNB were noted in the previous surveillance by KONSAR (Korean Nationwide Surveillance of Antimicrobial Resistance) group. The aim of this study was to determine that the increased resistance was a transient phenomenon.
Methods: The susceptibility test data in 2000 were collected from 32 of 68 KONSAR hospitals. Excluding hospitals with lower quality control performance, the data from 23 hospitals were analyzed and the trend was compared to the previous data.
Results: Seventy per cent of *S. aureus* and 20% of *E. faecium* were resistant to oxacillin and vancomycin, respectively. Relatively high resistance rates in GNB were: 27% of *E. coli* and 70% of *A. baumannii* to FQN; 25% of *K. pneumoniae*, 43% of *E. cloacae* and 68% of *A. baumannii* to ceftazidime; 9% of *E. coli* and 16% of *K. pneumoniae* to cefoxitin, and 5% of *A. baumannii* and 20% of *P. aeruginosa* to imipenem (Table 1).

Table 1 Antimicrobial resistance rates of GNB isolated in 2000

Species (no. of isolates tested)	Resistance rate (%)				
	Cefotaxime	Ceftazidime	Cefoxitin	Imipenem	Fluoroquinolone
E. coli (21268)	7	6	9	0	27
K. pneumoniae (11051)	15	25	16	0	9
E. cloacae (4536)	35	43	NA	<1	10
A. baumannii (9836)	68	68	NA	5	70
P. aeruginosa (17204)	NT	19	NA	20	42

The rates at different size or location of hospitals did not differ significantly, even in the VAN-resistant *E. faecium* and IMP-resistant *P. aeruginosa*. Compared to the previous data, gradual rise of resistance rates were noted in ceftazidime (17% in 1998 to 19% in 2000) and IMP-resistant (17% in 1998 to 20% in 2000) *P. aeruginosa*, and FQN-resistant *A. baumannii* (56% in 1997 to 70% in 2000). A previous study showed that approximately 1/2 and 10% of IMP-resistant *A. baumannii* and *P. aeruginosa*, respectively, had VIM-2 metallo-β-lactamase gene on mobile integrons.
Conclusion: VAN-resistant *E. faecium*, and IMP-resistant *A. baumannii* and *P. aeruginosa*, besides already problematic MRSAs and ESBL-producing *K. pneumoniae*, have become new threat in all levels of hospitals in Korea.

P437 High prevalence of PER-1-extended spectrum β-lactamase-producing *Acinetobacter baumannii* in Korea

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Objectives: PER-1 extended spectrum β-lactamase (ESBL), first reported in 1993, was prevalent in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

isolated from Turkey. As far as we know, it has not been reported in regions outside Europe. In this study, we examined the presence and prevalence of PER-1-producing *A. baumannii* and *P. aeruginosa* isolated in Korean hospitals.
Methods: In an initial study, 5 ESBL-positive *A. baumannii* isolates were collected from a tertiary care Korean hospital. In a subsequent study, 97 and 100 consecutive *A. baumannii* and *P. aeruginosa* isolates, respectively, were collected from another hospital. Double disk synergy was tested by placing amoxicillin–clavulanate disks and ceftazidime, cefotaxime and cefepime disks at an edge to edge distance of 1.5, 1.0 or 0.5 cm. PCR was used to detect presence of *blaTEM, blaSHV, blaOXA* and *blaPER-1* in the initial study. In the sebsequent study, all of the isolates were tested to detect presence of *blaPER-1*, and the results were compared to DDS. Representative *blaPER-1* PCR products were used to determine the nucleotide sequence by dideoxy chain termination method.
Results: In the initial study, *blaPER-1* was detected from all of the five *A. baumannii* isolates with positive DDS, but *blaTEM, blaSHV* and *blaOXA* were not detected from any of them. In the sebsequent study, *blaPER-1* was detected from 53 of 97 *A. baumannii* isolates, but none of the *P. aeruginosa*. Analysis showed that the nucleotide sequences of *blaPER-1* of the isolates were identical to that reported by Nordmann et al. All of the isolates with *blaPER-1* were resistant to cefotaxime, ceftazidime, aztreonam and cefepime. Only 14 of 53 of *blaPER-1*-positive isolates were DDS positive when the disk distance was 1.5 cm. Additional 12 and 16 positive isolates were detected when the distances were 1.0 and 0.5 cm, respectively. Cefepime disk gave more positive results.
Conclusions: Prevalence of PER-1-producing *A. baumannii* strains in Korean hospitals revealed that the resistance was also present in Far Eastern region. The *blaPER-1* sequence was identical to that reported in Europe. All of the *blaPER-1*-positive isolates were resistant to cefepime. It was difficult to detect PER-1-producer by DDS, but cefepime disk was more useful. Further studies are required to improve detection method and to determine clinical significance of the PER-1-producing isolates.

P438 Prevalence and phenotypic characteristics of extended spectrum β-lactamases (ESBL) in a medical center, Tel Aviv, Israel

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Objectives: The occurrence of ESBL producers and the common phenotype may vary across geographical areas. We examined the prevalence and phenotypic characteristics of ESBL producers among cefuroxime-resistant (Cefu-R) Enterobacteriaceae in Tel Aviv.
Methods: The MIC of nonurine clinical isolates to cefuroxime, cefotaxime (CTX) and ceftazidime (CTZ) was determined using automated microdilution system (MicroScan). Production of ESBL was confirmed among Cefu-R isolates by disk diffusion using cefpodoxime (CPD), CTX, and CTZ with and without clavulanate (Clav); a difference of 5 mm in zone of inhibition was considered as ESBL phenotype.
Results: Cefu-R rate was 60% among *E. coli*, 46% among *Klebsiella* spp., and 23% among *Proteus* spp. 432 Cefu-R isolates were examined for ESBL phenotype (51 blood isolates). Out of the 432 isolates tested 186 (43%) were confirmed as ESBL producers. Number of isolates tested and rate of ESBL producers among various species were: *Klebsiella* spp. (81) 79%, *Proteus* spp. (58) 62%, *E. coli* (64) 53%, *Enterobacter* spp. (69) 42%, *Serratia* spp. (70) 14%, *Citrobacter* spp. (25) 24%, *Providencia* spp. (21) 24%, *Morganella* spp. (41) 5%, and *Kluyvera* spp. (3) 0%. Among Cefu-R isolates 242 (56%) had MIC ≥ 2 for CTZ, and 70 (16%) for CTX. Of ESBL producers 39 (21%) had MIC < 2 for CTZ and 9 (4.8%) for CTX. Six of the ESBL-producing strains (3.2%) had MIC < 2 for both CTZ and CTX (*E. coli* 2, *Serratia marcescens* 2, *Citrobacter koseri, Morganella morganii*). The overall sensitivity of confirmatory tests were CPD/Clav, 79%; CTZ/Clav, 66%; CTX/Clav, 91% (compared to all three combined). Sensitivity of CTZ/Clav confirmatory test for *Klebsiella* spp., *Proteus* spp., *E. coli*, and *Enterobacter* spp. were 84, 22, 76, and 62%, respectively, and of CTX/Clav 95, 97, 94, and 83%, respectively. Sensitivities of combination of two tests were: CPD/Clav + CTZ/Clav 90%, CPD/Clav + CTX/Clav 95%, and CTZ/Clav + CTX/Clav 100%.
Conclusions: ESBL production is prevalent among Enterobacteriaceae in our institution. CTX/Clav was superior to CTZ/Clav in confirming ESBL producers, sensitivity varied between species. Combination of testing with CTZ/Clav + CTX/Clav detected all these strains, with no additional benefit from CPD/Clav. Screening isolates with elevated MIC to CTX + CTZ, would have resulted in missing 3% of the ESBL producers. Using Cefu-R as a criterion for ESBL screening is simple to apply and may improve the sensitivity of the test.

P439 Extended-spectrum β-lactamases (ESBL) production in clinical isolates of Enterobacteriaceae rods

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Objectives: The aim of this study was to determine the prevalence of ESBL-activity among isolates from various body sites of intensive-care units (ICUs) patients during the last years (1997–2000) in Poznań, Poland.
Methods: The analysis included 120 resistant to oxyimino-β-lactams strains belonging to Enterobacteriaceae family. ESBL profile of strains was detected using double-disc tests. The susceptibility of the isolates to 16 drugs (beta-lactams, aminoglycosides and quinolones) were determined by the disc-diffusion tests according to the recommendations of NCCLS.
Results: The results showed that ESBL-producing strains were dissemineted in ICUs (62% of strains demonstrated ESBL-activity), and *K. pneumoniae* (49 strains) and *E. coli* (12 srains) were the most common hosts of their expression. ESBL were found in strains of wide range of Enterobacteriaceae (*Serratia marcescens, Enterobacter cloaceae, Proteus mirabilis, Morganella morganic, Proteus penneri*).

Many of ESBL-producing isolates were not resistant to oxyimino-β-lactams in susceptibility testing in vitro and this have been asociated with clinical failures.
Conclusion: Our results confirmed the need of monitoring of ESBL-activity in Enterobacteriaceae isolates to prevent and control their dissemination and to avoid therapeutic failures.

P440 SHV ESBLs in the *Klebsiella pneumoniae* population in a regional hospital in Slovenia

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Objectives: To reveal the epidemiology of ESBL-producing *Klebsiella pneumoniae* in a large regional hospital in Slovenia.
Methods: Forty ESBL-producing *K. pneumoniae* isolates from a hospital in Celje were studied. They constituted a randomised sample of all 152 ESBL-producing isolates of the species collected from different patients in the center between January 1997 and May 2001. The isolates were typed by PFGE and subjected to the ESBL gene conjugative transfer experiment. Protein extracts of the isolates and the transconjugants were analyzed by IEF to visualise their β-lactamases, and ESBL activity was detected by the bioassay approach. ESBL-encoding genes were amplified by PCR with the use of plasmids purified from the transconjugants.
Results: Seven different PFGE types were discerned among the *K. pneumoniae* isolates analyzed. One of the types, split further into nine subtypes, was clearly predominant, being represented by 22 isolates. Each of the other PFGE types with multiple isolates was differentiated into several subtypes too. No correlation between the PFGE type and the hospital ward was observed. Thirty-four isolates of all PFGE types were characterised by a single β-lactamase IEF band with a p*I* of 7.6, which was also the case of all 15 transconjugants produced by these isolates. The bioassay revealed that this band contained an enzyme with cefotaxime- but not ceftazidime-hydrolysing activity. PCR revealed that the β-lactamase belonged to the SHV family. The remaining six isolates (all of the major PFGE type) expressed multiple β-lactamases with p*I*s of 8.2, 7.6, 6.8 and 5.4, and genes for the p*I* 8.2, 6.8 and 5.4 enzymes were located in transmissible plasmids. Only, the p*I* 8.2 β-lactamases demonstrated ESBL activity, hydrolysing cefotaxime and ceftazidime, and, according to PCR, these enzymes were classified into the SHV family too.
Conclusions: This work revealed a complicated epidemiology of ESBL-producing *K. pneumoniae* in the center. The remarkable representation of isolates of a single PFGE type indicated the clonal outbreak of a strain, which expressed either the p*I* 7.6 or p*I* 8.2 SHV-type ESBL. The presence of numerous other PFGE types and subtypes suggested the possibility of the transmission of the p*I* 7.6 ESBL gene-carrying plasmids among distinct strains. It could also indicate occurrences of the independent acquisition of the ESBL by the strains and/or the gradual evolution of the outbreak into a complex endemic situation.

P441 Antibiotic resistance of Gram-negative rods isolated from hospitalized children

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Objectives: Surveillance on antibiotic ($n = 9$–18) resistance of Gram-negative rods isolated from children hospitalized in Clinics of Paediatric (P.)Surgery (S) and P. Nephrology (N) in 1998–2000.
Material and methods: By the disc diffusion method (NCCLS) antibiograms for 1583 Gram-negative rods (611 from S and 972 from N) were performed.
Results: Strains isolated from children showed low level of resistance to fluoroquinolones (5% S & N), carbapenems (1–2% N) and piperacillin/tazobactam (9% N). High level resistance (> 50%) for S strains was found to *ceftriaxone* (56%), *aztreonam* (57%), *cefotaxime* (57%), *piperacillin* (60%), *cefuroxime* (61%), *cephalotin* (70%) and *ampicillin* (79%) while for N only to *ampicillin* (64%). In 4 groups of Gram-negative rods highly resistant to majority of antibiotics were *Klebsiella* (S & N) and *P. aeruginosa* (S). The last was also more resistant to imipenem – IPM (31%) and meropenem – MEM (43%); the most frequent pattern for carbapenem-resistant strains was simultaneous resistance to piperacillin/tazobactam, cefoperazone, gentamicin, netilmicin with – susceptibility to ceftazidime, amikacin and fluoroquinolones. Between 1998 and 2000 a great increase in resistance of *P. aeruginosa* to carbapenems (IPM from 9 to 55%, MEM – 16–62%) was noticed in S. ESBL(+) strains were more often isolated from S than N (32 & 12%), among them there were mainly *Klebsiella* (56 & 47%), *E. coli* (27 & 31%), *Enterobacter* (16 & 14%). The increase of ESBL(+) isolates was noticed in S among *Klebsiella* (70–85%) and *E. coli* (14–40%), while in N there was a decrease – *Klebsiella* (55–45%), *E. coli* (20–5%), *Enterobacter* (31–22%). ESBL(+) strains from S were a little more resistant to amoxicillin/clavulanic acid, piperacillin/tazobactam and netilimicin, while strains from N to gentamicin and cotrimoxazole.
Conclusions: (1) Increasing antibiotic resistance of Gram-negative rods isolated from S & N may be explained by higher number of ESBL(+) strains in S. (2) Because of increasing trend in ESBL(+) frequency cephalosporins usage should be limited. (3) Cross-resistance of ESBL(+) strains to aminoglycosides excludes them from antibacterial therapy. (4) Appearing and spreading of carbapenem-resistant *P. aeruginosa* in S may be the result of carbapenems overusage in hospital. (5) Low level resistance to fluoroquinolones is probably connected with limited use of those drugs in the treatment of children.

P442 Uropathogens and their susceptibilities to antibiotics

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Objectives: To analyze the frequency structure of urinary tract infection (UTI) and antimicrobial resistance of the most common important urinary pathogen isolated from in-patients in urology and nephrology center.
Methods: All urine isolates from urine cultures were collected from in-patients in Urology and Nephrology Center in Mansoura University. The analysis was based on the microbiology laboratory data. Urine cultures were done in laboratory according to the standard techniques. The identification of isolates and antimicrobial susceptibility tests were performed using the MicroScan WalkAway40 dried MIC/ID panels for Gram negative/positive.
Results: A total of 235 consecutive isolates collected from urine cultures were analyzed. There were 89.4% Gram-negative pathogens among them and 9.4% were Gram-positive pathogens. The most common isolates was *Escherichia coli* represented 29.4% of the isolates. The isolates that followed were *Klebsiella* spp. 19.6%, *Pseudomonas aeruginosa* 11.9%, *Enterobacter* spp. 8.9%, *Serratia* spp. 8.5%, *Citrobacter* spp. 5.5% and coagulase negative staphylococci 4.7%. These seven pathogens accounted (98.8%) of the isolates.

The resistance for *E. coli* was as follows; trimethoprim/sulphamethoxazole 73.9%, *gentamicin* 52.2%, *norfloxacin* 49.3%, *cephalothin* 43.5% and tobramicin 42%.
Conclusions: *E. coli* was identified as the commonest pathogen of UTI. There is a high degree resistance of trimethoprim/sulphamethoxazole, gentamicin and norfloxacin that can be caused by complicated UTIs cases. Amoxicillin/clavulanate or nitrofurantoin can be used for empyric treatment of UTI

P443 Antimicrobial resistance patterns in a military medical academy, Turkey

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Objectives: Here in we report antibiotic susceptibility patterns of different isolates from various clinical samples, sent from different departments to our bacteriology laboratory through January to December in 2000.
Methods: The antibiotic susceptibility tests have been made by disc diffusion method, according to the NCCLS criteria.
Results: Totally 29 374 specimens were received and 6395 pathogens were isolated. The frequency of specimens were as follows: 42.2% genitourinary system, 29.9% respiratory system, 11.8% body fluids and wound, 9.7% blood, 4.6% gastrointestinal system, and 0.7% CSF. Overall, the most important bacteria isolated were *Staphylococcus aureus* (24%), *Escherichia coli* (22%), *Pseudomonas aeruginosa* (9%), *Klebsiella pneumoniae* (9%), *Acinetobacter* spp. (5%). Forty-eight percent of *S. aureus* isolates were resistant to oxacillin. All *S. aureus* isolates were fully susceptible to teicoplanin and vancomycin. *E. coli* was the most common isolate of all Gram-negative isolates (38%) from Department of Urology. For *E. coli*, the most active antimicrobial tested were imipenem and meropenem (99%) and the most resistant antimicrobials were ampicillin, trimethophrim-sulfamethoxazole, and mezlocillin with 60, 51, and 47%, respectively. For *K. pneumoniae*, the most active antimicrobial tested were imipenem and meropenem (100%) and the most resistant antimicrobials were ampicillin (79%). *P. aeruginosa* was the most frequently isolated pathogen from wound specimens. For *P. aeruginosa*, the most active antimicrobial tested were amikacin (32%) and the most resistant antimicrobials were ampicillin, trimetophrim-sulfamethoxazole, and mezlocillin with 60, 51, and 47%, respectively. The antimicrobial agents most resistant against isolates were ticarcillin/clavulanic acid mezlocillin, and netilmicin with 76, 75, and 61% of isolates being resistant, respectively. Imipenem- and meropenem-resistance rates were high (39.41%). For *Acinetobacter* spp. almost all antimicrobial resistance rates were higher than 50% and for this isolate imipenem and meropenem-resistance rates were 51.53%.
Conclusion: Here, there are still sufficient treatment options for patients infected with the most important bacterial species. However, the situation for patients with *Acinetobacter* spp. infections is still critical.

P444 *Bacteremia involving antibiotic-resistant Gram-negative bacilli in critically ill patients: clinical outcome and length of hospitalization in comparison with bacteraemia involving antibiotic-susceptible isolates*

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Objective: To evaluate the clinical impact of antibiotic-resistance in Gram-negative bacteremia in critically ill patients.
Methods: Retrospective, single-center cohort study (January 1992–December 2000). An outcome comparison was performed between ICU patients with bacteremia involving antibiotic-resistant (AB-R) ($n = 120$) and antibiotic-susceptible (AB-S) ($n = 208$) bacilli. Bacilli were defined AB-R by in vitro resistance to ceftazidim. *P. aeruginosa* isolates were considered AB-R when resistant to one of the following antibiotics: piperacillin, ciprofloxacin, ceftazidim, imipenem. In all patients only the first episode of Gram-negative bacteremia was taken into account.
Results: Between patients with AB-R and AB-S bacteremia no important differences were found in age (resp. 52 ± 16.9 vs. 54 ± 17.4 years; $P = 0.303$), APACHE II score (resp. 23 ± 8.6 vs. 23 ± 9.0; $P = 0.814$), APACHE II related expected mortality (44 ± 27.3 vs. $41 \pm 28.2\%$; $P = 0.305$), acute renal failure (resp. 27% vs. 25%; $P = 0.431$), hemodynamic instability (resp. 85% vs. 78%; $P = 0.117$) and acute respiratory failure (resp. 96% vs. 92%; $P = 0.227$). Patients with AB-R bacteremia were mechanically ventilated for a more extended period (29 ± 25.9 vs. 20 ± 18.6 days; $P < 0.001$). They also had a longer duration of stay in the hospital (88 ± 83.8 vs. 70 ± 71.1 days; $P = 0.007$). This seems to be the consequence of a longer duration of hospitalization prior to the onset of the bacteremia (30 ± 30.3 vs. 18 ± 27.5 days; $P < 0.001$) whereas duration of hospitalization after onset of the bacteremia did not differ (55 ± 60.3 vs. 50 ± 62.4 days; $P = 0.333$). Also, when length of ICU stay was compared, patients with AB-R bacteremia

were found to have a longer ICU stay prior to the onset of the bacteremia (24 ± 20.5 vs. 13 ± 14.0 days; $P < 0.001$) whereas legth of ICU-stay after onset of the bacteremia did not differ (17 ± 19.7 vs. 16 ± 17.9 days; $P = 0.321$). No difference was noted between patients with AB-R and AB-S bacteremia in 14-day mortality (resp. 27% vs. 26%; $P = 0.550$), 28-day mortality (resp. 36% vs. 34%; $P = 0.756$) and in hospital mortality (45% vs. 42%; $P = 0.576$).
Conclusion: Antibiotic-resistance in Gram-negative bacteremia did neitherot adversely affect the outcome in our ICU population, nor does it increase the duration of stay in the ICU and the hospital.

S. pneumoniae

P445 Macrolide-resistance in pneumococci in the North-east of England

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Objectives: To determine the mechanism of macrolide resistance amongst *Streptococcus pneumoniae* isolated in the North-east of England and to assess the activity of a range of antibiotics against these resistant strains.
Methods: There are two principle types of macrolide resistance seen in pneumococci, resistance of the M phenotype (low level resistance to 14-, 15-member macrolides) is mediated by an efflux pump encoded by the *Mef E* gene. The MLS phenotype results in high level macrolide resistance (and resistance to clindamycin and steptogramins) and is due to methylation of ribosomal RNA peptidyl transferase encoded by the *erm B* gene. MICs were performed on 61 isolates from the North-east of England using the NCCLS agar breakpoint method. Erythromycin and clindamycin were tested along with clarithromycin, azithromycin, penicillin, cefotaxime, josamycin, spiramycin, telithromycin, linezolid and moxifloxacin. All macrolide-resistant strains were further analyzed by PCR to determine genotype.
Results: Thirty-seven erythromycin-resistant strains were detected (MIC ranged from 4 to >128 mg/L). Nine of these (24%) were resistant to clindamycin (MLS phenotype), the remaining 28 were susceptible to clindamycin (M phenotype). All of the MLS isolates displayed high level resistance (MIC > 128 mg/L) to erythromycin. All showed reduced susceptibility to penicillin, four having MICs > 1 mg/L. Pneumococci of the M phenotype had MICs to erythromycin in the range of 4–32 mg/L, of note, only five were penicillin resistant, and a further two were of intermediate sensitivity. Resistance to telithromycin, linezolid and moxifloxacin was uncommon. Of the M phenotype, 27/28 isolates were positive by *Mef E* gene PCR; the *erm B* gene was detected in 7/9 isolates displaying the MLS phenotype.
Conclusions: In Europe, resistance in >90% of cases is of the MLS phenotype and is due to the *erm B* gene whereas in USA and Canada, 56–63% of isolates carry the *Mef E* gene. No data on mechanisms of resistance is available in the UK. In this study we found 76% of the macrolide-resistant pneumococci to be of the M phenotype owing to the presence of the *Mef E* gene. Isolates of the MLS phenotype were more frequently penicillin resistant.

P446 Genotypes of invasive pneumococcal isolates recently recovered from Italian patients

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Objectives: To assess serotype variability, antibiotic resistance, and genetic relatedness among recent clinical isolates of invasive *Streptococcus pneumoniae* recovered in different areas of Italy.
Methods: Pneumococcal isolates were subjected to antibiotic-resistance testing and serotyping. Isolates were genotyped using pulsed-field gel electrophoresis (PFGE), multilocus-sequence typing (MLST), and restriction fragment length polymorphism (RFLP) analysis of penicillin-binding protein (*PBP*) genes and *pspA*.
Results: Nine genetically related sets of isolates, defined by PFGE and/or MLST, were identified among the 22 penicillin non-susceptible isolates (PNSP), and five of these sets were represented by two to seven isolates. Twenty-six genetically related sets were represented among the 59 penicillin susceptible pneumococci (PSP), six of which were represented by two to five isolates. In general, restriction fragment length polymorphism profiles of *pbp2b*, *pbp2x*, and *pspA* were conserved within isolates shown to be related through PFGE and/or MLST. Three of the genetically related sets were represented by multiple serotypes (9 A + 9 V + 14 + 24F, 19 A + 15 A, and 18C + 4). In addition, one genetically indistinguishable set of 3 type 24F PNSP shared a multilocus sequence type, only seen previously in intermediately resistant type 14 isolates. Three new PNSP multilocus sequence types and three new alleles were deposited into the pneumococcal MLST database.
Conclusions: This work reports new penicillin non-susceptible strains, and evidence of capsular switching to serotypes not previously reported to arise through horizontal recombination events. The MLST database at http://www.mlst.net is a useful resource for tracking the epidemiology of pneumococcal strains worldwide.

P447 β-Lactam-macrolide resistance and multiresistance of *Streptococcus pneumoniae* in severe infections

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Objectives: Study of antibiotics resistance and sensibility of invasive *S. pneumoniae* isolated between 1991 and 2000 in the Clinic of Infections Diseases of Iasi in order to adaptive the first-line antibiotherapy.
Methods: The study included 136 patients with severe *Pneumococcus* infections: 72.7% meningitis, 6.6% septicemia's and 20.5% infections of the inferior respiratory tracts. *S. pneumoniae* isolated and identified through conventional methods, were tested by the diffusion method (NCCLS Standards) using disks of oxacillin (1 μ), ampicillin, amoxicillin/clavulanic acid, ceftriaxone, cefotaxime, imipenem, vancomycyn, erythromycin, rifampin, chloramphenicol, tetracycline and ofloxacin. A part of the strains were tested through the dilution method for the MIC of penicillin.
Results: *S. pneumoniae* was isolated from cerebrospinal fluid (65.4% cases), hemocultures (13.2%), sputum (19.8%) pleural and pericardic liquid (0.7% cases). Our study showed a medium resistance to penicillin of 22.8% with yearly variation. The resistance to rifampin, ampicillin, chloramphenicol and erythromycin was of: 4.4, 5.1, 7.8 and 8.0%, respectively. About 11.8% strains were multiresistant (more or equal to three antibiotics). Resistance was uncommon to cephalosporines, imipenem, vancomycin and 63.4% strains were susceptible to all anti-*Pneumococcus* antibiotics. There were 15 different antibiotypes evident.
Conclusions: The prevalence of 22.8% strains of invasive *S. pneumoniae* resistant to penicillin, 8.0% resistant to erythromycin and 11.3% multiresistant with 15 antibiotypes of resistance and with yearly and regional variations, determine the adaptability of antibiotherapy of severe pneumococcae infections to the actual reality.

P448 Antimicrobial susceptibility pattern and serotype distribution of nasopharyngeal *Streptococcus pneumoniae* isolates from children with respiratory tract infections

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Objective: Because there is little information on the epidemiology of infections due to *Streptococcus pneumoniae* in Turkish children, most cases of bacterial pneumonia and meningitis are treated empirically. Conjugated pneumococcal vaccine coverage has not been evaluated with large scale studies in Turkey. As the pharynx is the primary reservoir for potentially invasive pneumococci, we performed a pharyngeal culture surveillance in 322 children with respiratory tract infections.
Methods: The swab samples obtained from lower nasopharynx were immediately put into Stuart transport medium and transferred to laboratory within 2 h. Each swab sample was inoculated onto blood agar medium and incubated overnight at 35 °C under 5% CO_2. Pneumococci were initially identified on the basis of alpha hemolysis and colony morphology, identification was

confirmed by optochin susceptibility and bile solubility. Minimal inhibitory concentrations (MIC) to penicillin were determined by the E-test. Susceptibility to other antimicrobial drugs was determined by disk diffusion test. Serotyping was performed by the Quellung reaction, with the use of antisera from the Statens Serum Institute, Copenhagen.
Results: *S. pneumoniae* was isolated from 92(28.7%) of the patients. Overall, 25 (27.1%) were resistant to penicillin G, 24(26.1%) with intermediate resistance (MIC 0.12–1 mg/L) and 1(1%) with high level resistance (MIC 2 mg/L). Percentages of resistance to other tested antimicrobials were as follows: erythromycine (9.6), clindamycin (3.2), chloramphenicol (6.4), ceftriaxone (1), trimethoprim–sulfametho–xazole (30.9), rifampin (0). The first three mostly isolated serotypes were 19F, 6B, 3 and 7, 9 and 11-valent conjugate pneumococcal vaccines covered 34.8, 38.1 and 42.4%, respectively, of our *S. pneumoniae* isolates.
Conclusion: Although penicillin-resistance rate was relatively high compared to majority of Western Europe, high level resistance was 1% only. Conjugate pneumococcal vaccine coverage is lower in Turkey than it is in Europe and is consistent with most Asian countries.

P449 Nationwide surveillance of antimicrobial resistance in *Streptococcus pneumoniae* in Slovenia

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Objectives: To determine rates of resistance to selected antimicrobial agents and phenotypes of resistance to macrolides and lincosamides.
Methods: Eight microbiology laboratories from all regions of Slovenia isolated 666 non-replicated strains of *Streptococcus pneumoniae* between January and September 2001, mostly from respiratory tract. Strains were isolated from outpatients and patients from general, but not from tertiary care hospitals. *S. pneumoniae* was identified by colony morphology and positive optochine or bile solubility test. Oxacillin screening test and susceptibility to five non-β-lactam antimicrobials (vancomycin, tetracycline, trimethoprime-sulfamethoxasole-SXT, erythromycin and clindamycin) was determined by standard disk-diffusion procedure according to NCCLS. Intermediate and resistant category (non-β-lactams) was interpreted as resistant. This procedure was used for all 666 strains. MICs for penicillin and cefotaxime of strains with oxacillin inhibition zone less than 20 mm were determined by E-test (AB Biodisk, Solna). Penicillin or erythromycin-resistant strains were tested with moxifloxacin (disk-diffusion, NCCLS). Phenotype of resistance to macrolides and lincosamides was determined by double disk induction method with disks of erythromycin and clindamycin.
Results: All strains were susceptible to vancomycin. Resistance to penicillin: 16% of strains were intermediate (MIC 0.12–1 mg/L), 1% resistant (MIC $\geq$ 2 mg/L). Resistance to cefotaxime: 4% of strains were intermediate (MIC 1 mg/L), 0.1% resistant (MIC $\geq$ 2 mg/L). Resistance rate to SXT was 28%, to tetracycline 14%, to erythromycin 12% and to clindamycin 8%. Two phenotypes were found in 77 erythromycin-resistant isolates: 70% constitutive MLSB phenotype and 30% M-phenotype. One hundred strains were tested with moxifloxacin, all strains were susceptible.
Conclusions: Rates of resistance to antinicrobials tested in *S. pneumoniae* is a cause of concern. Surveillance will continue. Erythromycin-resistant strains were frozen and should be studied further. Detailed epidemiological analysis of collected data is also necessary.

P450 Macrolide-resistant *Streptococcus pneumoniae* in Poland – molecular analysis

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Objectives: To investigate molecular determinants of macrolide-resistance among pneumococci isolated in Poland and to study the clonal structure of this population.
Methods: Among 726 isolates of pneumococci collected in 1994–1999, 96 (13%) showed resistance to erythromycin. For these strains, minimal inhibitory concentrations (MIC) for penicillin, erythromycin, clindamycin and spiramycin were determined by broth microdilution method according to NCCLS. Serotypes of strains were determined at the Statens Serum Institute, Copenhagen, Denmark by the Quellung reaction. Pulsed-field gel electrophoresis (PFGE) of *SmaI* restriction fragments of bacterial chromosome was used to study the population structure of macrolide-resistant *Streptococcus pneumoniae* and the strains were considered related when they shared at least 80% of bands in their PFGE patterns. The presence of *ermB* and *mefA* resistance genes, encoding ribosomal methylase and efflux pump, respectively, was tested by PCR with specific primers. Sequencing of the L4 protein gene *rplD* was performed in the case of isolates lacking any of the two resistance genes.
Results: The most prevalent serotypes among macrolide-resistant pneumococci were 23F and 6B (55 and 17 isolates, respectively). PFGE analysis revealed the presence of 34 clonal groups among analyzed strains. Different mechanisms were identified to be responsible for the resistance phenotype: rRNA methylation (both constitutive and inducible) by the ermB gene product, mutations in the *rplD* gene and drug efflux associated with the presence of *mefA*. The most prevalent mechanism was associated with the constitutive expression of the *ermB* gene.
Conclusion: Dissemination of macrolide resistance among *S. pneumoniae* in Poland is a result of independent acquisition of different resistance genes (mainly *ermB*), which is followed by successful expansion of some clones harboring the resistance genes.

P451 Surveillance of pneumococcal resistance in Belgium during winter 2000–2001

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A total of 318 pneumococcal isolates, collected by 10 labs, were tested for their susceptibility to penicillin (PEN), ampicillin (AMP), amoxicillin (AMX), amoxicillin/clavulanate (AMC), cefaclor (CFC), cefuroxime (CRX), cefotaxime (CTX), imipenem (IMI), ciprofloxacin (CIP)*, gemifloxacin (GEM), levofloxacin (LEV), erythromycin (ERY), clarithromycin (CLA), Azithromycin (AZI), miocamycin (MIO), clindamycin (CLI) and tetracycline (TET) using NCCLS standardized microdilution. Insusceptibility rates (IR; following NCCLS) were as follows: PEN 22.0% [of which 11.3% high-level (HL)], AMP 24.2% (HL 14.1%), AMX 2.2% (HL 0%), AMC 1.9% (HL 0%), CFC 20.7% (HL 18.2%), CRX 17.9% (HL 16.3%), CRX-axetil 16.3% (HL 14.4%)**, CTX 8.5% (HL 4.4%), IMI 4.4% (HL 0%), CIP 11.3% (HL 2.8%), GEM 0%, LEV 2.5% (HL 0%), ERY 30.8% (HL 27.3%), CLA 28.3% (HL 27.0%), AZI 28.3% (HL 25.2%), MIO 33% (HL 21.1%), CLI 11% (HL 10.4%), TET 39% (HL 38.1%). Overall, GEM was the most active compound with MIC_{50}, MIC_{90} and IR of 0.015 mg/L, 0.03 mg/L and 0%, respectively, followed by AMC, AMX and IMI: 0.015 and 1 mg/L, 1.9%/0.015 and 1 mg/L, 2.2%/0.015 and 0.12 mg/L, 4.4%, respectively, In PEN-non-susceptible isolates, MICs of the β-lactams rose with those of PEN. However, IMI, CTX, AMX and AMC were generally 4, 2, 1 and 1-doubling dilutions, respectively, more active on these isolates than PEN. AMP was equally active, whereas CRX and CFC were generally 2- and 3-doubling dilutions respectively, less potent. Furthermore, cross-resistance between PEN and other β-lactams in the PEN-non-susceptible isolates was not absolute. Indeed, most of the PEN-non-susceptible isolates remained fully susceptible to IMI (97.0%), AMC (91.4%) and AMX (90.0%). Compared to former surveillance studies we have noted the following variations in IR: PEN: 12.5% (1995), 12.3% (1997), 16.2% (1999) and 22.0% (2001); AMX and AMC: 0% (1995 and 1997), 1.0% (1999), and 2.2 and 1.9%, respectively, (2001); CTX: 6.2% (1995), 7.6% (1997), 12.7% (1999) and 8.5% (2001); IMI: 3.4% (1995), 3.3% (1997), 0.5% (1999) and 4.4% (2001); CIP: not tested in 1995 and 1997, 15.6% (1999) and 11.3% (2001); ERY: 21.6% (1995), 31.1% (1997), 36.1% (1999) and 30.8% (2001); TET: 27.3% (1995), 31.1% (1997), 22.9 (1999) and 39.0% (2001).

*: Pneumococcal infections not indicated; and **: breakpoints based on 250 mg.

P452 Mathematical modeling of the progression of bacterial resistance to penicillin G by decreased affinity in penicillin binding proteins

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Objectives: *Streptococcus pneumoniae* and *Neisseria meningitidis* share a very similar penicillin G-resistance mechanism mediated by decreased affinity in penicillin binding proteins. However, epidemiological studies report that whereas resistance is now common in *S. pneumoniae*, it is still rare in

N. meningitidis. The objectives of this study were to investigate the factors that may account for this difference and to anticipate trends in the meningococcal resistance to penicillin.

Methods: A mathematical model is presented for the emergence and spread of bacterial resistance to penicillin in the community, through intra-individual-resistance selection and inter-individual transmission. It is assumed that antibiotic exposure is unrelated to bacterial carriage. The resistance levels of bacterial strains are described by the MIC. The model depends on several parameters, which were estimated from the literature: the duration of carriage (2.2 months for *S. pneumoniae* and 10 months for *N. meningitidis*); the frequency of antibiotic treatment in the concerned population, mostly composed of children for *S. pneumoniae* and of young adults for *N. meningitidis* (from once a year to once every 2 years); the contact rates, etc.

Results: Used with parameters reflecting the natural history of colonization of *S. pneumoniae*, the model forecasts the emergence of resistant bacteria in an all sensitive population (MIC < 0.06) after approximately 20 years. The predicted repartition of resistance levels then becomes bimodal with a majority of resistant strains a few years after emergence. In the case of *N. meningitidis*, the model also predicts an evolution towards a bimodal repartition of resistance levels, but much slower.

Conclusion: The predictions concerning the emergence of pneumococcal resistance are consistent with what has been observed. Moreover, yearly data from the French National Reference Center of Pneumococci exhibit an evolution towards a bimodal repartition of MIC in agreement with the predictions. The model accounts for the differing epidemiology of resistance of *S. pneumoniae* and *N. meningitidis*: when introduced as model parameters, the different characteristics of pneumococcal and meningococcal colonization in terms of transmissibility, of carriage duration or of antibiotic use produce widely different outcomes. Finally, the model predicts that even if antibiotic use remains constant, meningococcal resistance is bound to increase in the next decades.

P453 *S. pneumoniae*: antimicrobial resistance and serotypes of isolates in adult patients with bacteremic pneumonia

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Objectives: During 1989–1993 we detected an incidence rate of 9.3 bacteremic pneumococcal pneumonia per 100 000 persons/year in Oviedo (Austria, Spain). Resistance to antibiotics and serotyping have been monitored since 1989. We analyzed data over a 12-year period (1989–2001).

Methods: The strains and the patients were identified by computerized microbiological and clinical records. The serotyping was performed in a Reference Center. The oxacillin disk screening test, the broth microdilution method (NCCLS M100-S10) and the Chi-square test were used.

Results: We identify 254 isolates, in blood cultures of adult patients (196 males, 58 females). 132 (51.9%) patients were ≥65 years old. The most common serotypes (%) were: 3 (22.8), 14 (11.4), 9 (8.5), 8 (8.5), 4 (7.7) and 19 (7.3); 95.4 and 96.0% were related to the 23-valent vaccine for all patients and the older patients (≥65 years), respectively. Resistance rate (%) were: penicillin 27.1 (20.4 intermediate, 6.7 resistant), erythromycin, 12.0, clindamycin 11.2 (2 strains had the M phenotype), chloramphenicol 15.2, tetracycline, 28.0, cotrimoxazol 36.2, ciprofloxacin 1.9, vancomycin 0. Penicillin-non-susceptible strains were 85.5 and 52.1% susceptible to amoxicillin and cefotaxime, respectively. Relevant trends (%) between 1989 and 94 ($n = 149$) and 1995–2001 ($n = 105$) were: resistance to erythromycin, 6.8 versus 19.4 ($P = 0.003$), resistance to tetracycline, 31.0 versus 23.8 ($P = 0.209$), resistance to chloramphenicol, 21.3 versus 6.6 ($P = 0.004$). No significance was found in resistance to penicillin (26.1 vs. 28.5, $P = 0.672$). The global mortality was 19.7 and 23.2% for the older patients ($P = 0.170$). No significant increase in mortality was detected in the penicillin-non-susceptible group (22.4% vs. 18.6%, $P = 0.542$).

Conclusions: Relevant data for treatment and preventive strategies are documented. Resistance to penicillin remain stable in our geographic area. For macrolides, studies of sensitivity in vitro are imperative.

P454 Antibiotic resistance in Croatian *Streptococcus pneumoniae* isolates

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Objectives: Most respiratory tract infections are treated empirically and we wanted to investigate the resistance rate in *Streptococcus pneumoniae* so to be able to recommend adequate therapy for community acquired respiratory tract infections.

Methods: We analyzed 174 pneumococcal isolates collected in two Croatian hospitals: University Hospital for Infectious Diseases in Zagreb and General Hospital in Cakovec. Isolates were obtained from blood cultures (11), cerebrospinal fluid (6), bronchoalveolar lavage (1), tracheal aspirate (27), pleural aspirate (2), wound swab (1), eye swab (7) and nasopharyngeal swab (37). Sensitivity to penicillin, amoxicillin and erythromycin was determined by detecting minimal inhibitory concentrations (MICs) using microdilution method.

Results: Sensitivity to penicillin was 63%; 20% of isolates were moderately and 17% highly resistant to penicillin. Sensitivity to amoxicillin was 99% and to erythromycin 85%. The macrolide-resistant strains showed M type (5%) and MLS-B type (10%) of resistance. Macrolide-resistant isolates were more frequent among penicillin moderately resistant (38%) and highly resistant (20%) isolates than among penicillin sensitive (7%) isolates.

Conclusions: In comparison with previous results, we have noted an increase in penicillin resistance, especially on the account of the shift towards highly resistant isolates.

P455 Penicillin-resistant *Streptococcus pneumoniae* isolated from infected children in Athens, Greece: resistance patterns, serotyping and penicillin-binding protein 2B mutation characterization by PCR

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Streptococcus pneumoniae remains a major cause of community acquired infections. Over recent years, the increasing emergence of penicillin-resistant *S. pneumoniae* (PRSP) is causing serious clinical problems worldwide. We report the experience gained in the Penteli Children Hospital of Athens, with respect to PRSP isolated from infected children, in terms of their resistance patterns to other antibiotics, serotyping and characterization of penicillin-binding protein 2B genes (pbp 2b) mutation by PCR, throughout 1 year (September 1999 to October 2000). From a total of 207 *S. pneumoniae* strains isolated from nasopharynx, mastoid pus, middle ear, sputum, conjunctiva and blood, 27 (13%) were resistant to penicillin (PRSP). The MICs of penicillin were for all PRSP ÀÝ 0.25 Ìg/mL. Significant resistance rates were found to cephalosporins and ciprofloxacin. All strains were susceptible to vancomycin and rifampicin and sparfloxacin. The multidrug-resistance rates were 25 out of 27 PRSP (92.6%). The 27 PRSP belonged to six different serotypes: O:23 (9), O:19 (9), O:9 (4), O:15 (2), O:14 (2) and O:6 (1). There was no link between serotype and a certain resistance phenotype. All strains were pbp 2b class B mutants. Our results support the general concept, that special preventive and therapeutic strategies should be implemented for the management of pneumococcal infections. These should include vaccination programs, as well as therapeutic options offered by strategies concerning newer quinolone derivatives, as well as other antimicrobial agents like ketolides, oxazolidinones and streptogramins.

Vancomycin status	$DIOC_5(3)$*	Bisoxonol*	Rhodamine-123**
Susceptible	4.60 ± 1.55	1.60 ± 0.12	1.47 ± 1.17
Heterogeneous GISA	1.72 ± 0.67	1.07 ± 0.15	0.66 ± 0.20
Homogeneous GISA	1.10 ± 0.60	1.06 ± 0.26	0.77 ± 0.60

P456 Tracking *Streptococcus pneumoniae* isolates with decreased antimicrobial susceptibility by MIC distribution analysis: SENTRY, Europe 1997–2000

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On behalf of the SENTRY Participants Group

Objective: To study the evolution of antimicrobial resistance of *Streptococcus pneumoniae* isolates obtained in Europe during the SENTRY Antimicrobial Surveillance Program over a 4-year period (1997–2000).
Methods: A total of 1600 *S. pneumoniae* isolates causing respiratory tract infections were obtained in 16 (1997), 18 (1998), 8 (1999), 15 (2000) laboratories from 15 European countries. Susceptibility (NCCLS) testing was centralized (Eijkman–Winkler Institute and The Jones Group).
Results: MIC ranges (μg/mL) and resistance rates (intermediate + resistant; NCCLS breakpoints) were: yearly differences in β-lactams and erythromycin resistance were owing to the inclusion of sites with widely different resistance rates (i.e. penicillin <3–55%). As expected, erythromycin resistance was higher among penicillin resistant isolates (53% during 1999 and 2000) and linked (>75%) to Erm phenotype. Decreased quinolone susceptibility was scarcely observed (0.3 in 1998 and 0.5 in 1999). Only four isolates (two from Spain) displayed levofloxacin MICs of ≥2 μg/mL during 1999 and 2000. All these four isolates were inhibited by 0.12–0.5 μg/mL of BMS284756.
Conclusion: MIC distribution analysis was useful to detect isolates with decreased antimicrobial susceptibilities (Table 1).

Table 1 MIC distribution analysis (Abstract P456)

	1997 ($n=316$)		1998 ($n=535$)		1999 ($n=213$)		2000 ($n=536$)	
	Range	%R	Range	%R	Range	%R	Range	%R
Penicillin	≤0.03 to 4	26.2	≤0.03 to 4	26.9	≤0.03 to 4	41.4	≤0.03 to 4	34.0
Cefotaxime	≤0.008 to 8	6.6	≤0.008 to 2	8.5	≤0.008 to 4	20.6	–	–
Ceftriaxone	–	–	–	–	–	–	≤0.008 to 8	21.1
Cefepime	≤0.06 to >8	10.4	≤0.06 to 8	10.0	≤0.06 to 2	18.8	≤0.06 to 2	19.1
Erythromycin	≤0.25 to >32	18.3	≤0.25 to >32	23.7	≤0.25 to >32	19.7	≤0.25 to >32	29.9
Quinu./dalfo.	≤0.25 to 2	6.0	≤0.25 to 2	0.7	≤0.25 to 1	0	≤0.25 to 2	0.4
Chloramphenicol	≤2 to 16	8.5	≤2 to 16	8.9	≤2 to >16	12.7	≤2 to >16	11.7
Cotrimoxazole	≤0.5 to >4	24.6	≤0.5 to >4	27.1	≤0.5 to >4	35.7	≤0.5 to >4	39.4
Tetracycline	≤2 to >16	25.9	≤2 to >16	24.6	≤2 to >16	20.7	≤2 to >16	31.8
Levofloxacin	≤0.5 to 2	0	≤0.5 to >4	0.3	≤0.5 to >4	0.5	≤0.5 to 2	0
BMS284756*	–	–	–	–	≤0.03 to 0.5	0	≤0.03 to 1	0

*The levogloxacin criteria was used (I + R > 2 μg/mL).

P457 Different properties of *Streptococcus pneumoniae* isolates carrying macrolide efflux genes *mef(A)* or *mef(E)*

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Objectives: One of the main determinants of macrolide resistance in *Streptococcus pneumoniae* is the *mef* gene which encodes for a drug efflux pump. The characteristics of the *mef*-containing elements and the properties of strains carrying the two different *mef* genes, *mef(A)* and *mef(E)*, were studied in a large series of erythromycin-resistant isolates.
Methods: The *mef* gene was detected by PCR. *mef(A)* and *mef(E)* were distinguished by PCR–RFLP and sequencing. Molecular typing was obtained by pulsed-field gel electrophoresis (PFGE). Multilocus sequence typing (MLST) of a *mef(A)* isolate was performed. Transformation assays and mating experiments were performed.
Results: Out of 187 erythromycin-resistant strains, 40 (21%) showed an M phenotype and carried *mef*: 33 carried *mef(A)* and seven *mef(E)*. All isolates carrying *mef(A)* belonged to serotype 14 and were susceptible to the antibiotics tested, except erythromycin. 17 *mef(A)*-positive strains were examined by PFGE. All shared very similar profiles suggesting they belong to the same clone. The allelic profile of one isolate, obtained by MLST (ST 9) corresponds to that of clone England 14–9, a major antimicrobial-resistant clone. The *mef(E)* strains belonged to six different serotypes, the majority were resistant to other antibiotics besides erythromycin, including penicillin. Three *mef(E)* strains examined by PFGE did not appear to be clonally related. The sequences of a fragment of the element, including *mef* and *msr(A)* homologue, were, respectively, identical in three *mef(A)* strains and in three *mef(E)* strains, although there were differences at 168 positions between the two groups. In all the *mef(A)* isolates, the *mef* element was inserted in *celB*, that led to impairment of the competence for transformation of the strains. The *mef(E)* element was inserted at different chromosomal sites, therefore, the competence ability of the *mef(E)* strains was maintained. Transfer of erythromycin resistance by conjugation was obtained from two out of three *mef(A)* strains, whereas no transfer was seen from the three *mef(E)* strains studied.
Conclusions: In Italy, erythromycin resistance in *S. pneumoniae* is conferred by *mef* genes in 21% of the resistant isolates and the *mef(A)* gene is prevailing. This situation is different from that in North America, where *mef(E)* is the most common erythromycin-resistance determinant. Owing to the important different characteristics of the strains carrying *mef(A)* or *mef(E)*, we suggest to maintain the distinction between these two genes.

P458 In vitro activity of β-lactam antibiotics against respiratory tract pathogens isolated in France during 2000–2001

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Objectives: The objective of this study was to evaluate the in vitro activity of oral β-lactam antibiotics (BL) commonly used in France against recent respiratory tract pathogens.
Methods: A total of 1694 French clinical strains of adult respiratory tract infections were isolated from November 2000 to April 2001 in 30 French participant centers. MICs of penicillin (PEN), amoxicillin (AMX), amoxiclav (AMC), cefpodoxime (CPD) and cefuroxime (CXM) were determined by the agar dilution method in a central laboratory. Susceptibility rates were calculated according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie.
Results: *Streptococcus pneumoniae* (SP): 49% of the strains were susceptible (PSS), 28.3% intermediate (PIS) and 22.7% resistant (PRS) to PEN. MICs 50% (mg/L)/MICs 90% (mg/L) of BL were the following: Overall SP strains ($n=675$): CPD: 0.06/4; AMX: 0.06/2; CXM: 0.25/4; PSS ($n=331$): CPD: 0.03/0.06; AMX: 0.015/0.03; CXM: 0.03/0.12; PIS ($n=191$): CPD: 0.5/2; AMX: 0.5/1; CXM: 2/4; PRS ($n=153$): CPD: 2/4; AMX: 2/4; CXM: 4/16. 4% of the strains were of high level of resistance (MICs ≥ = 4 mg/L) to AMX. *Haemophilus influenzae* (HI): 33.2% of the strains were β-lactamase (bla) producers. MICs 50% (mg/L)/MICs 90% (mg/L)/percentages of susceptibility were the following: overall HI strains ($n=751$): CPD: 0.12/0.25/99.9; AMC: 0.5/2/98.8; CXM: 1/4/66; non-bla producers ($n=493$): CPD: 0.12/0.25/99.8; AMC: 0.5/2/98.4; CXM: 1/4/66.7; bla producers ($n=245$): CPD: 0.12/0.25/100; AMC: 1/2/99.6; CXM: 1/8/64.5. *Moraxella catarrhalis* (MC) ($n=268$): 92.1% of the strains were β-lactamase (bla) producers. MICs 50% (mg/L)/MICs 90% (mg/L)/percentages of susceptibility were the following: CPD: 0.5/1/99.3; AMC: 0.03/0.12/100; CXM: 0.5/1/91.4.
Conclusions: The results of this study show that a high rate of penicillin resistance is found among French SP; AMX and CPD have a better activity than CXM against SP, especially against PIS and PRS–CPD has the best activity against HI, followed by AMC ant CXM which has a lower percentage of susceptibility – AMC has lower MICs against MC than cephalosporins although most strains are susceptible to the three compounds. Overall, CPD and AMC appear to be the best candidates for the treatment of community acquired respiratory-tract infections.

P459 Antimicrobial susceptibilities and serotypes (STs) of 364 *Streptococcus pneumoniae* isolates in the Comunidad Valenciana (Spain)

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Objectives: The importance of providing vaccination programs has increased with the emergence of antibiotic-resistant strains of *S. pneumoniae*.

Surveillance for drug resistance and ST distribution and its variation along time in the different geographical areas is critical for evaluating the coverage of pneumococcal vaccines.
Methods: We evaluated 364 strains of *S. pneumoniae*, isolated from the in- and out-patients of two hospitals of the Comunidad Valenciana. STs were determined by Quellung reaction, with reagents provided by the Statum Serum Institute. MICs of penicillin (PV), ampicillin (AM), amoxycillin/clavulanic acid (XL), cefuroxime (XM), cefotaxime (CT), erythromycin (EM), clindamycin (CM), rifampicin (RI), vancomycin (VA) and levofloxacin (LE) were determined by E-test method, and the breakpoints applied were NCCLS 2000 (M7-A5).
Results: From our 364 strains, 70 (19.2%) were invasive (INV) (mainly blood, CSF and pleural) and 294 (80.8%) were non-invasive (NON-INV). The most frequent STs were: 19 (22.5%), 6 (14.0%), 3 (11.8%), 23 (11.8%), 14 (9.1%) and 9 (6.9%). The prevalence of highly resistant strains (INV/Non-INV) were: PV 8.6% (9.4%/8.5%), AM 0.9%(0%/1.2%), XL 0%, XM 3.4% (7.7%/2.3%), CT 1.9% (1.5%/1.9%), EM 47.7% (38.1%/50.0%), CM 40.0% (23.8%/43.6%), RI 0%, VA 0% and LE 0.3% (0%/0.4%). For PV 36.1 and 69.0% of non-susceptible (NS) strains were NS to XM and EM, respectively; and 0.8 and 21.5% of susceptible (S) strains were NS to XM and EM, respectively. There were 83 (27.3%) strains S to all the antibiotics studied. Their ST (no) were: three (14), six (10), 19 (10), 23 (nine), 11 (six), 9 (five), 4 (four), 22 (three), 33 (three), non-typable (NT) (three), 14 (two), 16 (two), 17 (two), 18 (two), 24 (two), and one each of the following STs: 5, 10, 15, 21, 34 and 41. We found 77 (25.3%) strains NS to only one antibiotic. The ST (no) were 19 (19), three (12), 6 (12), 23 (seven), 9 (three), 11 (three), 29 (three), NT (three), 4 (two), 14 (two), 18 (two) and one each of the following ST: 5, 7, 8, 10, 16, 20, 24, 33 and 35. There were six (2%) strains NS to five antibiotics, belonging to STs, 9, 14 and 19.
Conclusions: The levels of resistance to PV, EM and XM found are higher than other similar studies reported in our country. The heptavalent conjugated vaccine covers 73.3% of invasive strains from children <2 years and the 23-valent pneumococcal polysaccharide vaccine covers >90% in all the groups older than 2 years.

P460 Effectiveness of the 23-valent pneumococcal polysaccharide vaccine (PPV) in COPD-patients on steroids

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Objectives: The efficacy of the PPV is still unclear especially in patients with COPD. There are no valid data concerning time-dependent antibody production after vaccination and the effect of often concomitant steroid therapy. To evaluate the efficacy of the PPV the antibody production in patients with steroid therapy-dependent COPD was compared with that in healthy volunteers.
Methods: Patients with COPD on inhalative steroids ($n=29$, mean age 62 years) or on systemic steroids (receiving >10 mg prednisolone/d; $n=17$, mean age 63 years) and a control group of healthy volunteers ($n=23$, mean age 64 years) were vaccinated with PPV (0.5 mL Pneumopur®). Antibody titers against the serotypes 4, 6, 9, 12, 14, 18, 19 and 23 were measured by the CovaLink NH ELISA technique® before and 3 and 12 months after vaccination, respectively. An antibody concentration of >1.0 μg/mL was considered to be protective.
Results: We found a significant ($P<0.05$) rise of antibodies in all groups 3 months post vaccination. Twelve months after PPV the COPD-patients on systemic steroids had a lower mean antibody titers (not statistically significant) than the patients on inhalative steroids and control patients.
Conclusion: Until 12 months after the vaccination with the 23-valent pneumococcal polysaccharide vaccine the COPD-patients on systemic and inhalative steroids exhibit sufficient antibody concentrations.

P461 The carriage of *Streptococcus pneumoniae* in nasopharynx of infants and young children

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Objectives: To determine the rate and risk factors of nasopharyngeal carriage of *Streptococcus pneumoniae* and its susceptibility to antibiotics in children under 4 years of age in Warsaw.
Methods: Two hundred children were examined between November 2000 and May 2001 in selected settings in Warsaw (orphanage, 107; crèche, 56; and the family children, 37). The collection of demographic data, previous antibiotic therapy, respiratory tract infections and vaccination was performed on a special questionnaire. Nasopharynx swabs were taken and they were cultured on blood agar plates and incubated at 37 °C in 5% of CO_2 overnight. *S. pneumoniae* was identified by colony morphology, optochin susceptibility and bile solubility. The MICs of 13 antibiotics for *S. pneumoniae* were determined by broth microdilution method according to NCCLS guidelines.
Results: Altogether, 110 strains of *S. pneumoniae* were isolated; 63 strains from orphanage children, 36 from creche, and 11 from the family children, thus the carrier rate of *S. pneumoniae* was 58.9, 64.3 and 29.7%, respectively. Susceptibility testing revealed that 34 strains (30.9%) were intermediate (orphanage 24 strains, creche 10) and 7 strains (6.4%) were fully resistant to penicillin (orphanage six strains, creche one). No single penicillin non-susceptible strain (PNSP) was found among isolates from the family children. Susceptibility to other antibiotics in orphanage/creche was as follows: amoxicillin 90.5%/97.2%, cefuroxime 90.5%/97.2%, cefotaxime 90.5%/97.2%, cefepime 90.5%/97.2%, imipenem 92.1%/97.2%, erythromycin 46.0%/66.7%, clindamycin 46.0%/66.7%, cotrimoxazole 9.5%/13.9%, doxycycline 47.6%/50%, chloramphenicol 87.3%/55.6%, rifampin 96.8%/100.0%, vancomycin 100.0%/100.0%. Pneumococci isolated from the family children were fully susceptible to majority of antibiotics tested with the exception of cotrimoxazole, chloramphenicol and doxycycline; to which susceptibility was 63.6, 81.8, and 90.9%, respectively.
Conclusions: The general rate of carriage was high (55%) and significantly higher in closed communities. Among 41 (37.2%) PNSP 7 strains (6.4%) were highly resistant to penicillin and most of them were isolated from orphanage children (six strains). In the orphanage and creche, high resistance to erythromycin and clindamycin as well as to cotrimoxazole was observed. Clonal spread of resistant strains within a closed community could have taken place and its confirmation requires further molecular studies.

P462 Utility of urinary antigen detection in the diagnosis of pneumococcal pneumonia in children

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Objectives: (1) To establish the utility of the urinary antigen detection by a new immunochromatographic membrane test (ICT) (Binax Inc., Portland, Maine) in the pneumococcal pneumonia diagnosis in pediatric patients. (2) Assessment of the ICT usefulness in healthy nasopharyngeal carrier children.
Methods: Twelve urine samples from children with pneumococcal pneumonia diagnosed by hemoculture and pneumococcal polysaccharide capsular antigen detection by counter-immunoelectrophoresis in urine were studied. We also evaluated urine samples from 34 pediatric patients with *Mycoplasma pneumoniae* pneumonia. Urine samples were studied both non-concentrated and concentrated by selective ultrafiltration. We included urine samples from healthy children but with pneumococcal nasopharyngeal carriage. The ICT was performed according to the manufacturer's instructions.
Results: *S. pneumoniae* antigen was detected in 11 out of 12 non-concentrated urine from patients with pneumococcal pneumonia (91.7%), and in 11 of the 11 concentrated urines (100%). The ICT specificity was 58.8% using non-concentrated urine and 6.1% using concentrated urine. Pneumococcal antigen was also detected in six non-concentrated urine from the 11 nasopharyngeal carriers and in eight cases out of the 10 concentrated urines.
Conclusions: (1) The ICT assay is a simple, sensitive, but a very nonspecific test for the pneumococcal pneumonia in children. (2) The use of the concentrated urine increases the sensitivity but decreases significantly the specificity. (3) The *S. pneumoniae* urinary antigen could be detected in nasopharyngeal carriers.

P463 *Streptococcus pneumoniae* (Sp) in community-acquired pneumonia (CAP): multicentre study

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Streptococcus pneumoniae (Sp) is one of the pathogens which is mainly responsible for respiratory tract infections in both, children and adults. The level of

prevalence of Sp resistant to penicillin (Pe), is well-documented worldwide in the literature. The aim of the present study is to determine: (a) the incidence of Sp in CAP, in adults and children; (b) the usefulness of the Sp antigen-detection test as a tool in presumptive lower respiratory tract infections; and (c) the prevalence of Sp resistant to Pe. Between April 2000 and October 2001 (two Winter seasons), we studied 179 patients with CAP, documented by clinical sings, symptoms and X-ray. Out of the total 179, 157 were adults and 22 children, aged between 1- and 2-year-olds. The specimens analyzed were expectorated sputum in adults, and lower respiratory tract aspiration in kids. Both samples were cultured between the 3 h post sample collection. Quantitative culture was performed on chocolate and sheep blood agar plates incubated at 35 °C in 5–10% CO_2 atmosphere for 48–72 h. At the same time Gram- and Ziehl–Neelsen stains was done. In 170/179 patients blood cultures were taken. The presumptive identifications of alfa-hemolytic colonies was done by conventional biochemical methods (optoquin susceptibility and bile solubility tests). Sp. antigen detection was done in each patients using Binax (in 109 urine samples) and Murex (70 urine, 70 serum and 21 sputum samples) according with the manufacturer's recommendations. The results obtain were:

- Total of Sp isolated (respiratory samples plus blood) 37/179 = 20.7%.
- Ag-positive by binax: 44%;
- Ag-positive by murex: 41.6%;
- PPV for binax: 86.2%;
- NPV for binax: 82.3%;
- PPV for murex: 80%;
- NPV for murex: 58.3%.

Pe-sensibility results were:

- 81.1% ≤ 0.06 μg/mL (30/37);
- 13.5% 0.12–1.0 μg/mL (5/37);
- 5.4% ≥ 2.0 μg/mL (2/37).

Conclusions:

1. The incidence of Sp as etiologic agent inn CAP in Argentina is within the international levels.
2. In our experience Sp antigen detection, could be used only as a presumptive rapid method.
3. The majority of the strains isolated in this study were highly sensitive and the prevalence of resistance tp Pe obtained was mainly of intermediate level.
4. The spread of Pe-resistant strains is increasing in the community worldwide, these makes the need to be determine the epidemiological situation in each country.

P464 Telithromycin is highly active against isolates of *S. pneumoniae* collected from patients with community-acquired respiratory tract infections in Italy (CARTIs; PROTEKT 1999–2001)

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Objectives: PROTEKT is a global longitudinal surveillance study designed to track the spread of antibacterial resistance among bacterial pathogens from CARTIs. Data are now available for isolates collected in Italy during two consecutive winter seasons: 1999–2000 (2000) and 2000–2001 (2001; preliminary data).

Methods: Isolates were collected from two centers in 2000 and five centres in 2001. MICs of antibacterial were determined centrally by NCCLS broth microdilution methods and interpreted using available NCCLS breakpoints.

Results: Prevalence of resistance to penicillin G and erythromycin A among isolates of *S. pneumoniae* during 2000 ($n = 119$) was as follows: penicillin G intermediate (PenI, MIC 0.12–1 mg/L), 6 (5%); penicillin G resistant (PenR, MIC > or = 2 mg/L), 12 (10.1%); erythromycin A resistant (EryR, MIC > or = 1 mg/L), 51 (42.9%). Corresponding data for 2001 ($n = 69$) were: PenI, 2 (2.9%); PenR, 6 (8.7%); EryR, 20 (29%). Mode MIC, MIC_{90} and susceptibility data for a selection of the antibacterials tested against *S. pneumoniae* are presented in Table 1. Telithromycin was the most potent of the oral agents tested, having a mode MIC, MIC50 and MIC_{90} of 0.015, 0.015 and 0.25 mg/L, respectively, in both study years. Importantly, telithromycin retained potent activity against pneumococci resistant to other antibacterials. Telithromycin was also highly active against isolates of *H. influenzae* (2000: mode MIC, 1 mg/L; MIC_{90}, 2 mg/L; 2001: mode MIC, 2 mg/L; MIC_{90}, 2 mg/L) and *M. catarrhalis* (2000: mode MIC, 0.12 mg/L; MIC_{90}, 0.12 mg/L; 2001: mode MIC, 0.06 mg/L, MIC_{90}, 0.06 mg/L).

Table 1 In vitro activity of test antibacterials against *S. pneumoniae* (2000)

Antibacterial	Mode MIC (mg/L)	MIC_{50} (mg/L)	90 susceptibility
Telithomycin	0.15	0.35	–[a]
Penicillin G	0.03	2	84.9
Cefuroxime	0.06	4	88.9
Erythromycin A	0.06	>64	57.1
Arithromycin	0.12	>64	57.1
Clarithromycin	0.03	>32	57.1
Cotrimoxazole	0.23	4	47.1
Levofloxacin	1	1	100
Linezolid	1	2	100
	0.06	0.12	100
	0.3	1	99.2

Conclusions: PROTEKT 1999–2001 confirms the high prevalence of macrolide resistance amongst pneumococci in Italy and the potent activity of telithromycin against isolates of the major bacterial pathogens implicated in CARTIs, irrespective of resistance phenotype.

P465 Breakthrough pneumococcal bacteremia in patients being treated with clarithromycin or amoxicillin/cClavulanate

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Objective: To evaluate the correlation between failure of antibiotic treatment and resistance of *S. pneumoniae* isolated from blood cultures.

Patients and methods: The patient population was selected using three criteria: >18 years, pneumococcal bacteremia and hospitalization at the University Hospitals Leuven. The records were retrospectively reviewed by means of standardized protocol. The identification of *S. pneumoniae* and the susceptibility testing was performed using conventional methods.

Results: 136 pneumococcal bacteremia (39% women and 61% men) were diagnosed in the period 1998–2000 (age group 18–50: 21%; 51–70: 34%, 71–80: 21%; and >81: 24%). Seventy-eight per cent had a pneumonia. The others had upper-respiratory tract infections (6%), bronchitis (5%), abdominal infections (3%) or catheter-related infections (2%). There was one case of cellulitis and OPSI and for 5% the source of infection was unknown. The susceptibility for penicillin was intermediate for 11 isolates and two were resistant (MICpeni 1.5 and 3 mg/L). 21% was resistant to erythromycin. 31 patients died (23%).

Thirteen patients took antibiotics a few days before admission to the hospital where pneumococcal bacteremia was diagnosed. Pre-hospitalization antibiotic therapy was clarithromycin (4×), amoxicillin/clavulanate (5×), cefadroxil (2×), cefaclor (1×) and amoxicillin (1×). Four patients received clarithromycin (2 × 500 mg/day) for 3–14 days. *S. pneumoniae* from these patients were sensitive to penicillin but resistant to erythromycin. Four patients received amoxicillin/clavulanate (2 × 500 mg/day) for 3–18 days and one patient amoxicillin for 7 days. All *S. pneumoniae* from these patients were sensitive to penicillin.

Conclusions: The four patients with breakthrough bacteremia under clarithromycin treatment showed erythromycin-resistant *S. pneumoniae*. In Belgium, >90% of the erythromycin-resistant pneumococci have the *ermAM* gene and express the MLSB-phenotype resistance (MICs erythromycin >256 mg/L). The pre-hospitalization therapy with amoxicillin ± clavulanate could not avoid a breakthrough bacteremia in six patients, but there was no association with penicillin resistance.

Resistance mechanisms in *S. pneumoniae*

P466 A preliminary comparison of the Macrolide resistance (Macr) mechanism distribution in *Streptococcus pneumoniae* (SPN) isolated from PROTEKT 2000 and PROTEKT 2001

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Objectives: PROTEKT is a global, longitudinal study of the antimicrobial susceptibility of bacterial pathogens associated with community-acquired lower respiratory tract infections. The program is currently completing its second year of investigation (year 1 = 2000, year 2 = 2001). The objective of this study was to compare the Macr mechanism distribution for SPN tested so far from countries with sufficient isolates tested so far in the PROTEKT 2001 study with isolates from the same countries in PROTEKT 2001.
Methods: The presence of Macr genes [*erm(A)*, *erm(A)* subclass *erm(TR)*, *erm(B)*, *erm(C)* and *mef(A)*] was determined using a rapid-cycle multiplex PCR assay with microwell-format gene specific probe detection as previously described [1].
Results: Comparative data is shown in Table 1.

Table 1 Comparative data

			Genotype (% of Macr isolates)							
	Isolates tested (*n*)		*Mef* (A)		*Erm* (B)		*Mef* (A) & *erm* (B)		Negative[a]	
Country	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
France	106	43	2.8	0	97.2	100	0	0	0	0
Germany	50	40	50	47.5	40	52.5	0	0	10	0
Italy	53	57	41.5	36.8	58.5	63.2	0	0	0	0
Spain	38	128	5.3	5.5	94.7	93.7	0	0	0	0
Canada	57	46	52.6	69.8	35.1	28.3	3.5	2.2	8.8	4.3
USA	105	58	63.8	67.2	22.9	17.2	12.4	15.5	1	0
Japan	242	467	43	34.3	52.1	63.4	3.3	1.9	1.7	0.4

[a]Negative for all the mechanisms tested, resistance owing to 23S rRNA and/or riboprotein mutations.

Discussion: The distribution of Macr genes has remained relatively unchanged in France, Italy and Spain. A significant increase in the percentage of *erm(B)* occurred in Japan. Conversely, data from the USA and Canada suggests an increase in *mef(A)* distribution and the USA still shows a significant percentage of isolates with dual mechanisms. Interestingly, the number of isolates negative for the mechanisms tested has decreased.

Reference

1. Farrell DJ *et al. J. Antimicrob Chemother* 2001 48:541.

P467 Activities of 16-membered macrolides on Italian *Streptococcus pneumoniae* isolates with different levels of erythromycin susceptibility

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Macrolide resistance among pneumococci is a major cause of concern in Italy, always affecting erythromycin, the new macrolide compounds, namely clarithromycin and roxithromycin, as well as the azalide azithromycin. More often than not, the resistance affects also the 16-membered compounds and the lincosamides, too. A total of 375 *S. pneumoniae* isolated from different Italian geographic areas were tested for their susceptibility to the 16-membered macrolides, the 14-membered macrolides erythromycin, clarithromycin and roxithromycin, the azalide azithromycin and other reference antibiotics. The presence of either the erm(A) or the mef(A) gene was investigated by PCR on all erythromycin-resistant strains. A total of 38.7% of the isolates proved erythromycin-resistant. All the erythromycin-resistant strains were also resistant to the other 14-membered macrolides tested and to azithromycin. Of all the erythromycin-resistant isolates, 60% revealed a MLS B constitutive resistance (MLS B CR), 21.4% a MLS B inducible resistance (MLS B IR), and a 18.6% an M-type resistance. The MLS B CR isolates showed the widest MIC range of 16-membered macrolides (all $\leq$0.06 to >256 mg/mL), almost 90% of these strains proving highly resistant to spiramycin, josamycin and midecamycin. Indeed Rokitamycin showed high-level resistance only in the 50% of strains. MLS B IR isolates showed a greater susceptibility to 16-membered macrolides: spiramycin (MIC_{50} 8 mg/mL, Mic_{90} country 256 mg/mL, range: $\leq$0.06–>256 mg/mL) being the least effective, whilst josamycin and midecamicine (MIC_{50} = 1 mg/mL and 0.5 mg/mL, respectively, MIC_{90} = 8 mg/mL, range: $\leq$0.06–32 mg/mL) and rokytamycin (MIC_{50} = $\leq$ 0.06 mg/mL, MIC_{90} = 0.5 mg/mL, range: $\leq$0.06–4 mg/mL) were definitely more active. Virtually all the M isolates proved susceptible to 16-membered macrolides. Thus, different phenotypes of erythromycin resistance show a different pattern of resistance to 16-membered compound macrolides, which in turn proved endowed with different levels of activity even on isolates sharing the same phenotype. Intrinsic potency and inducing capability might account for such differences.

P468 Multicentre evaluation and genotypic analysis of macrolide and ketolide activities on Italian *Streptococcus pneumoniae* isolates

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Macrolide resistance among pneumococci is a major cause of concern in Italy, where high rates of pneumococci that are highly resistant to macrolides have been reported, even in the absence of the high rates of penicillin resistance that are usually found at the same time. The activities of the novel ketolide telithromycin and of other reference antibiotics were assayed on 427 *Streptococcus pneumoniae* isolated from 12 different Italian centers over the period 1999–2001. The genotypes of all erythromycin-resistant strains were investigated by PCR. A total of 38% of the isolates proved erythromycin-resistant, whereas 17% were not susceptible to penicillin (5.3% resistant and 11.7% intermediate). All the erythromycin-resistant strains were also resistant to azithromycin, clarithromycin and roxithromycin. Genotype analysis revealed the presence of the erm(A) gene in 90.2% and of the *mef(A)* gene in 9.8% of the erythromycin-resistant isolates, respectively. According to the breakpoints, 98.4 of the isolates were susceptible to telithromycin, with MICs ranging from 0.002 to >32 µg/mL (MIC_{50} = 0.012 µg/mL, MIC_{90} = 0.064 µg/mL. Telithromycin was uniformly active on strains with either the Mef or the MLSB phenotype, including the constitutively resistant MLSB isolates whose erythromycin MICs exceeded 256 µg/mL. Telithromycin proved very active on all Italian *S. pneumoniae* isolates examined in the present study. No cross-resistance with erythromycin or other macrolides was detected; thus telithromycin deserves further attention as a potential therapy for *S. pneumoniae* infections (Tables 1 to 3).

Table 1 MIC_a (µg/mL) of *S. pneumoniae* (427 isolates)

Antibiotics	MIC_{50}	MIC_{90}	Range
Erythromycin	0.064	>256	<0.016–>256
Telithromycin	0.012	0.064	<0.002–>32
Azithromycin	0.19	>256	<0.016–>256
Clarithromycin	0.047	>256	<0.016–>256
Roxythromycin	0.125	>256	<0.016–>256
Clindamycin	0.064	>256	<0.016–>256
Penicillin G	0.023	0.5	<0.002–>12
Levofloxacin	–	–	–

Table 2 Susceptibilities (%) of *S. pneumoniae* (427 isolates)

Antibiotics	Susceptible	Intermediate	Resistant
Erythromycin	62	0	38
Telithromycin	98.4	1.4	0.2
Azithromycin	55	7	38
Clarithromycin	61.4	0.4	38.2
Clindamycin	69.6	0.2	30.2
Penicillin G	83	11.7	5.3
Levofloxacin	–	–	–

Table 3 Genotype distribution (%) of erythromycin-resistant *S. pneumoniaes*

	mef(A)	*erm(A)* [*erm(TR)*]	*erm*(B) [*erm(AM)*]
MLS_B (CR)	–		
MLS_B (IR)	–		
M			

P469 Macrolide resistance mechanisms among *Streptococcus pneumniae* nasopharyngeal strains isolated from children in Athens, Greece: a preliminary report

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Objectives: In Greece, macrolide-resistant pneumococci have been isolated at a rate of 25% in 2000. Knowledge of the prevalence and type of macrolide resistance mechanism may have important therapeutic implications as it may guide empirical antimicrobial treatment for common community acquired infections.
Methods: A total of 409 *S. pneumoniae* isolates were obtained during a surveillance study involving 1000 children (age 1–5 years) attending day-care centers of Athens metropolitan area from February to April 2000. These strains were characterized by in vitro susceptibility testing that was carried out by disk diffusion and *E*-test. The strains found to be erythromycin resistant, according to the NCCLS guidelines have been phenotypically and genotypically characterized. Fifty-one out of 101 erythromycin-resistant isolates have already been studied for the resistance phenotype by disk diffusion using 2 μg clindamycin, 15 μg erythromycin and 7.5 μg quinopristin disks (Johnston et al. 1998, AAC, 42: 2425–26) and for the presence of macrolide resistance genes (*ermAM* and *mefE*) by PCR (Sutcliffe et al. 1996, AAC, 40: 2562–6).
Results: Twenty-four isolates (47%) were assigned to the constitutive macrolide, lincosamide and streptogramin B resistance (MLSB) phenotype and all carried the *ermAM* gene. Twenty-seven isolates (53%) were resistant to erythromycin but susceptible to clindamycin (M-phenotype) and all possessed the *mefE* gene.
Conclusions: Although the study is still ongoing *mefE* and *ermAM* seem to be equally distributed in our community. Recommendations for the empiric treatment of pneumococcal infections based on these data cannot be made.

P470 Pneumococcal strains containing an *erm(B)* methylase can show heterogeneous resistance to ketolide antibiotics

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Ketolide antibiotics are a new macrolide-related class of compounds which have activity against strains inducibly resistant to erythromycin. In particular, these compounds have activity in vitro against isolates of *S. pneumoniae* containing the *erm(B)* methylase. Further study shows that some *S. pneumoniae* strains contain significant subpopulations which are able to form colonies on agar plates with amounts of telithromycin that are at least 10-fold higher than the MIC to this drug. Seventy clinical isolates of *erm(B)* pneumococci were tested for resistance to telithromycin and 29 gave this heterogeneous resistance response. Additionally, a smaller subpopulation of highly resistant clones from these strains could be selected on higher concentrations of telithromycin, which had an elevated MIC to ketolides. These isolates retained their resistance to ketolides even after repeated passage in the absence of drug. Strains which are heterogeneous to telithromycin give a comparable response to other ketolide antibiotics. Sequencing of the *ermB* gene and its immediate upstream region does not show any sequence that is consistently correlated with heterogeneity. However, heterogeneity is clearly linked to the *erm(B)* gene, because 21 of 21 *erm(B)* + transformants using donor DNA from five heterogeneous strains were heterogeneous. The transformants using donor DNA from nonheterogeneous (homogeneous) strains all were homogeneous in their response to telithromycin. Further DNA sequencing is in progress. Northern blot analysis showed that heterogeneous *erm(B)* strains produce more mRNA than homogeneous strains do, even without induction. In addition, they produce multiple *erm(B)*-hybridizing transcripts, implying that the heterogeneity determinant is located upstream of and that alters the transcription of the *erm(B)* locus. In vivo, heterogeneity has been shown to be important in murine infection models. Drug treatment enhances the recovery of resistant strains from both lung and blood. Furthermore, combining heterogeneous *erm(B)* genes with a mutation in ribosomal protein L4 leads to very high MICs to ketolides. This combination has recently been identified in a clinical isolate. Use of ketolides in the clinic may lead to rapid emergence of resistance.

Resistance mechanisms in *Streptococcus Pyogenes*

P471 A novel efflux mechanism in *Streptococcus Pyogenes* strains inducibly resistant to erythromycin

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Objectives: *Streptococcus pyogenes* strains inducibly resistant to MLS antibiotics can be subdivided into three phenotypes: iMLS-A, iMLS-B, and iMLS-C. Although iMLS-A isolates carry the methylase gene *ermAM*, both iMLS-B (higher-level resistant) and iMLS-C (lower-level resistant) isolates carry the methylase gene *ermTR*. The *mefA* gene, associated with the efflux-mediated erythromycin resistance typical of the M phenotype, is also detectable by PCR in most iMLS-B and iMLS-C isolates.
Methods: Erythromycin efflux was established by determining the intracellular accumulation of 14C-erythromycin in the absence and presence of an energy uncoupler (CCCP). Erythromycin-resistance genes were detected by PCR using specific primer pairs. The expression of *mefA* was determined by RT-PCR.
Results: Unlike control isolates of the M phenotype, none of the eight inducibly resistant strains tested with identical genotype (*ermTR mefA*), but different phenotype (four iMLS-B and four iMLS-C) expressed the *mefA* gene. Other macrolide efflux genes described in Gram-positive cocci (*mreA* and *msrA*) were not detected in the eight isolates. However, erythromycin efflux was recorded in all iMLS-B, but in no iMLS-C isolates. In conjugation experiments using an iMLS-B (*ermTR mefA*) isolate as a donor and a *RifR FusR* mutant of an iMLS-C (*ermTR*) isolate as a recipient, *mefA*-negative *ermTR*-positive transconjugants were obtained which had the novel efflux pump and showed the iMLS-B phenotype.
Conclusions: An efflux system other than that mediated by *MefA* contributes to MLS resistance in iMLS-B, but not iMLS-C, isolates of

erythromycin-resistant *S. pyogenes*. The lower-level resistance of the iMLS-C phenotype is apparently owing to *ermTR*-mediated methylase activity, whereas the higher-level resistance of the iMLS-B phenotype appears to depend on the same methylase activity plus the new efflux pump.

P472 Close association of *erm(B)* with *tet(M)* and of *erm(A)* and *mef(A)* with *tet(O)* in erythromycin- and tetracycline-resistant isolates of *Streptococcus pyogenes*

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Objectives: In *Streptococcus pyogenes*, erythromycin resistance is owing to either methylase *erm(A)*, *erm(B)*, or efflux *mef(A)* genes. Tetracycline resistance is found in *c.* 70% of the *erm(B)*-positive isolates with constitutive erythromycin resistance (those with inducible erythromycin resistance are usually susceptible to tetracycline); in >90% of the *erm(A)*-positive isolates; and in *c.* 70% of the *mef(A)*-positive isolates with efflux-mediated erythromycin resistance (M phenotype). The *tet(M)* is so far the most common tetracycline resistance gene found in this species.
Methods: Sixty-one, recent and independent Italian clinical strains of *S. pyogenes* resistant to both erythromycin (MIC >1 μg/mL) and tetracycline (MIC, >8 μg/mL) were tested. Resistance genes were detected by PCR using specific primer pairs.
Results: All *erm(B)*-positive isolates ($n = 16$) had the *tet(M)* gene. All *erm(A)*-positive isolates ($n = 27$) had the *tet(O)* gene [two were also positive for *tet(M)*]. All isolates carrying the *mef(A)* gene as the only erythromycin resistance gene ($n = 18$) had the *tet(O)* determinant.
Conclusions: We found the association between *tet(M)* and *erm(B)* in this species. However, also *tet(O)* was found to be extensively involved in tetracycline resistance among erythromycin-resistant isolates of *S. pyogenes*. The *tet(O)* gene was associated (i) with *mef(A)* in the tetracycline-resistant isolates with efflux-mediated erythromycin resistance; and (ii) with *erm(A)* in the tetracycline-resistant isolates carrying this gene, which mediates an inducible type of erythromycin resistance. This is the first time that the *tet(O)* gene has been found in association with any other antibiotic resistance genes.

P473 Telithromycin activity is reduced by efflux in *Streptococcus pyogenes*

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Telithromycin, a ketolide antibiotic, is active on many *Streptococcus* strains that are resistant to erythromycin by virtue of *erm* genes, encoding an 23S rRNA methylase. Differences in binding of the two antibiotics to the H35 helix of the domain II probably do not account for their different activities (Schlünzen et al., Nature 413 : 814, October 2001), which may be also related to different inducing activities. It has been also claimed that efflux mechanisms (namely *mefA* in *S. pyogenes*, producing an M phenotype) were not active on telithromycin. In an Italian collection of 269 *S. pyogenes* strains, genetically characterized by PCR, the range MIC for telithromycin in erythromycin susceptible strains was 0.004–0.06 μg/mL, and 0.19–0.5 μg/mL for strains endowed with the M phenotype and expressing the *mefA* gene. Those results were fully confirmed in a Spanish series of 152 genetically characterized erythromycin-susceptible (*mefA* minus) *S. pyogenes* (telithromycin MIC range 0.004–0.12; mode 0.03 μg/mL) and 40 strains containing the *mefA* gene (MIC range 0.06–4; mode 1 μg/mL). In summary, the MICs of strains with the *mefA* efflux gene were on average 15 times higher than those of fully susceptible strains. The effect of the *mefA* pump was then investigated by using radiolabeled telithromycin in strains with different resistance genotypes. A clear telithromycin efflux could be demonstrated in the strains expressing the *mefA* gene, but not in those expressing an *erm* gene nor in the susceptible strains (in which no *mef* or *erm* gene could be found). The efflux reversibility by addition of an inhibiting compound, namely sodium arsenate, could be also demonstrated.

P474 Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains isolated in Poland

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Objectives: The antibiotic resistance patterns and the distribution of erythromycin resistance genes of *Streptococcus pyogenes* with different resistance phenotypes were investigated
Methods: Fourteen local microbiology laboratories in Poland collected 69 isolates of erythromycin-resistant *S. pyogenes* from 1997 to October 2001. The isolates were recovered from throat swabs (43%), pus samples (53%) and blood (4%). Identification was performed by routine laboratory techniques including latex agglutination test. MICs were determined by the standard microdilution procedure and interpreted according to NCCLS criteria. The following antimicrobial drugs were tested: penicillin G, erythromycin, clarithromycin, roxithromycin, azithromycin, spiramycin, clindamycin, quinopristin/dalfopristin, telithromycin, linezolid, moxifloxacin and tetracycline. The erythromycin and clindamycin double-disc test was performed in order to classify the erythromycin resistance phenotype. The presence of the erythromycin resistance genes *ermB*, *ermTR* and *mefA* was detected by PCR.
Results: According to the double-disc test, 52 isolates (75%) exhibited the inducible and 14 (20%) the constitutive MLSB phenotype. The M-phenotype was presented by only three (5%) isolates. The PCR amplification showed that all the isolates with inducible MLSB and M phenotypes harbored the *ermTR* and *mefA* genes, respectively. Of the 14 constitutive phenotype isolates, eight were positive with primers specific for *ermTR* gene and the remaining isolates were positive with primers specific for the *ermB* gene. All the strains were susceptible to penicillin ($MIC_{90} < 0.003$ mg/L), linezolid ($MIC_{90} = 1$ mg/L), quinopristin/dalfopristin ($MIC_{90} = 1$ mg/L), moxifloxacin ($MIC_{90} = 0.12$ mg/L) and telithromycin ($MIC_{90} = 0.06$ mg/L). All constitutive MLS phenotype isolates were resistant to spiramycin but 44 (85%) of the inducible phenotype isolates were susceptible to 16-membered macrolide. Tetracycline resistance was observed in 67 (97.1%) strains.
Conclusions: All *S. pyogenes* were fully susceptible to penicillin with very low MICs. The great majority of resistant strains (85%) had the MLSB phenotype conferred by the presence of *ermTR* gene. The novel drugs such linezolid, quinopristin/dalfopristin, moxifloxacin and telithromycin retained good activity against erythromycin-resistant strains.

P475 In vitro susceptibility of β-hemolytic streptococci to penicillin, erythromycin, clindamycin and tetracycline: a 2-year study

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We studied the susceptibility of β-hemolytic streptococci isolated in our hospital from January 2000 to November 2001. Of 583 total β-hemolytic streptococci, 87.4% were group A; 11.2% group B and 4.1% were groups C and G. Most of group A streptococci were isolated from throat (94.9%) and most group B (69.4%) were from urine. Similar to group A, most of groups C and G (85.2%) were from throat cultures.
Methods: Susceptibility to penicillin was determined by E-test technique; and to other antibiotics by NCCLS disc diffusion method.
Results: Penicillin MICs against different serogroups of β-hemolytic streptococci demonstrated that all serogroups are still susceptible to penicillin. MIC_{50} and MIC_{90} of groups A, B and C–G were 0.008–0.012 μg/mL, 0.047–0.064 μg/mL and 0.012–0.016 μg/mL, respectively. The highest MIC_{90} (0.064 μg/mL) was observed for group B streptococci. In group A streptococci, 13.6, 2, and 0.8% of the strains were intermediately susceptible or resistant to tetracycline, erythromycin and clindamycin correspondingly. In group B, these rates were 80, 9.2, and 9.2%. Erythromycin resistance (27%) was relatively higher in group G, whereas none of the group C was resistant to this antibiotic.
Conclusion: This study exhibits that penicillin should still be the first choice of treatment against β-hemolytic streptococci. However, detection of one group A isolate with borderline penicillin MIC (0.125 μg/mL) and relatively higher MIC against group B streptococci indicate that routine laboratories must continuously perform susceptibility testing in order

to identify possible changes in penicillin susceptibility of β-hemolytic streptococci.

P476 Telithromycin activity is reduced by efflux in *Streptococcus pyogenes*

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Telithromycin, a ketolide antibiotic, is active on many Streptococcus strains that are resistant to erythromycin by virtue of *erm* genes, encoding a 23S rRNA methylase. Differences in binding of the two antibiotics to the H35 helix of the domain II probably do not entirely account for their different activities, which may be also related to different inducing activities. It has been also claimed that efflux mechanisms (namely *mefA* in *S. pyogenes*, producing an M phenotype) were not active on telithromycin. In an Italian collection of 269 *S. pyogenes* strains, genetically characterized by PCR, the range MIC for telithromycin in erythromycin susceptible strains was 0.004–0.06 μg/mL, and 0.19–0.5 μg/mL for strains endowed with the M phenotype and expressing the *mefA* gene. Those results were fully confirmed in a Spanish series of 152 genetically characterized erythromycin-susceptible (*mefA* minus) *S. pyogenes* (telithromycin MIC range 0.004–0.12; mode 0.03 μg/mL) and 40 strains containing the *mefA* gene (MIC range 0.06–4; mode 1 μg/mL). In summary, the MICs of strains with the *mefA* efflux gene were on average 15 times higher than those of fully susceptible strains. The effect of the *mefA* pump was then investigated by using radiolabeled telithromycin in strains with different resistance genotypes. A clear telithromycin efflux could be demonstrated in the strains expressing the *mefA* gene, but not in those expressing an *erm* gene nor in the susceptible strains (in which no mef or *erm* gene could be found). The efflux reversibility by addition of an inhibiting compound, namely sodium arsenate, could be also demonstrated.

P477 A 2-year multicenter evaluation of telithromycin activity on Italian *Streptococcus pyogenes* isolates with different genotypes of erythromycin resistance

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The activities of the novel ketolide telithromycin, of the 14-membered macrolides erythromycin, clindamycin and roxithromycin, of the azalide azithromycin and of other reference antibiotics were assayed on 495 *S. pyogenes* isolated in 12 different Italian centers over the period 1999–2001. The presence of either the *erm(A)* or the *mef(A)* gene was investigated by PCR on all erythromycin-resistant strains.

A total of 32.1% of the isolates proved resistant to erythromycin as well as to the other 14-membered macrolides tested and to azithromycin. The genotypic analysis revealed the presence of an *erm* gene in 68.7%, and of the *mef(A)* gene in 31.3% of the erythromycin-resistant isolates, respectively. The *erm* and the *mef(A)* gene did not coexist in any strain of *S. pyogenes*. According to the breakpoints, 94.1% of the isolates were susceptible to telithromycin ($MIC_{50} = 0.012$ μg/mL; $MIC_{90} = 0.38$ μg/mL). Telithromycin was evenly active on strains with either the M or the MLSB phenotype, included constitutively resistant MLS B isolates whose erythromycin MICs exceeded 256 μg/mL.

Telithromycin proved very active on most *S. pyogenes* isolates examined in the present study, including most strains resistant to erythromycin or other macrolides. It may represent a suitable alternative to the first-choice penicillin treatment of *S. pyogenes* infections when an alternative therapy is needed.

P478 Evaluation of 16-membered macrolides on Italian *Streptococcus pyogenes* isolates with different levels of sensitivity to erythromycin

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Half a decade after the dramatic increase of erythromycin-resistant *Streptococcus pyogenes* in Italy, the resistance levels observed in this country are still among the highest ever measured world-wide. Unlike the experience in other countries, in Italy the resistance is often not confined to the 14- and 15-membered macrolides only, but affects the 16-membered compounds and the lincosamides, too. Thus, the resistance would not appear to depend on the active efflux of antibiotic from the bacterial cell (the so-called 'M' phenotype) in the majority of cases.

A total of 495 cases from different Italian geographic areas were tested for their susceptibility to the 16-membered macrolides, the 14-membered macrolides erythromycin, clarithromycin and roxithromycin, the azalide azithromycin and other reference antibiotics. The presence of either the *erm(A)* or the *mef(A)* gene was investigated by PCR on all erythromycin-resistant strains.

All the erythromycin-resistant strains (namely 33.1% of the total isolates) were also resistant to all other macrolides tested. 25% of the erythromycin-resistant isolates revealed a MLSB constitutive resistance (MLSB CR), 43.7% a MLSB-inducible resistance (MLSB IR) and a 31.3% an M (efflux-related) resistance.

Resistance to 16-membered macrolides was high among virtually all the MLSB CR isolates, whilst the MLSB IR isolates showed different pattern of susceptibility. On the latter isolates, spiramycin and josamycin showed the lowest activity (MIC_{50} and $MIC_{90} \geq 256$ mg/mL, range: ≤0.06 to >256 mg/mL); midecamycin ($MIC_{50} = 8$ mg/mL, $MIC_{90} \geq 256$ mg/mL, range: ≤0.06 to >256 mg/mL) and rokitamycin ($MIC_{50} = 0.5$ mg/mL, $MIC_{90} \geq 256$ mg/mL, range: ≤0.06 to >256 mg/mL) were more effective. Virtually, all the M isolates proved susceptibility to all six-membered macrolides.

Thus, different 16-membered macrolides are endowed with different levels of activity against erythromycin-resistant *S. pyogenes* isolates. Such differences clearly relates with the different phenotypes of erythromycin resistance, too.

P479 Short course treatment: 5 days clarithromycin versus 5 days cefuroxime axetil and amoxicillin/clavulanate in patients with streptococcal tonsillopharyngitis

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Objectives: In order to investigate efficacy and safety of short course therapy in streptococcal tonsillopharyngitis a large clinical trial has been conducted to investigate 5-day treatment with various antibiotics in comparison to 10-day penicillin V. In the following evaluation results of the different short course treatment options were compared.

Methods: In this prospective, randomized, multicenter study in children aged 1–18 years with acute tonsillopharyngitis and positive culture for group A β-hemolytic streptococci (GABHS) efficacy and safety of clarithromycin (CLA: $n = 511$, 15 mg/kg/day, bid), cefuroxime axetil (CAE: $n = 498$, 20 mg/kg/day, bid) and amoxicillin/clavulanate (AC: $n = 515$, 37.5 mg/kg/day, tid) were compared. All patients were treated for 5 days.

Results: A total of 1524 patients were evaluated for intent-to-treat analysis. Microbiological testing demonstrated susceptibility of 100% for CAE and AC, whereas susceptibility for CLA was only 88.9%. Microbiological eradication 2–4 days after end of therapy was significantly higher ($P < 0.001$) for both CAE (89.9%) and AC (92.9%) in comparison with CLA (82.6%). Clinical efficacy 2–4 days after end of therapy was 97.2% (CAE), 96.9% (AC) and 94.1% (CLA); 7–9 days after end of treatment 89.9% (CAE), 92.9% (AC) and 82.6% (CLA), *P*-value 0.0077 and <0.001, respectively. The clinical and bacteriological response was significantly better in children with CLA-sensitive strains than in strains with CLA-resistant streptococci.

Conclusion: Regarding patients' compliance, analysis demonstrated that after 5-days treatment, compliance decreased dramatically. CAE and AC offer reliable options of successful short course therapy for tonsillopharyngitis of 5 days. Owing to the inferior efficacy of CLA it cannot be recommended as a first-line-treatment of GABHS tonsillopharyngitis.

MRSA

P480 Investigation of methicillin-resistant *Staphylococcus aureus* hand and nasal carriage among patients' helpers and visitors

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Hand and nasal carriage of *Staphylococcus aureus* is an important risk factor for infection by this organism in both community and hospital settings. Methicillin-resistant *S. aureus* (MRSA), is a major nosocomial pathogen. Regarding the recent reports, strains of MRSA, which had been largely confined to hospitals and long-term care facilities, are also emerging in the community. Several studies of *S. aureus* carriage have been performed among different groups of health care workers or among healthy individuals. However, other close relatives of the patients may act as a reservoir in *S. aureus* transmission to others. In this study, we investigated the hand and nasal carriage of MRSA among patients' accomplices and visitors to determine the role in the epidemiology of infection. The study group is apparently healthy, nonhospital workers who have temporarily been in the hospital somehow in relation with patients at risk. A total of 280 hand and nose cultures of 140 individuals were performed. Methicillin resistance of *S. aureus* strains were determined with disk diffusion assay according to the National Committee for Clinical Laboratory Standards Guidelines *S. aureus* ATCC25923 (29213) strain was used for control. *S. aureus* was found in 30 (21.4%) of nose and 19 (13.6%) of hand samples. Therefore, nasal carriage was found in 30 (21.4%) of nose and hand carriage was found in 19 (19/149; 13.6%) of these 140 subjects. Eight (16.3%) of the total (30 + 19) 49 strains were found resistant to methicillin in disk diffusion method. Three of the eight MRSA strains isolated from 140 individuals were obtained from nose and five of them were obtained from hands. Therefore, MRSA nasal-carriage rate was determined as 2.1% (3/140) and hand carriage was defined 3.6% (5/140). These results may be epidemically significant in spreading of MRSA both among hospitalized patients and in community.

P481 Prevalence of methicillin-resistant *Staphylococcus aureus* in different regions of Russia: results of multicentre study

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens. The spread of methicillin-resistant staphylococci has become an alarming problem throughout the world, but no data concerning the prevalence of MRSA in Russia have been published yet. The aim of this study was to determine the frequency and distribution of MRSA in different regions of Russia.
Methods: A total of 879 clinical strains of *S. aureus* isolated in 2000–2001 from patients hospitalized in 17 hospitals in different parts of Russia – four in Central region (Moscow, Ryazan, Smolensk), two in North–West region (St. Petersburg), three in South region (Krasnodar, Stavropol), two in Volga region (N. Novgorod, Kasan), three in Ural region (Ekaterinburg, Ufa), three in Siberian region (Krasnoyarsk, Novosibirsk, Tomsk), were included in the study. Antimicrobial susceptibility testing was performed by agar dilution method. The susceptibility testing and interpretation of the results were performed according to the NCCLS guidelines. *S. aureus* ATCC29213 strain was used for quality control.
Results: Among 879 *S. aureus* strains 295 (33.6%) have been found to be methicillin resistant. The prevalence of MRSA varied significantly between different hospitals – from 0 to 89.5%. No association between geographic location of hospital and rates of resistance to methicillin was found. The highest rates of resistance to methicillin (36.6–89.5%) were found in hospitals where patients with infected burns and orthopedic infections were predominant. No or much lower resistance rates to methicillin (0–27.3%) were found in the general service hospitals and pediatric hospitals. The highest percentage of MRSA was observed in burn, orthopedic and intensive care units of all hospitals included in this survey.
Conclusions: The prevalence of MRSA in hospitalized patients in Russia was found to be more dependent on the profile of clinical ward than on the geographical location of a hospital.

P482 Epidemiology of MRSA-resistance phenotypes in a Greek tertiary care hospital

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Objectives: To investigate the distribution of resistance phenotypes of methicillin-resistant *S. aureus* (MRSA) in our hospital during the period 1994–2001.
Materials and methods: A total of 1212 nonreplicate MRSA strains out of a total of 3178 *S. aureus*, isolated from 1994 to 2001 in 'Laikon' General Hospital, a tertiary care hospital, in Athens, Greece, were included in the study. Identification was based on standard methodology. Susceptibility to gentamicin (G), erythromycin (E), vancomycin (V), cotrimoxazole (S), ciprofloxacin (C), rifampicin (R), fusidic acid (F), clindamycin (L) and chloramphenicol (H) was performed with the Kirby–Bauer disk diffusion method according to NCCLS. A total of 127 MRSA strains, isolated during the last 2 years were investigated by the agar-screening test (4 μg/mL) for Vancomycin Intermediate *Staphylococcus aureus* (VISA). Resistant phenotypes were analyzed with the WHONET software.
Results: The incidence of MRSA increased from 33% in 1994, to 50% in 2001. MRSA strains, susceptible to all antibiotics tested, decreased from 40% in 1994 to 13.5% in 2001. Two multiresistant phenotypes were the most common in 2001, representing 48.3% of MRSA isolates. Phenotype GE_SCRFL_ (resistant to all antibiotics, except vancomycin and chloramphenicol) had a rate of 40.7%, and phenotype GE_SCRFLH (resistant to all antibiotics, except vancomycin) had a rate of 7.6%. These two phenotypes were found increased in comparison to 1994 (rates of 1 and 0%, respectively). Phenotype GE_SCRFL_ was mainly detected in ICU and surgical department (38% of the phenotype's isolates), whilst phenotype GE_SCRFLH was detected mainly in two of the three medical departments (55% of the phenotype's isolates). No differences were detected with respect to the distribution of susceptible MRSA isolates, with 11, 11.6 and 13.2%, among ICU, medical and surgical wards, respectively. No VISA strains were detected. Only one isolate was confirmed by E-test to have MIC 3 μg/mL.
Conclusions: (1) No VISA strain was isolated in our hospital. (2) A progressive increase in the MRSA isolation rate was documented. (3) Two main multiresistant phenotypes emerged and predominated in the hospital. (4) Differences were detected with respect to the distribution of these two phenotypes among different departments. (5) These phenotypic results suggest a possible clonal dissemination of multiresistant strains among wards of the hospital, which needs further evaluation with genotypic techniques.

P483 Investigation of slime factor (production) and methicillin resistance in coagulase-negative staphylococci isolated from patients' helpers and visitors

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Increased incidence of coagulase-negative staphylococcal infections due to the progress of invasive medical methods become a major clinical problem since they have an ecological niche on human skin from which they are difficult to eradicate, and they have also adapted themselves to survive on inert devices. Slime production is considered useful as a marker for clinically significant infections with coagulase-negative staphylococci (CNS). The two major clinical importances of CNS are, either the resistance against many antibiotics used for therapy and particularly transfer of antibiotic resistance to the other more pathogen bacteria via plasmids (conjugation). Methicillin resistance in staphylococci are resistant to many other antibiotics and have being recognized increasingly among immunocompromised patients. In this study, we investigated methicillin resistance and slime production in CNS isolated from patients' accomplices and visitors considering their close relationship between patients at risk. Slime production of CNS strains was investigated using Congo-red agar method. Methicillin-resistance of CNS isolates was determined with disk diffusion assay recording to the National Committee for Clinical Laboratory Standards guidelines. A total of 137 strains were isolated from 280 hand and nose cultures of 140 individuals. Slime factor was found positive in about 66 (48.1%) of the isolates. Forty-three (31.4%) of the strains

showed resistance to methicillin. Of these 38 (88.3%) produced slime. The amount of slime positive strains (38/43) were more than slime negative ones (5/43). Slime-positive CNS isolates were found more resistant to methicillin than slime negative-ones. The incidence-of resistance to oxacillin and slime positivity detected in the strains from patients' accomplices and visitors was found epidemiologically remarkable.

P484 Characterization of MRSA isolates involved in outbreaks by conventional and molecular typing

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Objectives: Characterization of MRSA isolates involved in outbreaks by different epidemiological typing and to determine the contribution of the typing methods based on PCR.
Methods: We studied the four MRSA outbreaks originated at the Hospital Universitario de Canarias from 1997 to 2000. We studied 26 MRSA isolates from nasal carriage and clinical samples of 15 patients and 7 MRSA isolates from health-care workers. Also we studied four MRSA isolates from three patients and one staff involved in an outbreak at the Hospital Universitario Nuestra Señora de La Candelaria placed also in Tenerife. The typing methods used were antibiogram, macrorestriction (*Sma*I) and pulsed-field gel electrophoresis (CHEF II), and protein A gene and coagulase gene polymorphisms.
Results: The molecular typing methods used anable us to identify the epidemic strain in all the outbreaks. While PGFE is time consuming (7 days) and laborious, the techniques based on PCR are rapid (24–48 h) and simple to carry out. The antibiogram anable us to identify the epidemic strain except for one patient. However, the molecular markers results and the epidemiological data, for this patient, related it with the outbreak. The two hospitals shared the same epidemic strain.
Conclusions: The molecular markers based in PCR were a good method in detecting outbreak-related strains and are useful in the preliminary studies of outbreaks because of their easy and rapid accomplishment.

P485 Epidemiologic typing of methicillin-resistant *Staphylococcus aureus* (MRSA) by pulsed-field gel electrophoresis (PFGE) and the association with the intra- or extra-hospital origin of the isolates

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Objectives: Epidemiologic typing of MRSA isolates by pulsed-field gel electrophoresis (PFGE) and the association of the restriction patterns obtained with the intra- or extra-hospitalary origin of the isolates.
Methods: We studied 128 MRSA isolates obtained from the Hospital Universitario de Canarias between 1997 and 2000, corresponding to both intra- ($n = 95$) and extra-hospitalary ($n = 33$) infections and colonizations. We also studied eight MRSA isolates from health-care worker that we consider intra-hospitalary acquired. Criteria for infections were as defined by Center for Disease Control and Prevention (CDC) guidelines. Isolates not associated with infection were classified as colonizing. MRSA was defined as nosocomial if the isolate was obtained after 48 h of admission. The methicillin resistance was confirmed by an oxacillin disc (5 μm) and the amplification by PCR of the *mecA* gene. Macrorestriction with *Sma*I and PFGE (Chef II, Bio-Rad) was performed. For statistical analysis we used χ^2 and Fisher's correction.
Results: We detected three pulsotypes: pulsotype A (12 subtypes), B (2 subtypes) and C, according to Tenover's criteria and the fingerprinting analysis of the PFGE profiles. The 89% of the isolates were pulsotype A, the 10% pulsotype B and the 0.8% pulsotype C. The pulsotype A (81.6%) was statistically more frequent among intra-hospitalarily acquired isolates and was the pulsotype that caused outbreaks while B (84.6%) and C (100%) pulsotypes dominated the extra-hospitalary isolates ($P < 0.001$).
Conclusions: In our experience, the PFGE is a good molecular typing to ascertain the intra- or extra-hospitalary origin of the isolates.

P486 Economic evaluation of linezolid for the treatment of infections caused by MRSA in Spain

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Linezolid is a new antibiotic agent, active against Gram-positives including MRSA with intravenous and oral presentations. It has demonstrated a similar clinical efficacy compared to that of Vancomycin for the treatment of different infections produced by MRSA.
Objective: To carry out an economic evaluation of using linezolid versus vancomycin in the treatment of MRSA infections.
Methods: A cost-minimization analysis was performed by building a decision analytic model. Health resources utilization, after the use of both antibiotics was obtained from published articles and only direct medical costs were included in the analysis. Cost acquisition of both products was obtained from official sources while the cost of each day of hospitalization (8 days for linezolid and 12 days for vancomycin), analytical tests and administration of both drugs were taken from a national database of health-care costs (Soikos).The perspective selected for this analysis was hospital assistance and the time horizon chosen was for 12 days, the maximum time that dischargeable patients are hospitalized in clinical trials.
Results: In the table, all the resources consumed after the administration of linezolid or vancomycin and the total cost/patient are shown (in Euros).

Costs	Linezolid	Vancomycin
Drug acquisition	1.524 E	422 E
Days of hospitalization	2.308 E	3.462 E
Administration i.v.	16 E	78 E
Plasmatic levels	0 E	30 E
Analytical tests	6 E	12 E
Total cost/patient	3.854 E	4.004 E

Conclusions: The use of linezolid instead of vancomycin in the treatment of infections caused by MRSA will save resources for the Spanish National Health Service producing similar clinical results with lower associated costs. In addition, linezolid will increase the quality of life of patients as it will permit them finalize their treatment at home.

P487 Agar dilution MIC and time kill assays on *Staphylococcus aureus* focusing on nonmultiresistant MRSA strains

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Objectives: To assess the antibacterial activity of various antibacterial agents with reference to their MICs and time-kill assays on *Staphylococcus aureus*, particularly, non-multiresistant MRSA strains.
Methods: Agar dilution MICs (NCCLS) were performed on 27 isolates of methicillin-susceptible *S. aureus*, 34 isolates of non-multiresistant MRSA (susceptible to 0–2 non-β-lactams) and 24 isolates of multiresistant MRSA. The following antibiotics were tested: chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gatifloxacin, gentamicin, linezolid, oritavancin, mupirocin, oxacillin at 35 °C, oxacillin at 30 °C, quinupristin/dalfopristin, rifampicin, teicoplanin and vancomycin. Time kill assays were performed with a nonmultiresistant Western Samoan Phage Pattern-2 and ATCC25923 (fully sensitive *S. aureus*) strains. Ciprofloxacin, galifloxacin, clindamycin, rifampicin, fusidic acid, trimethoprim and vancomycin were tested alone and in various combinations.
Results: Ciprofloxacin and gatifloxacin were the most bactericidal compounds tested; rifampicin and vancomycin were only bactericidal at 24 h. Resistance emerged readily *in vitro* to ciprofloxacin, rifampicin, fusidic acid and trimethoprim. No combination tested exhibited synergy. The combinations of rifampicin/fusidic acid, rifampicin/ciprofloxacin, rifampicin/

clindamycin and ciprofloxacin/trimethoprim exhibited indifference. The combinations of ciprofloxacin/clindamycin, ciprofloxacin/fusidic acid, ciprofloxacin/trimethoprim and rifampicin/trimethoprim were antagonistic.
Conclusion: Selection of antibiotics to treat nonmultiresistant MRSA remains problematic.

P488 Utility of the automated VITEK system in detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in a general hospital

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Staphylococcus aureus (SA) is one of the most common pathogens in community and hospital acquire infections. The prompt investigation of the methicillin resistance is a challenge for microbiologists. Detection of *mecA* gene is the gold standard method but it is difficult and time consuming. To find a reliable and quick method, easy to perform daily in a health care center, we studied the utility of the automated VITEK system (bioMérieux), for detection of MRSA in clinical isolates in comparison to disk diffusion and oxacillin agar screening.
Methods: A total of 748 SA strains of hospitalized patients, isolated from skin and soft tissue, respiratory samples, body fluids, bone and blood., obtained from 01 January 1997 to 31 October 2001 were tested. We compared disk diffusion (following NCCLS recommendations) results with those obtained with GPS-SA card to 31 December 2000 and GPS-102 from 01 January 2001(bioMérieux). The strains were confirmed with the oxacillin agar screening.
Results: We found 49% of SAMR and 51% of susceptible strains. Only one strain was susceptible to oxacillin for VITEK and resistant by disk diffusion, the agar screening result was positive. Ten strains were resistant with VITEK but susceptible with disk diffusion and the agar screening negative. Seven strains were susceptible with VITEK and resistant with disk diffusion, with the agar screening negative and finally only two strains were found resistant with VITEK but susceptible with disk diffusion and the screening result was positive.
Conclusions: According to our results, the automated VITEK system is a very useful, quick and reliable tool for routine labor for detection of MRSA. We suggest to follow-up of the Expert System and the confirmation of suspicious strains by oxacillin agar screening.

P489 Patterns of susceptibility of methicillin-resistant *Staphylococcus aureus* in Beirut, Lebanon

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Objective: In many countries, oxacillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) has become a significant nosocomial pathogen. Changes in susceptibility of MRSA were recorded during last years where more susceptibility is observed to gentamycin, erythromycin and clindamysin. The aim of this study is to analyze the patterns of susceptibility of the clinical isolates of methicillin-resistant *Staphylococcus aureus* to other antimicrobial agents in two general hospitals in Beirut.
Material and methods: This study was conducted from 01 January 1998 till 01 January 2001 (3 years). All reported antibiotic susceptibility data were derived from the routine clinical laboratory database at two general hospitals located in the area of Beirut. The antibiotic susceptibility testing was determined using Kirby–Bauer technique or flooding technique. A total number of 316 strains os MRSA were isolated in hospital 1 and 260 strains in hospital 2.
Results: The results are shown in Tables 1–3.

Table 1 Percentages of susceptibility of MRSA at the studied hospitals

	MR	Gen	Eryt	Clin	Ofl	Pef	Van	Tei
Hospital 1	14.5%	41.9	39.4	57.6	45.5	45.5	1001	100
Hospital 2	14.2	54.0	51.4	62.2	–	–	100	100

Gen: gentamycin; Ery: erythromycin; Clin: clindamycin; Ofl: ofloxacin; Pef: pefloxacin; Van: vacomycin; Tei: teicoplenin.

Table 2 Different resistance phenotypes represented as number of strains (corresponding percentage)

	Eryth R/clinda R	Eryth S/Clind S	Eryth R/Clind S
Hospital 1 ($N=46$)	19 (41.3%)	23 (50.0%)	4 (8.7%)
Hospital 2 ($N=37$)	14 (37.8%)	19 (51.4%)	4 (10.8%)

Table 3 Evolution of the percentages of susceptibility to gentamycin between 1995 and 2000

	1995	2001
Hospital 1	31.6%	41.9%
Hospital 2	–	54.0%

Discussion: The emergence of new epidemic MRSA strains more susceptible to antibiotics has been recently reported by several authors. A marked decrease in use of gentamicin was suspected to be a factor contributing to the emergence of gentamycin-susceptible MRSA. In our results, we can note an increase in the susceptibility to gentamicin between 1995 and 2000, as well as high prevalence of erythromycin–clindamycin-susceptible strains. These results show similar patterns in both hospitals. This is most probably due to the fact that the population is the same. All MRSA were susceptible to glycopeptides and showed a relatively low susceptibility to fluoroquinolones (45%).

P490 Resistance of methicillin- and ciprofloxacin-resistant *Staphylococcus aureus* to the newer quinolones levofloxacin, moxifloxacin and to linezolid, quinupristin/dalfopristin, vancomycin and teicoplanin

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Objectives: *S. aureus* resistant to methicillin (MRSA) are not only resistant to all the β-lactam antibiotics, but are also often resistant to other classes of antibiotics, limiting therapeutical possibilities in case of necessary treatment. In order to determine the efficacy of vancomycin, teicoplanin and the newer antibiotics levofloxacin, moxifloxacin, linezolid and quinupristin/dalfopristin, each agent was tested to 112 strains of clinical MRSA.
Methods: A total of 112 non-repeat clinical strains of MRSA were tested to the glycopeptide antibiotics vancomycin and teicoplanin, as well as to the newer quinolones levofloxacin and moxifloxacin, further to linezolid, an oxazolidinon and to quinupristin/dalfopristin, an antibiotic of the macrolid/streptogramin-group. The minimal inhibitory concentrations (MICs) of the drugs were determined according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).
Results: MICs for the glycopeptide antibiotics vancomycin and teicoplanin ranged from 0.5 to 4 mg/L and from 0.75 to 8 mg/L, respectively. Linezolid and quinupristin/dalfopristin exhibited MIC levels ranging from 0.125 to 2 mg/L. The MICs for the newer quinolones exhibited from 2.0 mg/L to $\geq$32 mg/L for levofloxacin and from 0.5 to 8 mg/L for moxifloxacin.
Conclusions: Both glycopeptides, vancomycin and teicoplanin remain uniformly susceptible to the tested strains. Linezolid and quinupristin/dalfopristin also show excellent activity to the tested MRSA strains, and may be alternatives in therapy or may be used in combination. All MRSA strains included in this study have been tested resistant to ciprofloxacin with MICs $\geq$ 16 mg/L. Even if the results for the newer quinolones show better in vitro results compared to ciprofloxacin, treatment of such strains with levofloxacin or moxifloxacin should be considered with care.

P491 Dramatic increase in methicillin-resistant *Staphylococcus aureus* (MRSA) due to a predominant clone in county jails between 1996 and 2001

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Objective: To describe the prevalence and distribution of MRSA genotypes among clinical isolates from San Francisco (SF) County jail inmates.

Methods: A total of 244 clinical isolates of *S. aureus* cultured from inmates in one hospital jail ward and five county jails were evaluated between January 1996 and October 2001. Isolates were characterized using antimicrobial susceptibility patterns and MRSA were analyzed using pulsed-field gel electrophoresis (PFGE) following *Sma*I digestion. Clinical information from the inmates hospitalized at San Francisco General Hospital (SFGH) was obtained from a computerized clinical record database.
Results: Of the 233 unique individuals, 70% were male, 23% were female, and 7% were unidentified. One hundred and one (41%) of all 244 isolates were resistant to methicillin. MRSA rates increased from 12% in 1996 to 68% through October 2001 (Fig. 1). Antimicrobial resistance among MRSA isolates from the jails versus the hospital jail ward were: gentamicin (3%/6%), ciprofloxacin (10%/11%), clindamycin (7%/11%), tetracycline (10%/17%), trimethoprim–sulfamethoxazole (4%/11%), and erythromycin (55%/50%). Over 80% of MRSA isolates originated from wound or soft tissue cultures. Among the 17 hospitalized inmates, 14 (82%) had prior admissions. Admitting diagnoses were primarily soft tissue infections (65%), followed by pneumonia (15%) and osteomyelitis (5%). MRSA isolates from 74 individuals were available for genotyping. Of the eight clonotypes identified, two clones (1 and 8) were present in all jails and the jail ward since 1996. Clone 8 initially prevalent at <15% in 1996–1997 accounted for >80% in 2000–2001 (Fig. 2). Only one of the five clones found in the inpatient jail ward was not seen in the county jails.

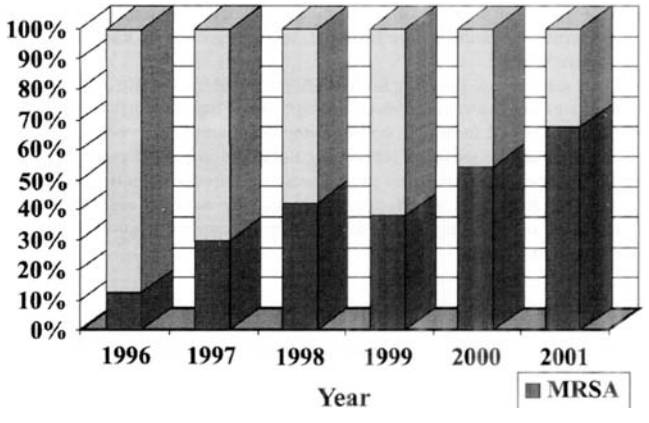

Figure 1 Percentage of MRSA over time.

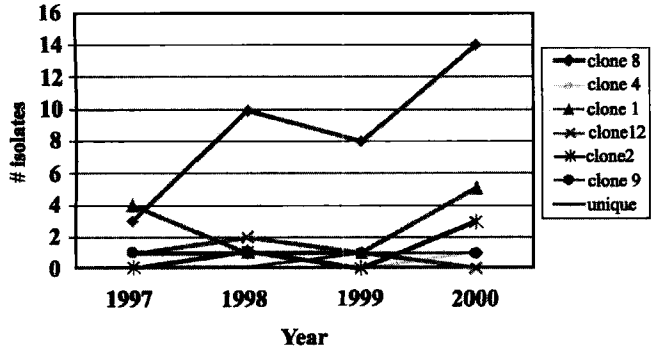

Figure 2 Clone distribution by year.

Conclusion: We found a high MRSA prevalence beyond nosocomial rates among clinical *S. aureus* isolates from county jails. The increase in MRSA prevalence between 1996 and 2001 was associated with the emergence of a predominant clone. Susceptibility patterns were consistent with community-acquired (CA) MRSA. This report identified a potentially aggressive CA-MRSA clone with enhanced survival in the community. Furthermore, jails may represent a significant reservoir for widespread transmission of CA-MRSA.

P492 Less than 1-h detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swabs by real-time PCR using the Smart Cycler®

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most significant pathogens, being responsible for significant patient morbidity, mortality and healthcare costs. Current control strategies to limit the emergence and rapid spread of this organism rely on the rapid and sensitive detection of MRSA carriers, so that appropriate barrier precaution may be used to reduce transmission. The objective of this study was to develop a real-time fluorescence-based assay which allows the detection of MRSA directly from nasal swabs in less than 1 h.
Methods: Primers specific to the right extremities of the staphylococcal cassette chromosome mec (SCCmec) sequences identified in the major epidemic clones of MRSA were used in a multiplex PCR assay in combination with a primer specific to the *orfX* gene sequence of the *S. aureus* chromosome located to the right extremity of the SCCmec integration site. A molecular beacon probe targeting the amplified region of *orfX* was integrated into the multiplex assay for real-time detection of MRSA using the Smart Cycler®. Nasal samples obtained from volunteers were spiked with MRSA cultures. Spiked samples were prepared by using a rapid DNA extraction protocol. A ready-to-use assay (AMPLIMRSA) has been designed (Infectio Diagnostic) for the routine detection of MRSA.
Results: There was a 96.7% correlation between the PCR results and the identification of MRSA. Two of 205 (1%) MRSA strains tested were not detected by PCR while 13 of 252 (5.2%) MSSA strains were misidentified as MRSA. The PCR assay allowed detection of less than 10 copies of MRSA per PCR reaction based on testing with spiked nasal samples. None of the 10 methicillin-resistant or 14 methicillin-sensitive coagulase-negative staphylococcal species tested were detected using the real-time PCR assay.
Conclusions: This multiplex PCR assay represents a rapid and powerful method for the specific detection of MRSA directly from nasal swabs.

P493 Determination of nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospital staff and outpatients

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Introduction: The aim of this study was to determine the carriage rates of nasal MRSA in hospital staff and outpatients.
Material and methods: The study included 500 hospital staff and 500 outpatients. Nasal samples cultured onto 5% sheep blood agar. Gram-staining, catalyze and coagulation activities were evaluated in samples showing typical colonial morphology. These colonies were also cultured onto DNAase and Mannitol salt agar. The protein profiles of the isolated strains were identified on polyacrylamide gel electrophoresis (SDS-PAGE).
Results: Nasal-carriage rate of MRSA was 6.6% (33/500) among hospital staff, while the same rate was 2.6% (13/500) among outpatients. The difference was statistically significant ($P < 0.001$). MRSA carrier staff were mainly working in the surgical wards where hospital infections due to MRSA were frequently detected. The protein profiles of the isolated MRSA strains from both hospital staff and outpatients were similar by SDS-PAGE.
Conclusion: MRSA nasal carriage rate among hospital staff was higher than the rate among outpatients ($P < 0.001$). The similarity between the protein profiles of these strains suggested that nasal MRSA strains of the outpatients might have been originated from the hospital.

P494 Staphylococcal scalded skin syndrome in an adult due to methicillin-resistant *Staphylococcus aureus*

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Objectives: Staphylococcal scalded skin syndrome (SSSS) is a generalized exfoliative dermatosis, caused by exfoliative toxin (ET) producing strains of *Staphylococcus aureus*. SSSS usually affects infants and children, but rarely occurs

in adults. SSSS in adults is usually associated with immunosupression and/or renal insufficiency, and mortality rate is high We report a case of SSSS in an adult due to ET producing methicillin resistant *S. aureus* (MRSA); the immunocompromised patient was successfully recovered.

A 71-year-old man, with a chronic history of diabetes mellitus, was admitted to our hospital with lumbago on July 17th, 2000. He was diagnosed as renal cell carcinoma with bone metastasis. In hospital, he had a sudden onset of high fever and erythema, followed by formation of flaccid bullae and exfoliation with a positive Nikolsky sign. Methicillin-resistant *S. aureus* (MRSA), producing exfoliative toxin B, was isolated from blood and bile cultures, and *Aeromonas hydrophila* was isolated from bile culture. Skin biopsy specimen showed a cleavage of the epidermis at the level of the granular layer. Soon after, the patient was recognized to have SSSS and cholecystitis, was treated with intravenous vancomycin and teicoplanin, and underwent percutaneous transhepatic gall bladder drainage, which led to recovery. SSSS in adults is usually seen in the host of immunosuppression. *A. hydrophila* is recognized as opportunistic pathogen.

Conclusions: SSSS should be considered in the differential diagnosis of immunocompromised adult patients with sudden onset of high fever and erythema. Rapid microbiological examination is necessary for appropriate antimicrobial therapy, because it is possible that SSSS in adults is caused by MRSA and complicated by opportunistic infection.

P495 Effective control measures for preventing transmission of methicillin-resistant *Staphylococcus aureus* in a medical intensive care unit

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Objective: Programs for controlling methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals with a high endemic level have not universally been accepted. Main concerns are high costs and low anticipated medical benefits of such programs. Goal of this study was to evaluate a strategy to prevent cross-transmission of MRSA in a 10-bed medical intensive care unit (ICU) of a university hospital with a high level of endemic MRSA.

Methods: The incidence of ICU-acquired MRSA cases was compared between a 6-month period before and a 6-month period after implementation of control measures at November 1, 2000. Routine screening for MRSA carriage (nose, throat, wounds, tracheal aspirate) was performed in all patients admitted during both periods. Control measures in the second period included (1) strict use of alcoholic hand rub by health care workers before and after each patient contact, before manipulating catheters, after contact with body fluids, and after removing gloves (2) preemptive skin and throat decolonization with chlorhexidine in the first 5 days after admission, and (3) barrier precautions and decolonization of patients with MRSA (including nasal mupirocin).

Results: Of 133 patients, admitted during the first 6-month period, MRSA was recovered from 23 patients, of which 14 cases (60.9%) were ICU-acquired. After implementation of infection control measures, 149 patients were admitted to the ICU in the following 6 months. MRSA was detected in 24 patients, of which 3 cases (12.5%) were ICU-acquired. The incidence of ICU-acquired cases decreased after implementation of control measures from 10.5 to 2.0% ($P = 0.003$), whereas the rate of imported cases increased in the same period from 6.8 to 14.0% ($P = 0.05$).

Conclusions: Infection control measures can significantly decrease the incidence of transmission of MRSA in an ICU with a high level of endemic MRSA in spite of increased rate of imported cases.

P496 Cost resulting from applying the German guidelines on hospital hygiene measures in patients carrying methicillin-resistant *Staphylococcus aureus* (MRSA)

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Objectives: In 1999, the German health authorities—Robert Koch Institute (RKI)—published a guideline on hospital hygiene measures in patients carrying MRSA (MRSA-pat.). Aim was to estimate surplus costs when carrying for MRSA-pat. according to these guidelines.

Methods: Records of all MRSA-pat. treated in a ward for septic surgery (daily costs for inpatients: 398.41€) during 1 year were assessed. For each measure's costs were estimated considering information from: medical, budget and house cleaning personnel. For each MRSA-pat. these costs were collected for the time spent in the ward. Costs concerning each measure during the year were then added together and divided by the total number of MRSA-Hospital-Day (MHD) assessed.

Results: 20 MRSA-pat. —80% male, age median: 74.5 years, mean 70.7 years, 80% with first time MRSA-positive microbiological swabs during the present hospital stay (MRSA-new) were assessed, resulting in a total of 498-MHD, 65% of these in MRSA-new pat.

- For each category of measure costs of one MHD were calculated.
- Time for information and training of personnel: on ward 0.25€, hygiene department: 3.17€.
- Isolation of MRSA-pat. (costs for 'barred' beds): 305.74€.
- Protective measures (time and material): 36.02€.
- Desinfection and cleaning (time and material): 2.00€.
- Refusal (material): 2.44 €.
- Microbiological screening (exclusive time of sample taking): 12.91€.
- Eradication (preparations): 7.55€.
- Measures upon discharge from hospital (time): 0.38€.
- Transportation concerning diagnostics (time and material): 1.26€.

Total costs for one MHD were 371.95€. During 1 year cost for MRSA-new patients were about 120 000.00€, in those previously known to be MRSA positive (MRSA-old) about 65 000.00€, assuming that costs for barred beds did not differ between MRSA-new and MRSA-old pat.

Conclusions:

- Most of the surplus costs concerning MRSA hygiene measures according to German guidelines arose from pat. without previously known MRSA positive status.
- Daily surplus costs in caring for MRSA-pat. alone were about as high as the total amount the ward received for one day of inpatient stay.
- Much discussed measures, e.g. wearing a cap, amounted only to minute costs but can be useful in reinforcing discipline in patient care.
- The most expensive measure per day was a separate room for these patients. This is categorized as IB (recommended) by national authorities and therefore of high importance as far as legally correct hospital hygiene is concerned.

P497 Computerized surveillance of methicillin-resistant *Staphylococcus aureus*

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Objectives: The aim of this study is to asses the usefulness of a software designed to detect MRSA colonized or infected patients and to send this information to the infection control unit.

Methods: The hospital de Basurto is an 800-bed general teaching hospital that gives care to the urban area of Bilbao. A software was implemented for surveillance of MRSA. When a patient previously colonized/infected with MRSA is admitted to the hospital or a new case is diagnosed in the Microbiology Laboratory an alarm is displayed in the Infection Control Unit. So if it is judged to be or epidemiological significance contact precautions are started and nasal and axilar swabs are cultured. If MRSA is recovered nasal mupirocine and clorhexidine soap are started. Microbiological methods for MRSA study include: agar screen, disk diffusion, BBL Crystal, Gen. mec detection, and molecular typing by means of PCR for epidemiological purposes.

Results: Since 1 January 1999 to 31 October 2001, 193 colonized/infected patients with MRSA have been diagnosed in our hospital. The software has found 106 previously infected and 87 newly diagnosed cases during present admission. MRSA have been recovered from wounds in 76 patients, from blood in 41, from respiratory tract in 28 and from urine in 18 cases. A total of 191 patients have been studied to find nasal and/or axilar colonization. 22.64% of previously infected patients and 48.27% of newly diagnosed were colonized with MRSA in nasal and/or axilar cultures so decontamination with nasal mupirocine and clorhexidine soap was carried out. Twenty-four (12.43%) out of 193 patients were admitted to the hospital from a nursing home and 16 of them (66.6%) were MRSA carriers. Of the 167 remaining patients only 30% were MRSA carriers. An outbreak (five patients) was detected in ICU in December 2000; 66 health-care workers were studied, no one of them was a MRSA carrier. Cross infection was diagnosed by molecular typing methods. This outbreak was finished with strict adherence to contact precautions, nasal mupirocin and clorhexidine soap.

Conclusions: The software has been useful to detect MRSA infected/colonized patients and outbreaks. Nasal carriers are more frequent among newly diagnosed cases and among patients admitted from nursing homes.

P498 Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in health care workers (HCWs) in an Iranian hospital

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Background and objectives: The carriers of *Staphylococcus aureus* are a major source of infections for hospitalized patients. Several studies have reported that the rate of nasal carrying is ranging from 16.8 to 50.1% in HCWs. The mortality rate of infections caused by MRSA is very high. It is estimated to be 20–84%. The aim of this study was to determine frequency of nasal carriage of *S. aureus* in HCWs of Imam Khomeini Hospital of Urmia, West Azarbayjan, Iran.
Methods: Hospital staff ($n = 230$; 115 male and 115 female; average age 30 years) were randomly selected. Cultures were obtained from the anterior nares of HCWs. Specimens for detection of MRSA were planted onto mannitol—salt agar and incubated at 35 °C for up to 48 h. Organism with a yellow color (mannitol fermenters) were identified as *Staphylococcus aureus* by standard methods, including Gram stain, catalase, DNAse and tube coagulase test. Agar screen test was used to detect MRSA by inoculating 104 colony forming units onto Muller–Hintons agar supplemented with 4% NaCl containing 6 mg of oxacillin per mL. Strains resistant to oxacillin were confirmed as methicillin-resistant. No changes in the method of identifying MRSA occurred during the course of the study. Antibiogram typing was performed by using disk-diffusion method according to the National Committee for Clinical Laboratory (NCCL) standards. In addition, resistance of *S. aureus* to penicillin, co-trimoxazole, vancomycin, ciprofloxacin, erythromycin, clindamycin and gentamicin were tested using the disk-diffusion method.
Results: A total of 90 (40%) of the 230 HCWs were positive for nasal carriage of *S. aureus* (53 male and 39 female). The frequency of MRSA in the staff of different wards in increasing order was as follows: physicians 8%, nurses 31.81%, service workers 33.3%, staff of emergency ward 42.9%, operation room technicians 83.3%, anesthesia technicians 100%. Resistance rate of *S. aureus* isolated from nose of HCWs to commonly used antibiotics was as follows: penicillin 67.4%, co-trimoxazol 41.3%, gentamycin 25%, clindamycin 18.3%, ciprofloxacin 14.1%, erythromycin 8.7%, and vancomycin (0%).
Conclusion: Our study indicated that the rate of MRSA nasal carriage is high in HCWs and that the rate of resistance to other antibiotic is increasing which may be a serious problem for the treatment hospitalized patients.

P499 Methicillin-resistant *Staphylococcus aureus*: differentiation by bacteriophage typing and by pulsed-field gel electrophoresis

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Objectives: Determine the diversity of bacteriophage types and pulsed-field gel electrophoresis types among methicillin-resistant *Staphylococcus aureus* strains recovered in a general Belgian hospital.
Methods: During the year 2000, 138 methicillin-resistant *Staphylococcus aureus* strains, collected in a 500-bed general Belgian hospital, were characterized with the traditional bacteriophage typing method and by a pulsed-field gel electrophoresis using the *Sma*I enzyme. All strains were checked for coagulase, oxacillin resistance and the *mecA* gene.
Results: All strains were coagulase positive. They were all *mecA* positive except four, which have been excluded from this study. The oxacillin MIC varied between 16 mg/L and more than 256 mg/L, except for one strain showing a MIC of 4 mg/L and two strains with a MIC of less than 1 mg/L. Nevertheless, the two latter strains, being *mecA* positive, produced the corresponding protein PBP 2′ (detected by a serological method). With the bacteriophage technique, only two strains were not typable. On the other hand, 110 strains (80%) were only sensitive at RTDX100. The majority of the strains belonged to a [O] type (61%), followed by a [J] type (12%). A total of 90% of the strains had the same pulsotype. Nearly all strains of the [O] and [J] phage type belonged to this pulsotype. A different band profile was found for the two not typable strains. The phage type 54/81 (RTDX100) corresponded also to a specific pulsotype.
Conclusions: Bacteriophage typing is the standard method for typing *S. aureus*. Analysis of the cell DNA by pulsed-field gel electrophoresis after macrorestriction is increasingly recognized as an improved method. Yet it is technically and financially more exacting. Among the 134 strains, we examined two main phage patterns were observed whereas only one pulsotype was dominant. Both phage types belonged to this pulsotype. So, it appears that a subdivision of pulsotypes by a method like phage typing can provide supplementary information. As to the Belgian strains phage typing remains highly sensitive, at least at the 100-fold concentration.

P500 Molecular epidemiology of MRSA isolates in intensive care unit during a 4-year period

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Objective: Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) pose a serious problem in many countries. Patients admitted to intensive care unit (ICU) are especially prone to develop infections caused by common nosocomial pathogens like MRSA due to special risk factors (invasive procedures, antibiotic use, age, chronic disease, etc.). We analyzed 56 MRSA strains isolated from ICU patients between 1998 and 2001 by arbitrarily primed (AP)-PCR in order to evaluate strain relatedness.
Methods: MRSA isolates were identified by standard methods. DNA extraction was performed by Nuclespin DNA extraction kit (Macherey-Nagel, Germany). AP-PCR analysis was performed with M13 and ERIC 2 primers. Amplicons were analyzed by electrophoresis through agarose gels. On the basis of different amplified DNA band patterns strains could be divided into various PCR groups by visual inspection.
Results: Evaluation of band patterns revealed that there was an evidence of transmission of strains between patients and, during whole study period there were 11 incidences of two or more different patients with isolates having identical fingerprints. Fortunately, a given genotype affected four patients at most and the longest persistence period was 2 months.
Conclusion: AP-PCR method can be applied as a part of routine laboratory procedures for earlier detection of epidemics in ICU in order for immediate determination of common source and take infection control measures.

P501 Isolates of methicillin-resistant *Staphylococcus* aureus (MRSA) from stool of patients with acute gastroenterocolitis (AGEC) often produce enterotoxins

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Background: Enterotoxins are important virulence factors of *Staphylococcus aureus*. We studied the frequency of enterotoxin positive isolates of *S. aureus* isolated from patients with AGEC.
Methods: In the period from January 2000 to August 2001, 3877 stool specimens of patients with AGEC or diarrhea, were analyzed for pathogenic bacteria. We did not routinely examine stools for *C. difficile*. Isolates of *S. aureus* were tested for staphylococcal enterotoxin (SE) A, B, C and D production using SET-RPLA (Staphylococcal enterotoxin test kit, OXOID). In all primoisolates of *S. aureus* antimicrobial susceptibility testing was performed by use of disk-diffusion method. We determined *mecA* gene by PCR and performed molecular typing by pulsed-field gel electrophoresis.
Results: In 6.5% out of 3877 stool samples, we isolated *Campylobacter* spp.; in 4.7% *Salmonella* serogroup B, C, and D; in 0.3% *Yersinia enterocolitica*; in 0.1% *Shigella* spp. *S. aureus* was found in 27 (0.7%) stool specimens. Eleven isolates were MRSA and belonged to six patients hospitalized in two regional hospitals. All MRSA isolates produced SE A. One isolate of MSSA produced SE B, other MSSA were SE negative. Although MRSA isolates were sporadic, they were identical morphologically, according to biochemical tests, and susceptibility to antibiotics. All MRSA were *mecA* gene positive. PFGE confirmed the same genotype. For that reason we suppose they represent the same strain.
Conclusions: More than half of *S. aureus* isolates obtained from stool of patients with AGEC produced SE. All MRSA produced SE. All MRSA

positive stool samples were sent from two hospitals. Four out of six MRSA were isolated within 2 months and could be epidemiologically connected.

As SE is an important virulence factor of *S. aureus*, we think that detection of SE production in connection with the clinical picture argues for the clinical importance of the isolate. In addition, the pattern of SE production may contribute to the epidemiological analysis of MRSA epidemics.

P502 An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) with a strain that is negative in latex agglutination tests (LAT) and does not express capsular polysaccharide

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Objectives: Clonal study of LAT negative MRSA strains and its relationship with capsular polysaccharides.
Methods: Eighteen MRSA were isolated from 16 patients and two nasal carriers in the Fátima Clinic between 1999 and 2000. All the MRSA were identified by tube coagulase test, amplification of the *mecA* and *nucA* genes, API-STAPH (bioMérieux), and oxacillin disc diffusion. Four LAT were tested: Slidex-Staph Plus and Staph-Kit (bioMérieux), Dryspot Staphytect Plus (OXOID), Pastorex Staph-Plus STAPH (Sanofi-Pasteur). Capsule serotyping was performed by immunodiffusion assays. The epidemiological relatedness was studied by repetitive element sequence-based PCR, random-amplified polymorphic DNA, *coa* gene restriction fragment length polymorphism and *spa* gene polymorphism analysis.
Results: All 18 of the isolates represented a single epidemic clone and were LAT negative. Three representative strains did not react with antibodies (Ab) to any of the known CP serotypes (1, 2, 5, or 8), although they did react with Ab to ribitol teichoic acid. PCR amplification of the cap8-specific genes, but not the cap5-specific genes, suggests that the strains do not carry a new capsule locus. The three strains were positive in a biofilm assay.
Conclusion: Nonreactivity of these MRSA in the LAT is not likely due to the production of capsule, but it may be related to biofilm production. Alternatively, these strains may be regulatory mutants whose expression of surface antigens is down regulated. The development of any new LAT should include Abs to consitutively expressed staphylococcal antigens like ribitol teichoic acid in order to avoid false negative results.

Nosocomial infections: epidemiology

P503 Prevalence of infections in 18 intensive care units in an Italian region (Piemonte)

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Objectives: The aims of the study were to determine in our region the prevalence of infections among patients in the intensive care units (ICUs), the setting where infections were acquired (community, hospital, ICU), the most frequently reported infections and their relationship with specific risk factors.
Methods: A number of 18 of the 33 nonspecialized ICUs in Piemonte took part into the study. The patient population was defined as all patients occupying a bed over a 24-h period in 2 days: 27 June and 18 July 2001. For each patient, the investigator collected a comprehensive case report with demographics data, clinical status on admission (Simplified Acute Physiologic Score, SAPS II), diagnostic and therapeutic interventions up to 1 week preceding the study day. The definitions of infections followed the 1988 statements of the CDC. Patient outcome was assessed up to 3 weeks after the study day.
Results: Of the 183 participants, 126 were men and 57 female. The mean age was 61 years. Eighty-four (45.9%) patients had one or more infections. Out of the 109 total infections, 26 (23.9%) were community-acquired infections, 29 (26.6%) hospital-acquired and 54 (49.5%) ICU-acquired infections. Most frequent infections involved low respiratory tract and urinary tract (see Table 1).

Table 1

Type of infection	*n*	%
Pneumonia	40	36.7
Lower respiratory tract	20	18.3
Urinary tract	16	14.7
Gastrointestinal	11	10.1
Clinical sepsis	9	8.3
Bloodstream	8	7.3
Wound	2	1.8
Central nervous system	2	1.8
Skin and soft tissue	1	0.9
≥2 ICU-acquired	11	27.5
≥2 Hospital-acquired	4	16.0
≥2 Community-acquired	3	13.0

Increasing length of ICU stay (more than 8 days), tracheotomy, SAPS and total parenteral nutrition have been identified as risk factors for ICU-acquired infection on bivariate analysis, whereas only the length of ICU stay was yet significant on logistic regression analysis.
Conclusions: Infective problems are of great importance in the ICU setting and almost 50% of patients in ICUs has one or more infections. Overall, infection rates are similar to findings of other European reports (e.g. EPIC study) but ICU-acquired infections are less frequent than those previously reported in other Italian studies.

P504 A real-time computer-based nosocomial infection surveillance program (Infhos)

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Objectives: The aim of this study is to asses the effectiveness of a computer-based nosocomial infection (NI) surveillance program called INFHOS to detect NI by comparing it's results with the results of a prevalence survey (EPINE) carried out in our hospital in 2001.
Methods: The INFHOS program detects patients with NI by means of an expert software that merges data from microbiology, pharmacy, and admission. Patients are classified in four groups: without NI, IN possible, IN probably, NI certain. In the case of patients with NI, criteria for NI diagnosis (positive cultures, antibiotics, or clinic) and date of diagnosis are also displayed. This program has been built in our hospital. In this study we wish to compare the results of our annual prevalence survey (excluding psychiatry and short time admission ward were we didn't find any NI) with the results of the INFHOS.
Results: A total of 497 patients were studied by the two methods—130 in surgery, 30 in the ICU and 337 in other wards. In the prevalence survey we found 31 NI, 28 out of them were also classified by INFHOS as IN certain. In the other hand, the INFHOS program find 28 NI among the patients without nosocomial infection in the prevalence survey. When these 28 cases were analyzed, only three were active NI the day when the prevalence survey was carried out. Two of this three cases were classified as community-acquired infections in the prevalence survey and the other was a positive blood culture with coagulase-negative *Staphylococcus* in only one out of six blood culture bottles that we interpret as a contaminant. After this comparison we decided to change some rules to adjust the NI definition criteria.
Conclusions: The computer system allows all inpatients to be screened for either infection control or antibiotic management interventions on a daily

basis with minimal time being expend on data collection. The main advantage of this program is to move infection control surveillance to a real-time mode.

P505 Nosocomial infection after acute stroke

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Introduction: Despite the efforts to decreasing stroke incidence and mortality, nosocomial infection are an important problem after acute stroke.
Objective: To know the incidence, etiology, localization and factors associated with nosocomial infection during acute stroke.
Methods: Prospective study (from June 2000 to May 2001) of all patients admitted to the hospital because of nonhemorrhagic acute stroke. Diagnosis of nosocomial infection, after the first 72 h after admission, was based on the clinical manifestations, supported by hematological, microbiological and imaging findings.
Results: During this period, 201 patients were included. In 17.4% there was an infectious complication, 54.3% urinary tract infection (UTI) and 45.7% pneumonia (owing to aspiration). Diagnosis of stroke (vs. transient ischemic attack) increase the infectious risk (P 0.01) as well as a Canadian Neurological score <7 at admission (P 0.004) (a lower value increase the severity of the stroke). Infection happened a median of 6.8 days after admission (range, 3–15). The presence of fever and indwelling bladder catheter were associated to a higher infectious risk ($P = 0.001$). Statistical analysis ($P < 0.05$) showed that age >75 years, male, diabetes, Charlson score for co-morbidity >1, and a value at admission of glucose >126 mg/dL, iron <50 μg/dL, CRP > 10 mg/L, leukocyte count $>10 \times 10^3/\mu L$ and fibrinogen >350 mg/dL where associated to a higher infectious risk. Gram-negatives accounted for 76.5% of the episodes (mainly *E. coli*), and 11.8% were polymicrobial. Antimicrobial therapy was considered adequate in 61.5% of cases. The overall mortality is increased by the presence of an infection ($P = 0.001$).
Conclusions: After acute nonhemorrhagic stroke, 17.4% of patients present some infectious complication. The main etiology is UTI followed by pneumonia. Gram-negatives are the main microorganism implicated.

P506 Nosocomial urinary tract infection (Nuti) in the elderly admitted to internal medicine services

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Introduction: NUTI increase the morbidity and mortality of elderly patients admitted at hospital.
Objective: To assess the incidence, etiology and risk factors associated with NUTI in elderly.
Methods: Prospective study (June 2000 through May 2001) of patients older than 65 years admitted in Internal Medicine Services. We analyzed demographic characteristics, clinical data, associated factors, urinary drainage methods, type of catheter bladder, predisposing factors, and complications (including mortality). Urinary culture was realized in symptomatic patients (dysuria, frequency or urgency), including fever of unknown localization. A urine culture was considered to be positive if a quantitative culture growing a single organism in concentrations of >105 cfu/mL of urine, and no more than two isolated present (with exception of *Enterococcus* and *Candida* infections).
Results: During this period, 1342 elderly patients were admitted, 52% women, and a median age (SD) of 78.5 (7.4) years. The global incidence of NUTI was 3.8%; 60.4% women, and a median age (SD) of 80.3 (6) years. Risk factors associated to NUTI were: previous hospitalization ($P < 0.05$), nursing home resident ($P < 0.001$), higher severity of the McCabe & Jackson ($P < 0.005$), presence of exogenous risk factors (>2) (P 0.001), bedridden ($P < 0.001$), urinary incontinence ($P < 0.001$), and indwelling bladder catheter ($P < 0.001$) (in special Foley catheter; $P < 0.001$). Gram-negatives accounted for 54.7% of the episodes (mainly *E. coli*), and 7.5% were polymicrobial. Among patients with NUTI, bacteremia was present in 5.6%. NUTI increased the days of hospitalization ($P < 0.005$) but not the overall mortality.
Conclusions: Concomitant illness or risk factors, previous admission at hospital or nursing home resident, and a urinary incontinence or bladder catheter are risk factors associated to NUTI in elderly. Gram-negatives are the main microorganisms implicated. NUTI increase the hospitalization but not the mortality.

P507 Prevalence of nosocomial infections in Italy: results from the Lombardy survey in 2000

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Objectives: To obtain prevalence rates of nosocomial infections (NI) in Lombardy, to determine distribution of hospital acquired infections by hospital department and to identify the pathogens more frequently involved.
Methods: A descriptive study by point prevalence survey. The survey involved 88 out of the 113 public hospital of Lombardy, voluntarily participating. All adult patients (older than 15 years) admitted in the 24 h preceding the survey in any department, excluding those in psychiatric departments, were investigated. All patients in the same ward had to be investigated on the same day. The survey was conducted by local teams trained by Prevention Unit of Region Lombardia. NI infections were defined according to CDC criteria.
Results: A total of 18 667 patients was surveyed, representing 72% average daily total of occupied beds in public hospitals in Lombardy. A total of 916 NI were observed, thus the overall prevalence was 4.9%. Prevalence of bloodstream infections (BSI) was 0.6%; of pneumonia was 1.1%; of urinary tract infections (UTI) was 1.6% and of gastrointestinal infections (GI) was 0.4%; surgical wound infections (SWI) occurred in 2.7% of surgical patients. The highest rate of BSI and pneumonia was in intensive care unit (ICU), respectively, 7.0% and 17.2%. With respect to urinary tract infections (UTI), high prevalence was observed in spinal unit (20.0%), ICU (8.3%) and rehabilitation (6.4%).Operated patients had an increase of SWI, BSI and pneumonia directly related to increased surgical contamination. The prevalence rates of all sites of NI increased with ASA score. Microbiological confirmation was obtained for 50 cases of BSI (45.9%), for 87 of pneumonia (42.0%), for 209 of UTI (67.9%), for 23 of GI (33.8%) and for 50 of SWI (36.5%). MRSA accounted for the 23% of all isolated *S. aureus*; in intensive care units patients the proportion of MRSA represented 44% of *S. aureus* isolations. In BSI coagulase-negative staphylococci accounted for 22%. The proportion of methicillin resistance between coagulase-negative staphylococci was 38% in all isolates, and 43% between isolates from BSI.
Conclusions: The high participation in this study suggest that the method is suitable. The results are of undoubted descriptive interest for understanding the general dimension and features of the problem of NI in Lombardy, and provides baseline data for rational priorities in allocation of resources, for further studies and for infection control activities.

P508 The first national prevalence study of hospital infections

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Objectives: The hospital infections are for a long time very well-known public health problem with a certain of consequences like medical, economics, ethic, statutory, etc. The number of countries, which recognize the need of scientific cognition's repose on collecting data about the frequency of these infections by organized epidemiological surveillance, increase. The study of prevalence is the first necessary stage in insight of hospital infection frequency. This study enrolls hospital infection among the all patients present in hospital in the moment of study (usually 1 day). The aim of this study is to insight status and the size of the hospital infection problem in Republic of Serbia.
Methods:
- The type of study (prevalence study);
- The studied population;
- The infection and variable observe definition;
- The data collecting;
- The validity of data;
- The facing of data.

Results: Twenty-seven (27) hospitals took part with 7115 patients. Therefrom 21 (21) General Hospitals with 4131 (58.1%) patients and six (6) University Hospitals with 2984 (41.9%) patients. Study included 3107 (43.7%) men and 3107 (43.7%) women. The prevalence of the patients with the hospital infection was 6.3%, whereas the prevalence of hospital infections was 7.5%. Highest prevalence level of hospital infection was determined in university hospitals (9.9%), whereas that level in general hospitals was little

less (5.7%). The highest prevalence level was confirmed in neonatology (14.6%) than orthopedic and traumatology departments (10.4%). According to anatomic localization mainly there were infections of operating field, urinary system, respiratory system and infections of skin and soft tissues. Microbiological confirmation had only 50.8% of hospital infections. The main causes were *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella* spp. and *Escherichia coli*. During the study antibiotics has been taken from 35.4% of hospitalized patients. Antibiotics in prophylaxis has been taken from 36.3% of patients that was operated and among them 37.8% patients with uninfected operating field.
Conclusion: The study of prevalence pointed out the importance of epidemiological surveillance initiation at hospital infections.

P509 Three prevalence surveys of hospital-acquired infections in a Greek hospital

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As it is well known, surveillance of hospital-acquired infections (HAIs) is an important component of an effective nosocomial infection control program. Prevalence surveillance is a rapid and inexpensive way to estimate the problem of HAIs. To study the problem of nosocomial infections in our hospital, three prevalence studies were made from our team during the years 1994–1999. The first study included 288 patients, the second 288 too and the third 265 (the total number of hospitalized patients at the time of the study). In the first study a nosocomial infection was found in 20 patients, in the second in 15 patients and in the third study in 13 patients. The overall prevalence of HAIs was 6.9, 5.2 and 4.9% for the three studies, respectively. In the first study, among HAIs, urinary tract infections were 12 (60.0%), lower respiratory tract infections were six (30.0%) and surgical site infections were two (10.0%). In the second study, urinary tract infections were four (26.7%), lower respiratory tract infections were two (13.3%), surgical site infections were 5 (33.3%) and bloodstream infections were four (26.7%). In the third study, urinary tract infections were six (46.1%), lower respiratory tract infections were three (23.0%), surgical site infections were three (23.0%), and bloodstream infections was one (7.7%). The use of antibiotics among the hospitalized patients was found to be 47.2, 60.1 and 58.5% for the three studies, respectively. The incidence of multi-resistant bacteria was primarily *Enterococcus* spp. and secondary *Pseudomonas aeruginosa, Enterobacter* spp., *Klebsiella pneumoniae, Escherirchia coli, Staphylococcus aureus, Staphylococcus epidermidis*. Regarding age the highest incidence of HAIs occurred in the third age group. Unjustified prescription of prophylactic chemotherapy was found despite the suggestions of the infection control committee. Repeated prevalence surveillance is a valid way to estimate the problem of HAIs.

P510 Two point prevalence studies of hospital-acquired respiratory tract infections in 14 Greek hospitals

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Objectives: Hospital acquired respiratory tract infections (HARTIs) are associated with significant morbidity and mortality. The aim of this study was to investigate the epidemiological feature of HARTIs in Greek hospitals.
Methods: Two point prevalence studies of hospital-acquired infections (HAIs) were carried out in 14 Greek hospitals, scattered in all Greek territory, during the years 1999 and 2000. Respiratory-tract infections were recorded according to the CDC definitions.
Results: A total of 7120 hospitalized patients were registered during the studies. A number of 3767 (52.9%) were men. Their median age was 62 years (range: 0–99). During the study 610 (8.6%) patients of HAI were identified. Among them 197 (32.3%) were HARTIs [118 out of 366 (32.2%) HAIs, in 1999s study and 79 out of 244 (32.4%), in 2000 s]. One hundred and eleven out of 193 patients (56.3%) had a lower respiratory tract infection (LRTI), 67 (34.0%) patients had a pneumonia and 19 (9.7%) had an upper respiratory tract infection (URTI). Five out of 197 (2.5%) patients had a concomitant nosocomial surgical site infection (SSI), and four out of 197 (2%) had a nosocomial urinary tract infection (UTI). The greatest prevalence rate of NRTIs was found in the adult ICU (31.1%). Mechanical ventilation, as well as, the presence of intra-tracheal tube or tracheostomy were the main risk factors for NRTIs ($P < 0.0001$). Responsible microorganism was found in 80 out of 197 (40.6%) cases and 102 strains were isolated. The majority of them were Gram-negative bacteria (66.7%). The most frequently isolated microorganisms were: *P. aeruginosa* (21.6%), *Staphylococcus* spp. (21.6%), *Acinetobacter* spp. (19.6%) and *K. pneumoniae* (11.8%). With the exception of *K. pneumoniae* (17.2% in 1999's study and 4.5% in 2000 s), there was no difference in the frequency of microorganisms' isolation between the two prevalence studies.
Conclusion: Our data suggested that HARTIs are the most common HAI in Greek hospitals. Gram-negative microorganisms are the most frequently isolated pathogen. No substantial epidemiological differences in the two-point prevalence studies were noted.

P511 Microbiology of nosocomial infections and susceptibility to antibiotics in an intensive care unit in Greece

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Objectives: To determine microbial flora of nosocomial infections in ICU. To determine antimicrobial resistance patterns among aerobic Gram-positive and -negative microorganisms isolated from patients on a seven-bed ICU over a 3-year period.
Methods: During 1998–2000, 913 cultures from 1098 patients with various clinical manifestations of nosocomial infections were isolated. Disc diffusion method and breakpoint MICs were employed to detect the in vitro activity of antibiotics.
Results: A total number of 913 isolates were evaluated from respiratory tract (34.6%), blood (29%), intravascular catheters (13.8%), wounds (12.4%) and urinary tract (10.2%). The isolated microflora was Gram-positive cocci: CNS (34.7%), *S. aureus* (16%), *Enterococci* (5.8%) and Gram-negative rods: *P. aeruginosa* (17.3%), *A. baumannii* (10.4%), *Klebsiella* sp. (5%), *E. coli* (4%) and *Enterobacter* sp. (2.8%). The sensitivity of Gram-positive microorganisms was tested to ampicillin, amoxicillin/clavulanic acid, oxacillin, vancomycin, teicoplanin, gentamicin, ciprofloxacin, erythromycin and clindamycin. The high level of methicillin resistance among CNS (80%) was obtained. The sensitivity of Gram-negative microorganisms was tested to ampicillin/sulbactame, gentamicin, piperacillin/tazobactam, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, imipenem and meropenem. The solated Enterobacteriaceae had good susceptibility to imipenem (95%) instead of *P. aeruginosa* and *A. baumannii* susceptibility to the same drug (30%) and (50%), respectively.
Conclusions: In the ICU of our hospital β-lactam antibiotics are not the drugs of choice for empirical treatment in nosocomial CNS infections. For the treatment of infections caused by Gram-negative microbes the drugs of choice would be carbapenems and piperacillin/tazobactam.

P512 Control and temporal evolution of a multi-resistant *Acinetobacter baumannii* outbreak in an intensive care unit

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Objectives: In 1997, *Acinetobacter baumannii* became the most common cause of intensive-care bacteremia (ICU) in our hospital. The aims of this study were to describe the genotypic characteristics of these isolates, the temporal evolution of a detected multi-resistant cluster, and analyze the influence of control measures on the evolution of the outbreak.
Methods: Prospective study of all strains of *A. baumannii* isolated in bacteremic patients of an ICU in a teaching hospital, during the last 6 years. Analysis of genomic DNA, by PFGE and RFLP–PCR, and susceptibility study of the isolates. Clustering of the genotypic patterns obtained and analysis of their temporal evolution. Analysis of preventive and control measures adopted during the multi-resistant outbreak, including architectonic changes in the ICU.
Results: During the study period 312 blood and other samples from 72 patients with *A. baumannii* bacteremia were analyzed. Six clusters were obtained The multi-resistant outbreak was caused by the a cluster (C3), which grouped the 66.6% of cases. In this cluster, all the antibiotics tested, including carbapenems, were resistant, except polimixine and colimicine.

Multi-resistant-C3 caused the 100% of isolates in 1997. Despite preventive measures, including architectural changes in ICU, the C3 cluster remained as the main group of *A. baumannii* (67% of cases) in 1998. However others clusters that grouped susceptible-strains were emerging. Finally, from February 2001 the only present cluster was sensible-C5, and multi-resistant-C3 was eradicated.
Conclusions: The common preventive measures adopted for the control of a multi-resistant outbreak of *A. baumannii* in ICU were not enough effective. Only the architectonic changes had a remote effect in the outbreak eradication.

P513 Computer keyboards and nosocomial pathogens in a children's hospital

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Introduction: Nosocomial infections are important adverse events that complicate the hospitalization of patients and result in considerable morbidity and mortality, increased length of stay and increased health care cost. The role of the hospital environment as a reservoir of nasocomial pathogens is controversial and has not been thoroughly investigated. Computers are a relatively recent addition in hospital environment with frequently used keyboards. This study was undertaken to investigate the potential role of keyboards in the transmission of nosocomial pathogens
Methods: Sampling occurred on the same day from 156 computer keyboards. Forty-four out of 156 keyboards were located in the inpatient services, and from neonatal and pediatric intensive care units. Two keyboard samples from bone marrow transplantation unit and, 32 keyboard samples from doctors' stations were taken. In total 78 samples from in patient unit were collected. The other 78 were taken from doctor offices (46), secretary unit (28), parenteral nutrition unit (1) cardiac catheterization unit (2) and one from information desk. A sterile cotton tipped swab moistened with sterile saline solution was moved over the letter keys A, K, L, M (most commonly used letters in Turkish), space bar and enter key. After the collection, thioglycollacte broth medium and blood-agar medium was inoculated.
Results: A total of 24 environmental isolates were obtained with two sample sites yielding multiple organisms. *Staphylococcus aureus* was the most common pathogen identified. Seven out of 19 *S. aureus* isolates were MRSA. Other isolates were *Enterobacter* (5) and *E. coli* (2). Five of the MRSA was isolated from the outpatient units and two multiple organism isolated sites from the intensive care unit.
Conclusion: As a result of these findings, hand washing before and after the keyboards contact must be encouraged. Also plastic keyboard cover and daily cleaning procedures for keyboard must be installed in both services and outpatient units.

P514 Microbiological control of airborne contamination in hospitals with the use of an airIDEAL sampler

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Objectives: The aim of the study was to compare different methods for microbiological control of hospital air and to assess the utility of the airIDEAL impactor sampler (bioMerieux) in controlling air contamination in the hospitals environment.
Methods: Air samples were collected from 12 conventionally ventilated operating rooms (n-LAF) and from10 rooms of the hematology wards equipped with HEPA filtration system (LAF). Air contamination was measured by the sedimentation method, i.e. by 30-min exposure of plates spread with nutrient agar (TSA; TSA with neutralizing agents, and Sabouraud dextrose chloramphenicol), and by the airIDEAL sampler involving the same agar plates. Air samples from each of the investigated rooms were taken simultaneously by both the methods. Statistical analysis was performed using the Statistica PL.
Results: According to the measuring method applied, air contamination in n-LAF rooms varied from 68.1 to 1655.8 cfu/m^3 (sedimentation method), ranged between 342.4 and 9895.6 cfu/m^3 (air IDEAL sampler + TSA), or fell between 463.6 and 10314.6 cfu/m^3 (air IDEAL sampler + TSA with neutralizing agents). In LAF rooms air contamination varied from 0 to 74.7 cfu/m^3, from 5 to 33.3 cfu/m^3 and from 5 to 121.3 cfu/m^3 for the three methods, respectively. The investigations show that the cfu/m^3 values obtained with the sedimentation method averaged lower than those achieved with the impactor sampler. This difference was found to be statistically significant ($P = 0.03$) for n-LAF rooms. When use was made of TSA with neutralizing agents, the number of detected airborne microorganisms was greater ($P = 0.01$). The most commonly isolated pathogenic species were *S. aureus*, *Enterococcus* spp., *Streptococcus* spp., *P. aeruginosa*, *A. lwoffii* and *A. faecalis*, *Penicillium* spp. and *Cladosporium* spp.
Conclusions: (1) Microbiological control of air contamination in hospitals with the use of the airIDEAL impact sampler detected higher cfu/m^3 values as compared to those measured by the sedimentation method; for rooms with conventional ventilation this difference was of statistical significance. (2) The application of TSA with neutralizing agents increased the detectability of airborne microorganisms.

P515 A comparative study of the frequency and resistance of Gram-negative bacteria isolated from intensive care unit and the rest wards in a Greek hospital within a 3-year period

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Objectives: The recording of the kinds of Gram(–) bacteria which were isolated from Intensive care unit (ICU) and the rest wards of a Greek hospital within a 3-year period as well as a comparison of their frequency and resistance to antibiotics.
Materials and methods: During the 3-year period from 1998 to 2000, a total of 4279 clinical isolates of Gram(–) bacteria were collected from patients treated in our hospital. Of these 610 (14.3%) derived from ICU and 3669 (85.7%) from the rest of the hospital. The most common materials were in ICU: bronchial secretion 69%, catheter tips 11%, blood cultures 7.5% and in the rest: urine 45%, blood cultures 13%, wounds 6%. Cultures and identification of bacteria were performed by conventional methods. Susceptibility to antibiotics was detected by the diffusion method according to NCCLS guidelines.
Results: Of the strains tested the most frequent bacteria were in frequency order in ICU: *P. aeruginosa* 35%, *A. baumannii* 33%, *E. aerogenes* 10%, *K. pneumoniae* 8% and in the regular wards: *E. coli* 44.5%, *P. aeruginosa* 18%, *K. pneumoniae* 8.4%, *Proteus* spp. 7.5%. The percentage of resistance to antibiotics for Gram(–) bacteria derived from ICU and the regular wards were, respectively, for *P. aeruginosa*: ticarcillin 78–33%, imipenem 75–18%, ceftazidime 64–21%, tobramycin 73–31%, ciprofloxacin 72–20%, for *A. baumannii*: imipenem 12–7%, ceftazidime 94–86%, ciprofloxacin 88–75%, tobramycin 66–49%,for *E. coli*: ampicillin 71–49%, amoxicillin–clavulanic acid 43–24%, cefotaxime 24–7%, ciprofloxacin 19–7%, gentamicin 19–7%. As regards the sensitivity of the bacteria isolated, it is worth nothing that: The resistance of *P. aeruginosa* to aminoglycosides and Ceftazidime has increased during the 3-year period (tobramycin: in ICU from 70% in 1998 to 76% in 2000, in the rest from 22 to 32% ceftazidime in ICU from 52 to 63%). *A. baumannii* was multiresistant with increasing resistance to imipenem.
Conclusions: Gram(–) bacteria isolated from ICU were much more resistant to antibiotics than these of the rest wards of the hospital. The resistance of the majority of Gram(–) bacteria has increased significantly during the 3-year study period. The continual observation of bacteria and the policy of the restriction in the use of antibiotics can have beneficial effects.

P516 Emergence of multi-resistant *Acinetobacter baumannii* in the Leiden University Medical Center

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Multiresistant strains of the genus *Acinetobacter* have been reported to cause outbreaks in many hospitals worldwide. In the Leiden University Medical Center (LUMC), these organisms have been isolated only sporadically from clinical specimens. In November 2000, a sudden increase in the incidence of multiresistant *Acinetobacter* was noticed in patients on the ICU of the Internal Medicine Department (ICIG). Species identification by amplified ribosomal DNA restriction analysis (ARDRA), and strain identification (typing) by RAPD–PCR and AFLP showed that one single *Acinetobacter baumannii* strain was involved. The problems gave rise to several successive interventions in the period of November 2000–March 2001, including limited admission and strict isolation of patients, and an prevention of admission followed by

cleaning and disinfecting. Although these measures initially resulted in a decrease of cases, the organism was later found to have spread to the surgical ICU and was again – albeit at a lower rate – found in patients on the ICIG. Because its first manifestation, a total of 97 isolates were cultured from 23 patients on the two ICUs, mostly from respiratory tract specimens. Many environmental specimens were analyzed for presence of the epidemic organism, although it was recovered from a few of these – the sources and mode of transmission could not be elucidated. The AFLP fingerprint of the organism was compared to hundreds of *Acinetobacter* isolates of the AFLP library. It appeared that the LUMC strain was genetically neither closely related to *A. baumannii* strains from other European outbreaks, nor to the strains causing outbreaks in several Dutch hospitals in the same period. The fact that the strain persisted in the LUMC over a period of up to 10-months indicated that after an initial epidemic episode, it had become endemic in the hospital.

P517 Phenotyping and genotyping methods for epidemiological control of *Acinetobacter baumannii* hospital infections

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Objectives: *Acinetobacter baumannii* is Gram-negative bacillus that is responsible for an increasing number of nosocomial infections. It is difficult to control *A. baumannii* outbreaks, because usually they are caused by multiresistant strains. The aim of this study was to evaluate usefulness of different methods used for of *Acinetobacter* spp. typing.
Methods: Sixty *Acinetobacter* spp. isolates were recovered between January 1999 and December 2000 in different wards of the Regional Hospital in Czestochowa. The strains were identified to the species level using the ATB and Vitek systems (bioMeriéux) and the molecular ARDRA method. Susceptibility to a broad panel of antimicrobial agents was determined by the agar dilution method according to NCCLS. Similarity among the isolates was studied by the PCR-based approaches with RAPD-7, RAPD-1283 and ERIC1 primers.
Results: Using the ATB ID32GN test 88% of *Acinetobacter* isolates were identified as *A. baumannii*, one isolate as *A. haemolyticus* and only one isolate as *A. junii*. The seven remaining isolates were identified using the Vitek test as *A. calcoaceticus* bio. *anitratus*, and by the ARDRA method as *A. baumannii*, genospecies 4 and genospecies 5. Analysis of the biochemic profiles, the resistance and genetic patterns revealed similarity between *A. baumannii* strains which were collected in different wards of hospital.
Conclusion: The results suggested that *A. baumannii* infections that occurred in the hospital from January 1999 to December 2000 were caused by multiresistant strains. All of them were sensitive to carbapenems and 88% of strains were sensitive to netilmicin. The ARDRA method is more discriminatory than biochemical tests. Typing results revealed the homogenous structure of the population of *A. baumannii* in analyzed wards of hospital and this could have resulted from the patients transfer between these wards.

P518 PER-type β-lactamase-producing Proteus mirabilis strains causing outbreaks in a general hospital in northern Italy

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Introduction: An increase of β-lactam resistance rates, mostly owing to the acquired β-lactamases of TEM-type, has occurred in *P. mirabilis*, especially in nosocomial settings. Recently, also extended-spectrum β-lactamases (ESbetaLs) have started spreading in these species.
Objectives: the aims were to investigate the production of ESbetaLs and the epidemiological correlations in *P. mirabilis* isolates from an Italian hospital where increased resistance rates to expanded-spectrum cephalosporins have recently been observed.
Methods: we studied 294 *P. mirabilis* obtained from inpatients of the University Hospital of Pavia during the periods February 1997–January 1998 and January–November 2001. Isolates were identified by GNI cards and tested for antimicrobial susceptibility by GNS cards (Vitek System-BioMérieux). β-lactam susceptibilities were also determined by broth macrodilution procedure. Ampicillin resistant isolates were screened for ESbetaL production by the clavulanate sinergy test. Isoelectric focusing, activity on substrates, PCR amplification of bla alleles and sequencing were performed. Genotypic analysis of ESbetaL producers was carried out by pulsed field gel electrophoresis (PFGE) of genomic DNA and plasmid analyzes.
Results: A total of 294 *P. mirabilis* were collected from patients in medical and surgical wards and in a general ICU. 158 of the above strains resulted intermediate or resistant to ampicillin and 81 isolates produced an ESbetaL. Enzyme assays and molecular analysis revealed the presence of a determinant encoding PER-type ESbetaLs in 63 isolates, 52 strains were obtained in the first period, whereas 11 strains in the latter period, respectively. Genotyping by PFGE showed that all the PER-type producers isolated in the first period were clonally related, notwithstanding their origin from different wards, so a dissemination of strains had occurred. The isolates of the latter period harbored a conjugate plasmid codifying for a PER-type ESbetaL, so in this case, horizontal transfer of the resistance determinant could have played a relevant role in its dissemination. Analysis of digested genomic DNA by PFGE of *P. mirabilis* isolates unambiguously identified different bacterial clones responsible for the two epidemics.
Conclusions: The long persistence of PER-type producing *P. mirabilis* and their circulation in different hospital wards suggest their possible role as a long-term reservoir for further epidemic spread.

P519 Comparison of efficacy of alcohol-based hand antiseptics on reduction of release of skin flora

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Objectives: In the disinfection of the hand skin the most often used antiseptics are alcohol-based preparations. They are frequently combined with chlorhexidine (CH) because of their antimicrobial spectrum and residual activity. Recently there has been an increasing number of reports on allergic reactions to CH among the medical staff and the emergence of CH-resistant nosocomial bacteria. Therefore, the following question emerges: is it possible to reduce the application of antiseptics containing CH? The aim of the study was to compare in vivo the efficacy of selected alcohol-based antiseptics without and with CH.
Methods: We used three antiseptics containing: ethanol 52 g + 2-propanol 10 g (E); 2-propanol 36 g + 1-propanol 28 g (P); 2-propanol 30 g + 1-propanol 25 g + ethanol 20 g + CH 0.5 g (PCH). The tests were performed on 23 volunteers. According to EN 12791/97 the mean reduction factor (RF) of bacteria counts released from the fingertips before hand-wash and immediately after the hand-disinfection (2 × 3 mL, 3 min) as well as after wearing gloves for 3 h (sustained effect) were determined. The fingertips were rubbed for 1 min on the base of a petridish containing 10 mL of tryptone soya broth with neutralizer (3% polisorbate 80 + 2% lecitine L + 3% lubrol) to asses the release of skin bacteria. Statistical analysis was performed using Wilcoxon test.
Results: The immediate effect of rubbing of PCH ($\log RF = 1.2$; $SD = 0.8$) and P (0.9; 0.9) was equal, in contrast to the E (0.4; 0.7), which was significantly less effective ($P < 0.05$). There were no significant differences in reduction of skin flora assessed for PCH (1.1; 0.3) and P (0.9; 0.2) after 3 h of wearing gloves and both antiseptics showed sustained effect. In contrast activity of E against skin bacteria was significantly less evident (− 0.02; 0.8) and there was no sustained effect.
Conclusions: The propanon-based antiseptics demonstrate the rapid and strong effect on reduction of the release of skin flora from the hands and the significantly better efficacy than antiseptics containing ethanol. Antiseptics like P and PCH could be replaced one by another in contrast to ethanol-based antiseptics. It may give an argument for the limitation in application of antiseptics containing CH for hands disinfection.

P520 What is the medical staffs' knowledge and behavior for hand-washing in intensive care units?

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Objectives: Hand hygiene is considered the most important measure to reduce the transmission of nosocomial pathogens in healthcare settings. The

aim of this study is to investigate the hand-washing compliance of health care workers which working in neonatal unit and intensive care unit, and to compare their hand washing behavior with their answers in questionnaire.
Methods: Between February 1 and March 31, 2001 medical personals behavior was observed in intensive care and newborn units without telling any one by an infection control nurse. The observer recorded the patient contacts and activities for each participant during 3-h observation periods. After inspection of each person, a questionnaire form was requested and filled by all persons in second part of the study.
Results: The study group consists of 50 physicians, 38 nurses, 34 medical students and 19 nursing assistants. Hand-washing ratio was 7.8% before any application and 64.5% after application. Hand washing or rubbing occurred after 64.5% of total contacts. Nurses, physicians, students and nursing stuff washed their hands after any contacts 64.9, 68.0, 61.8 and 63.2%, respectively. Nurses had a significantly higher hand washing frequency before contact than the other groups ($P < 0.0001$). Liquid soap and water 76 (74.5%) was the most preferred hand washing method. None of the men washed their hands before any application, all of the hand washed persons before application were female ($P < 0.0001$). 52.0% of the hand washed people dried their hands, 13.2% of them with disposable towel. About 82.3% of the group believes that hand washing or disinfection is very important to reduce nosocomial infection and 86.5% of the staffs believes that theirs and medical personnel's hand-washing frequency is poor. The most important reason for poor hand-washing was inadequate hand washing and drying equipment.
Conclusions: The participants in this survey were well aware of this significance, but there is still a discrepancy between theory and practice. To increase hand-washing frequency education programs about this topic should be made.

P521 Choosing an optimal preservation system for some ointments

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Objectives: Ointments need protection against microbial spoilage, first of all in order to insure the consumer against potential danger arising from pathogenic germs contaminating the product, and second to guarantee long-term stability (shelf life) of the formula, even under use conditions. On the other hand, preservatives are often cited (just after perfumes) as the cosmetic ingredients responsible for skin irritation, allergies, and atopic reactions. To guarantee microbially 'clean' ointments, with long shelf life, and reduced amounts of preservative levels, is therefore the wish of every formulator.
Microbiological studies: To determine the efficacy of a preservative system, the so-called 'challenge tests' are carried out on the ointment formula: the product is artificially contaminated with a known amount (~1.105–1.106 microorganisms/g), and the decrease in total germ count with time (3, 5 or 7 days, 14 and up to 28 days) is measured. Unfortunately, no standard universally accepted method of challenge testing is agreed upon for cosmetic products (USP, BP, CTFA guidelines differ greatly from each other in scope and interpretation). Our own studies were carried out therefore in the following way the product to be tested (a typical cosmetic formula) is inoculated with, respectively:
1 a mixture of *E. coli* (IP52166), *S. aureus* (ATCC 9144), *P. aeruginosa* (IP5842) and *B. subtilis* (ATCC 6633) as representatives of Gram-positive and Gram-negative bacteria;
2 a mixture of *C. albicans* (IP 4872) and *S. cerevisiae* (ATCC 2601) to stand for yeasts; and
3 *Aspergillus niger* (ATCC 16604) as a member of the mold genus.

After 3, 5 or 7 days, 14 and up to 28 days, the total germ count of each contaminated product is determined by standard plate-count methods.
Conclusion: Mastering the risk of microbiological contamination of ointments during manufacturing, filling and over the time of storage and use is a tough challenge for the formulator and microbiologist. Even though it might be difficult today to establish a demonstration of illness contracted through the use of a contaminated off the shelf ointment, recalls (voluntary or forced by the authorities) owing to unacceptable levels of microbial loads, occur on a regular basis.

P522 Microbial contamination of oxygen manometers in selected hospitals of Tehran

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Objectives: Oxygen is used as an acceptable therapeutic option. One of the oxygen-therapy methods is application of humidifier, but unfortunately microbial contamination of these manometers may be leaded to patient's infection.
Methods: A total of 130 samples of water in humidifiers of three general hospitals in Tehran were studied. Incidence of microbial contamination was determined and its severity was assessed by colony count. These samples take from internal, ICU and CCU wards. All samples were inoculated on Brain–Heart Infusion agar and colony count were performed. The pure colonies were subcultured and isolated strains were determined by microbiological methods.
Results: Microbial contamination was 91.7%. Mixed microbial contamination was evident in 61.1%. The contamination rate is varied from 63.6 to 100% in different wards. Colony counts were changed in the range of 100 to More than 100 000 cfu/mL. There are more than 100 000 cfu/mL in 38.2‰ samples. Frequently isolated strain were *E. coli*, *Pseudomonas aeruginosa*, *Acinetobacter*, *Staphylococcus saprophyticus*, *Proteus*, *Diptheroid* and *Fungi*.
Conclusions: According to the high incidence of contamination in humidifiers, especial attention to infection transmission by these objects is necessary.

P523 Integrons as tools for epidemiological studies

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Objectives: Integrons, defined as genetic elements involved in the site-specific incorporation and excision of gene cassettes, are widespread among clinical isolates. This prevalence, coupled with the reported stability of entire structures over extended periods of time, brings to mind the potential use of integron detection as an epidemiological tool. In order to evaluate this contribution, we examined integron content of Gram-negative strains implicated in three distinct episodes of suspected cross-infection among inpatients.
Methods: Ribotyping of isolates and PCR directed to the conserved regions of integrons together with restriction-pattern analysis of amplification products were the adopted techniques.
Results: In the first episode, ribotyping identified a strain of *Acinetobacter* sp., isolated several times over a 3-month period, as responsible for an outbreak correlated with the use of mechanical ventilation in the intensive care unit. Sixteen patients and 18 isolates, including controls, were enrolled in this study. The second event concerned simultaneous isolations of *Pseudomonas aeruginosa* and Serratia marcescens from 13 patients submitted to bronchoscopic procedures. Molecular typing was able to characterize a pseudo-outbreak related to the inappropriate disinfection of the equipment. In both events, results obtained with integron detection and ribotyping were in agreement and correctly identified epidemiologically related strains. In the third episode, several isolates of *Acinetobacter* sp. were collected from distinct patients in the Intensive Care Unit over a 3-month period. The isolates belonged to the same genotype, as determined by ribotyping and PFGE. However, integron content was mostly distinct, indicating that no cross-infection had occurred.
Conclusion: The present results strongly suggest that integron detection can be considered an important tool for molecular epidemiology in hospital environments, helping in the quick elucidation of possible cross-infection cases, especially in critical wards such as intensive care units.

Resistance to TB drugs

P524 Molecular basis of isoniazid resistance in *Mycobacterium tuberculosis* strains from the St. Petersburg area of Russia

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Objective: To study the prevalence of isoniazid (INH) resistance associated mutation *katG* AGC(Ser)315 → ACC(Thr) in *Mycobacterium tuberculosis* clinical isolates from unrelated patients in St. Petersburg area of Russia in 1998–2001.
Methods: Susceptibility testing and *katG315* PCR-RFLP assay to detect phenotypically and genotypically INH resistance; IS6110-RFLP and spoligotyping to assess genetic relatedness of the strains.
Results: We revealed 95.2% prevalence of katG315ACC allele among 174 INH-resistant isolates. This mutation was not found among 39 INH-susceptible cases. The Beijing genotype was identified in 63% of INH-resistant strains. The katG 315AGC → ACC shift was more prevalent among the Beijing family strains versus strains of other genotypes: 100% versus 84.5% for new cases, 98% versus 86% for all cases.
Conclusions: High prevalence of this *katG315* mutation makes it useful genetical marker for detection of INH resistant tuberculosis (TB) in St. Petersburg area of Russia. Current TB epidemic in this region is due to the clonal dissemination of the MDR strains of the Beijing family. The ongoing transmission of these strains is likely to be the driving force of such a high prevalence of this mutation in *katG315*.

P525 Characterization of *rpoB* mutations by line probe assay and DNA sequencing in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates from Kuwait and Dubai

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Objectives: To identify mutations in rifampin-resistance-determining region (RRDR) of the *rpoB* gene in rifampin-resistant clinical isolates of *Mycobacterium tuberculosis* recovered from tuberculosis (TB) patients in Kuwait and Dubai.
Methods: A line probe assay and direct DNA sequencing of PCR-amplified RRDR were used to identify mutations in *rpoB* gene in clinical isolates of *M. tuberculosis*. A total of 29 rifampin-resistant and 12 rifampin-susceptible isolates were analyzed, recovered from TB patients from Kuwait and Dubai. The genotyping was performed by double-repetitive-element PCR (DRE-PCR) on the isolates which contained the similar kind of mutation.
Results: The line probe assay identified 28 resistant isolates as rifampin-resistant with specific detection of mutation in 22 isolates including one isolate that exhibited hetro-resistance containing both the wild-type as well as H526D mutation in RRDR while one of the isolate was identified as rifampin-susceptible. All the susceptible isolates were correctly identified. The DNA sequencing confirmed these results and led to further identification of five other mutations in the RRDR. These analyzes identified eight different mutations within RRDR with majority (14 of 29 or 48%) carrying mutations at codon 526 and one novel mutation (S522W) that has not been reported so far. The genotyping performed by DRE-PCR on the isolates carrying similar mutations showed that majority of these isolates exhibited variable DNA banding patterns.
Conclusions: The concordance of the line probe assay and DNA sequencing with phenotypic drug susceptibility testing was 96.5% (28 of 29). Mutations at codon 526 were most prevalent (14 of 29 or 48%), the highest reported so far. One new allele (S522W) of the *rpoB* gene was identified. The majority of isolates carrying similar mutations were genotypically different as they exhibited variable DNA banding patterns in DRE-PCR.
Acknowledgements: Supported by Kuwait University Research Administration grant MDI 301.

P526 Clinical and experimental reduction of mycobacteria resistance to TB drugs

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Objectives: To study possibilities to reduce resistance of mycobacteria to TB drugs. We treated a clinical strain resistant to streptomycin (S), rifampicin (R), isoniazid (H) and kanamycin (K) with dissolved ozone (PO_3) (0.5–4.0 μg/mL) during 1 h and inoculated it onto solid media containing different concentrations of TB drugs. After 3 weeks, we treated mycobacteria with PO_3 again; the cycle was repeated three times. After each treatment with PO_3 mycobacteria resistance to H was reduced and after the third treatment susceptibility to H was completely restored. Resistance to R was also reduced after each treatment, however, it remained high (640 μg/mL R). After the second treatment resistance to S was reduced, however, it was restored after the third treatment. Resistance was reduced due to eliminated hydrophobic properties of the external membrane and activation of intracellular catalase peroxidase complex. A total of 68 mice were infected with mycobacteria resistant to S, H, R, K and divided into five groups: 1 and 2—controls; group 3—treated with H; group 4—treated with H and PO_3 (0.5–4.0 μg/mL); group 5—treated with PO_3. The first mouse died in the fourth month of infection. Inoculation of the mycobacteria collected in the lungs of the mice treated with PO_3 resulted in growth of mycobacteria, susceptible to H. A total of 36 out of 56 sputum-positive patients with pulmonary TB, resistant to S, H, R, K received treatment with TB drugs plus intravenous injections of 400 mL PO_3 (0.5–4.0 μg/mL) every 5–7 days, 12–55 infusions. Within 4 months susceptibility to H and/or R was restored in 59.5% cases. By the fourth month susceptibility to H, R, K was restored in 47.2% cases. Out of 20 controls, resistance to one drug was reduced in 15% cases. Sputum conversion occurred in 76.7% cases, treated with H and/or R plus PO_3.

P528 Molecular mechanisms of isoniazid and rifampin resistance in *Mycobacterium tuberculosis* from Barcelona

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Objectives: To establish the presence and frequency of molecular mechanisms for *Mycobacterium tuberculosis* resistance to isoniazid (INH) and rifampin (RIF) in the urban area of Barcelona.
Methods: A sample of 53 INH-resistant strains of *M. tuberculosis* from Barcelona, 17 of which were also resistant to RIF, was analyzed by polymerase chain reaction (PCR) and direct sequencing analysis of the entire *katG* gene, *inhA* regulatory region, *oxyR–ahpC* intergenic region and *rpoB* core region. Each of these strains displayed a unique RFLP-IS6110 RFLP pattern.
Results: Of 53 INH-resistant strains, 29 (55%) had *katG* alterations consisting of small deletions (3–14 nucleotids) ($n = 3$) or nucleotid substitutions ($n = 26$) resulting in either aminoacid replacement or stop mutation. All strains but two had a single alteration. Although the nucleotid substitutions were detected all along the gene (codons 94, 172, 189, 204, 234, 315, 463, 560, 592, 678 and 728), the most common deduced alteration was a Ser-to-Thr (Arg) or (Asn) at postion 315 ($n = 17$). Seventeen (32%) of those 53 INH-resistant strains had a C to T ($n = 14$), T to C ($n = 2$) or G to T ($n = 1$) substitutions flanking the 5′ side of the presumed ribosome binding site, located in the region upstream of the *inhA* locus. In the intergenic region *oxyR–ahpC*, eight (15%) of those 53 INH-resistant strains had simple nucleotide substitutions (positions −6, −12, −37, −39) or insertions (one or two nucleotids; positions −38/−39 and −45/−46). Mutations in the core region of *rpoB* gene were detected for all 17 RIF-resistant strains but one. Six different *rpoB* mutations were identified at codons 441, 451, 456. The most common nucleotide variation was a Ser-to-Leu at position 456 ($n = 7$).
Conclusions: The finding that 64% of INH-resistant isolates have sequence alterations in either codon 315 of *katG* or *inhA* upstream region and that 94% RIF-resistant isolates have mutations in the core region of *rpoB* indicates the

need to develop assays to screen for the emergence of INH and RIF resistance in our area. Thus, diagnostic PCR-based methods using three target sequences spanning codon 315 of *katG*, upstream region of *inhA* and core region of *rpoB* would facilitate the rapid adjustment of treatment regimens in our area, in time to reduce the chance of developing further drug resistance and of transmitting resistant strains.

P529 The relationship between the isoniazid-resistant molecular mechanisms with minimum inhibitory concentration and the catalase activity in *Mycobacterium tuberculosis*

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Objectives: To study in the strains resistant to isoniazid the relationship between the minimum inhibitory concentrations (MIC), the catalase and peroxidase activity and the molecular mechanisms of resistance.
Methods: Fifty-three isoniazid-resistant strains of *Mycobacterium tuberculosis* were studied in the area of Barcelona. All of them were resistant to 0.1 μg/mL of isoniazid by radiometric system. The isoniazid MICs were obtained in the Middlebrook 7H10 medium. The catalase and peroxidase activities were determined with the Bogen reactive. The mutations were determined by PCR and were sequenced with ABI Prism (A.B.) Each of these strains displayed a different RFLP-IS6110 pattern.
Results: MICs of isoniazid were lower or equal than 2 μg/mL in 33 strains (62%), between 4 and 16 μg/mL in 17 (32%) and upper or equal than 32 μg/mL in three (6%). MICs of the 17 strains with mutation in the regulatory region of *inhA* ranged between 0.25 and 2 μg/mL, 71% of them having an MIC between 0.25 and 0.5 μg/mL. The MIC of the strains with mutation at codon 315 of gene *katG* was between 4 and 16 μg/mL. In nine of the 30 (30%) strains with MIC lower than 2 μg/mL and one of the three strains with MIC upper than 16 μg/mL no mutations were found. The three strains with MIC upper than 16 μg/mL lacked catalase and peroxidase activity. The presence of catalase activity was detected in 71% of the strains with mutation at codon 315 of *katG* but, however, the peroxidase at 1 h was negative in the 65% of these strains. Ninety percent of the strains with MIC lower than 2 μg/mL conserved the catalase and peroxidase activities.
Conclusions: The strains with MIC upper than 16 μg/mL lacked catalase and peroxidase activities. The strains with mutation at codon 315 of gen *katG* had a high level of resistance but the majority conserved the catalase. All the strains with MIC between 4 and 16 μg/mL had a mutation at the 315 codon of *katG*. The strains with mutations in the regulatory region of *inhA* had a low level of resistance and the majority conserved the catalase activity. The absence of catalase activity or the presence of a mutation at codon 315 of gene *katG* permits the rapid detection of the 96% of the *M. tuberculosis* strains with high level resistance.
Acknowledgements: This study was supported by the Grant 99/0049–01 from the FIS, Ministerio de Sanidad y Consumo, Madrid, Spain.

P530 Detection of mutations linked to rifampin- and isoniazid-resistance in *Mycobacterium tuberculosis* strains by molecular assays

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Objectives: Anti-*Mycobacterium tuberculosis* drug-resistance, mainly multidrug resistance (MDR-TB), represents an important public health problem in several countries. Recently, the World Health Organization and Center for Diseases Control and Prevention have elaborated anti-TB treatment guidelines: suggested regimens include primary (isoniazid, ethambutol, rifampin, streptomycin-capreomycin) and alternative drugs (cycloserine–terizidone, pyrazinamide–morfazinamide, kanamycin, *p*-amino salicylic acid). Rifampin and isoniazid resistance is related to mutations of *rpoB* and *katG* genes, respectively. Aim of our study is to identify the presence of these mutations in *M. tuberculosis* isoniazid- and rifampin-resistant strains, isolated in our Institute; to evaluate linkage between type of mutation and level of resistance; usefulness of easy molecular techniques for rapid detection of such mutations on body specimens.
Methods: Isoniazid- and rifampin-resistance was tested on 50 *M. tuberculosis* strains by single-strand conformation polymorphism (SSCP) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays, using HaeIII, PstuI, BsteII, BstuI enzymes. Drug-resistance of control strains was determined by cultural techniques (fluorimetry-BACTEC 9120).
Results: Cultural assay showed isoniazid- and rifampin-resistance in 6.12 and 2%, respectively (data confirmed by SSCP assay). Mutation of *katG*, linked to isoniazid resistance, was detected using BstuI enzyme, and mutation of *rpoB*, expression of reduced sensitivity to rifampin, using HaeIII. A total of 15 body specimens, *M. tuberculosis*-positive to conventional assays, were tested by SSCP technique. Similar results were found only where the mycobacterial load was elevated.
Conclusions: Epidemiologic reports of numerous cases of tuberculosis due to MDR strains induce to detect quickly both Mycobacteria and drug-resistance, in order to start rapidly an effective therapy. On this basis, molecular assays are useful for a rapid therapeutic decision.

P531 Activity of 11 antimicrobial agents against multidrug-resistant strains of *Mycobacterium tuberculosis* isolated during the 1998/1999 National Survey in Italy

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The SMIRA Study Group (Italian Multicentre Study on Resistance to Antituberculous Drugs)

Objectives: Determination of the in vitro activity to first- and second-line antituberculous agents of multidrug-resistant (MDR) strains (resistant to at least isoniazid, INH, and rifampicin, RMP) of *M. tuberculosis* (MTB) isolated during the 1998/1999 Italian Survey.
Methods: A network of 20 laboratories fulfilling the WHO/IUATLD quality criteria for determining the susceptibility to antituberculous agents isolated MTB strains from March 1998 to March 1999 and determined drug susceptibility to first-line agents. The strains were sent to the National Reference Laboratory of Villa Marelli, Milan, for re-testing drug susceptibility; MDR strains were then sent to the WHO Supranational Reference Laboratory of the Istituto Superiore di Sanità, Rome, for determination of the MICs of INH, RMP, streptomycin (SM), ethambutol (EMB) (first-line drugs), and ciprofloxacin (CIP), ofloxacin (OFL), sparfloxacin (SPA), moxifloxacin (MOX), rifabutin (RFB), rifapentine (RFP), amikacin (AK) (second-line drugs), in Middlebrook 7H11 agar. MIC was defined as the lowest drug concentration inhibiting >99% of the inoculum. Recommended critical concentrations (μg/mL) for separating susceptible from resistant strains were: INH, 0.2; RMP, 1; EMB, 8; SM, 2; tentative critical concentrations were: CIP, OFL, 2; SPA, MOX, RFB, RFP, 0.5; AK, 8.
Results: Out of 810 isolates, 649 were fully susceptible and 161 were resistance to at least one drug. Out of a total of 51 MDR strains, 18 strains were tested for MICs. All strains were resistant to INH (MIC range 1 to ≥64 μg/mL) and RMP (MIC range 2 to ≥64 μg/mL). Sixteen strains (89%) were resistant to SM (MIC range 4 to ≥64 μg/mL) and four (22%) to EMB (MIC 16 μg/mL). Three strains (17%) were resistant to CIP (MIC range 4 to ≥16 μg/mL) and SPA (1–4 μg/mL) and two (11%) to OFL (MIC range 8–16 μg/mL) and MOX (2–4 μg/mL). All strains were resistant to RFB (MIC range 2–32 μg/mL) and 17 (94%) to RFP (MIC range 2 to ≥0.64 μg/mL). Only one strain was resistant to AK (MIC ≥ 64 μg/mL). Although all drugs displayed a rather continuous MIC distribution, more than 50% of the strains resistant to RMP, RFP and AK showed MICs of ≥64 μg/mL.
Conclusions: These data showed that, among first-line drugs, EMB is active against most MDR strains tested. Among second-line drugs, all fluoroquinolones, but not rifamycins, were still active against most of these strains with none or low resistance levels. These observations can be of importance for treatment of MDR tuberculosis in Italy.

P532 In vitro resistance of *Mycobacterium tuberculosis* drugs in Bilbao, Spain

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Objectives: The aim of this study was to evaluate the current status of resistance of *Mycobacterium tuberculosis* to antituberculous drug in our hospital.
Methods: The in vitro sensitivity of 224 strains of *M. tuberculosis* isolated from 1977 to 2001 in 37 (16.5%) HIV (+) patients and 187 (83.5%) patients of general population against isoniazid (0.2 mg/L), streptomycin (6 mg/L),

ethambutol (7.5 mg/L) rifampicin (2 mg/L) and PAS (0.5 mg/L) was studied by the Canetti's proportions method.
Results: A total of 78 (35%) out of the 224 strains studied were resistant to at least one of the drugs tested. Among them, 74 (33%) showed resistance to streptomycin, 15 (6.7%) isoniazid, two (0.9%) PAS, 0 ethambutol (7.5 mg/L) and 0 rifampicin. Single drug resistance was observed in 66 (29.5%) strain with the following distribution: 62 (27.7%) to streptomycin, three (1.3%) to isoniazid and one (0.5%) to PAS. Resistance to two or three drugs was found in 12 (5.4%) strains with the following distribution: 11 (4.9%) were resistant to streptomycin + isoniazid, and one strain to streptomycin + isoniazid + PAS.
Conclusions: (1) The rate of resistance to INH was higher than 4%. (2) There was not multirresistance to the INH + RIF so combined therapy is useful. (3) There were no differences between both seropositives and seronegatives patients.

P533 Molecular epidemiology study of isoniazid-resistant tuberculosis in Barcelona, Spain

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Objective: To determine the diversity of DNA fingerprint patterns of isoniazid (INH)-resistant *Mycobacterium tuberculosis* strains from Barcelona and to evaluate their community transmission.
Methods: Between October 1995 and September 1997, a survey of drug-resistant tuberculosis in Barcelona area found a rate of 5.7% primary and 20.5% acquired drug resistance, 3.8 and 17.3% of which was to INH. One *M. tuberculosis* isolate was available for RFLP-IS6110 analysis from 61 (64%) INH-resistant strains. A 'cluster' is defined as two or more *M. tuberculosis* isolates with completely identical RFLP patterns when six or more bands are present.
Results: Of the 61 INH-resistant strains, 46 (75%) had a unique IS6110 RFLP pattern and 15 (25%) matched at least one other isolate, forming seven clusters. All clusters contained RFLP patterns with six bands or more. Twelve of fifteen clustered patients were in small clusters of two and the rest were in a cluster of three. Epidemiological relatedness was demonstrated in only two patients paired by RFLP.
Conclusion: RFLP analysis indicated that transmission of INH-resistant strains contributes substantially to the emergence of INH-resistant tuberculosis in Barcelona through short transmission chains. Therefore, in addition to the effective control tuberculosis programs adopted in our area, further improvement in early identification and treatment might prevent the development and transmission of INH resistance.

P534 Molecular fingerprinting of isoniazid-resistant clinical *Mycobacterium tuberculosis* isolates from Kuwait

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Objectives: To determine genotypic relatedness among isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* recovered from tuberculosis (TB) patients in Kuwait during a 3-year period (1998–2000).
Methods: Touchdown double-repetitive-element PCR (DRE-PCR), a rapid typing method based on specific amplification of segments of DNA located between two repetitive elements (insertion sequence, IS6110 and poly GC-rich sequences, PGRS) on the *M. tuberculosis* genome, was carried out for molecular fingerprinting of 53 isoniazid-resistant clinical isolates. The polymorphism at codon 463 in the *katG* gene was also determined and correlated with genotypic relationships among the isolates.
Results: The DRE-PCR classified 53 isoniazid-resistant *M. tuberculosis* isolates as having 28 distinct patterns with 24 being unique patterns. Majority of isolates (17 of 25) yielding a single DNA fragment in DRE-PCR were recovered from patients of South Asian origin. Two isolates recovered from some of the patients within a span of 2 months yielded the same typing pattern. Most of the isolates (70–90%) from South Asian and South-east Asian patients contained L463 in the *katG* gene. Nearly 85% of the isolates recovered from patients of Middle Eastern origin also contained L463 instead of R463 in the *katG* gene.
Conclusions: Nearly half of the isoniazid-resistant *M. tuberculosis* isolates recovered from TB patients in Kuwait yielded unique DNA banding patterns. The presence of a single DNA fragment of the same size in DRE-PCR from majority of isolates recovered from South Asian patients, known to carry <2 copies of IS6110 element, was most likely due to low copy number of IS6110 rather than any specific relationship among these isolates. The prevalence of L463 in the *katG* gene was higher than expected in isolates from Middle Eastern (mostly Kuwaiti) patients. The genotypic relatedness of two isolates, recovered from a Kuwaiti patient and a Filipino patient, was strongly indicated.
Acknowledgement: Supported by Kuwait University Research Administration Grant MI 116.

P535 Comparative evaluation of different molecular typing methods for multidrug-resistant *Mycobacterium tuberculosis* isolated in Italy

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The resurgence of tuberculosis and the emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* has stimulated the development of improved molecular typing methods, valuable instruments for tuberculosis surveillance, control and prevention. The insertion sequence (IS) IS6110 is the strain-specific marker of choice for the epidemiological typing of *M. tuberculosis* isolates, and the IS6110 restriction fragment length polymorphisms (RFLP) analysis is the "gold standard" for *M. tuberculosis* fingerprinting. For strains with few insertional sequences pulse field gel electrophoresis is the technique of choice. Both techniques are labor-intensive and time consuming and require large amount of high molecular weight chromosomal DNA. The aim of this study is to compare a rapid and easy to perform PCR-based typing strategy (Heminested Inverse PCR) to conventional methods for molecular characterization of MDR *M. tuberculosis* strains. We typed 61 strains (53 patients) isolated in Italy in a 2 years time frame (1998–2000 SMIRA Project) by Heminested Inverse PCR (HIP) of IS6110, IS6110 RFLP analysis and Pulsed Field Gel Electrophoresis (PFGE). HIP typing was 100% reproducible, showing identical patterns from the same isolate in different experiments. A total of 40 easily distinguishable fingerprints consisting in 2–10 fragments were identified by HIP. One strain gave only one fragment and was considered not typeable by this method. Among the 61 strains examined, only three small clusters of two, two and three patients were recognized. Multiple strains from the same patient gave identical pattern indicating short-term treatment failure. These results were confirmed by PFGE and IS6110 RFLP showing that sensitivity between the three methods was comparable. The strain not typeable by HIP was well characterized by the other two techniques. No evidence of predominant MDR strain circulating in Italy was found by any of the three typing methods. In conclusion, Heminested Inverse PCR is a reproducible, easy to perform method for molecular typing of MDR *M. tuberculosis* strains with high potential for rapid identification and monitoring of outbreaks.

P536 Drug resistance in *Mycobacterium tuberculosis* in Turkey: a retrospective study by BACTEC TB system

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Objectives: With the recent evolution of multidrug-resistant strains of *Mycobacterium tuberculosis*, the need for prompt and accurate antimicrobial susceptibility testing has become a real necessity. So, we retrospectively evaluated the rate of drug susceptibility of *M. tuberculosis* to four primary antituberculosis drugs in Gülhane Military Medical Academy between January 1998 and October 2001.
Methods: Drug susceptibility tests to isoniazid (INH), streptomycin (S), ethambutol (E), and rifampin (R) were performed by using the radiometric BACTEC 460 TB system and the records of the laboratory were evaluated.
Results: The drug susceptibility tests of 1032 *M. tuberculosis* complex isolates of 454 patients were evaluated. We found that 85.3% of isolates were susceptible to all four major drugs, whereas 14.8% were resistant to at least one drug. Mono resistance rate was 10.8% and three strains (0.7%) were resistant to all four drugs. Multidrug resistance (MDR) was accounted for 1.6% of isolates while polyresistance rate other than MDR was observed in 15 (3.3%) isolates.
Conclusions: Although our results don't represent the drug resistance of entire country, the present study is one of the few retrospective studies about the

prevelance of the drug resistance of *M. tuberculosis* in Turkey, but gives good information about antimycobacterial drug resistance rates in our country. Continuous monitoring of drug resistance in *M. tuberculosis* is essential in order to support national tuberculosis control programs although our resistance rates are below the dangerous zone.

P537 Multidrug-resistant tuberculosis in Portugal: molecular characterization

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Objectives: An alarming increase in multidrug-resistant tuberculosis (MDR-TB) has been reported in several countries including Portugal, particularly among patients infected with human immunodeficiency virus (HIV). In Portugal, nosocomial outbreaks of MDR-TB related to HIV-infected patients have been described. A particular MDR-TB cluster (cluster A) including 76% of the analyzed strains was identified in 1998. These isolates had been collected in several hospital units all over the country. Although strains isolated in hospital and public health laboratories are currently notified to Portuguese authorities, the true magnitude of the problem is unknown. Our objectives were to evaluate the prevalence and clustering of MDR-TB strains isolated in Portuguese laboratories, in particular cluster A, and to set up a national database of MDR-TB strains genotypes.
Methods: MDR-TB strains were collected in several hospital and public health laboratories all over the country, in the year 2000. We included all the strains (susceptible and resistant strains) from two hospital units in Lisbon. We performed Restriction Fragment Length Polymorphism analysis using IS6110 as a probe (RFLP-IS6110) to all the isolated strains.
Results: RFLP-IS6110 analysis revealed that cluster A strains are still responsible for 49% of all the multidrug resistance tuberculosis analyzed in this study. We have also found 12 new RFLP patterns corresponding to 12 never detected strains. We also found for the first time the existence of cluster A patterns in susceptible strains (19% of cluster A strains).
Conclusion: In view of the above findings, we can conclude that cluster A strains are still spread all over the country and continues to be responsible for the main resistant tuberculosis. RFLP-IS6110 analysis is not sufficient to molecularly define MDR-TB strains in Portugal, as we have found identical patterns in susceptible and resistant strains. The new detected patterns may correspond to new cases of MDR-TB and epidemiological investigation must be conducted in order to evaluate this situation.

P538 Genotypic and phenotypic characterization of rifampin-resistant *Mycobacterium tuberculosis* strains

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Objectives: The characterization of *Mycobacterium tuberculosis* strains resistant to antituberculous drugs is a relevant issue. Detection of resistance to rifampin (RMP) is of particular importance, as it is a marker for multidrug-resistant MTB strains.
Methods: A total of 11 RMP-resistant and 11 RMP-susceptible strains (as determined by the agar proportion method) were selected for analysis. The genotypic characterization of the rpobeta core region was performed by DNA sequencing and single-stranded conformation polymorphism (SSCP). For the phenotypic characterization, the RMP minimal inhibitory concentrations (MICs) were obtained by E-test.
Results: All resistant strains showed a single mutation and all susceptible control strains showed no mutation in the rpobeta core region. A high mutation variability was detected, seven different mutations recorded among the 11 resistant strains. The mutations were located at the three codons more frequently associated with rifampin resistance: 516 (two strains), 526 (four strains), 531 (five strains). The sensitivity and specificity of genotypic screening by SSCP was 54 and 100%. SSCP allowed mutations to be detected at codon 526 but not all at 531 or 516. The phenotypic characterization indicated a great difference in rifampin MICs for resistant (12 to >256 μg/mL) and susceptible (<0.05 μg/mL) strains. The highest MIC values were not linked to mutations in any specific codon.
Conclusions: (1) All RMP-resistant MTB strains showed single mutations in the rpobeta core region. (2) A high variability of mutations was recorded. (3) SSCP has a high specificity but lacks sensitivity, requiring additional DNA sequencing. (4) E-test showed a good correlation with DNA sequencing.

Candida

P539 Enzymatic properties of *Candida* spp. strains isolated from various clinical specimens

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Objectives: The aim of this study was to examine enzymatic properties of *Candida* spp. strains which were isolated from various clinical specimens.
Methods: A total of 420 yeast strains belonging to *Candida* spp. were examined. These strains were isolated from the following clinical specimens: vaginal swabs, oral and throat swabs, urine samples, tissue samples from patients after surgery for larynx carcinoma, from peritoneal fluids, from large intestine samples, from nail samples, from sputum, bile, sperm and pus samples, and from groin swabs. The yeasts were identified on the basis of their morphological characteristics and according to their biochemical properties examined by means of API 20C AUX kits (bioMérieux). The ability to release lipases and proteases was examined as well. The release of lipases was examined after incubation of 30 and 37 °C. The proteases release was examined using media with human albumin and bovine caseine.
Results: Among the 420 *Candida* spp. strains examined, the following were most commonly observed: *C. albicans*, 317 strains (75.5%) and the next: *C. krusei*, 52 strains (12.4%), and *C. tropicalis*, 33 strains (7.8%). However, the species: *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, *C. pelliculosa*, *C. gulliermondii*, *C. humicola* were less frequently observed. All strains of *Candida* spp. were divided into 13 biotypes according to their lipolytic and proteolytic activity. The strains of *C. albicans* were divided in eight different biotypes. Biotype I was dominant among *C. albicans* and it included 150 strains examined. This biotype was characterized by lipolytic activity both in 30 and 37 °C and proteolytic in the presence of albumin and caseine. Other strains were assigned to different biotypes.
Conclusions: The enzymatic examination of yeasts may be helpful in diagnosing *C. albicans* and in epidemiological characterization of candidosis.

P540 Mechanisms of antifungal activity of vaginal diamines on *Candida* species

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Putrescine and cadaverine are bacterial diamines that are produced in significant amounts by the overgrowing bacterial population found in the vaginal fluid of women with BV [1]. Both amines showed a dose-dependent inhibition of germ tube formation by *C. albicans* as well on budding of non-albicans strains [2].
Objectives: To evaluate the mechanisms of antifungal activity of putrescine and cadaverine, estimated as the minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) on the most commonly pathogenic *Candida* species.
Methods: Seven clinical vaginal isolates of *Candida* were tested at serial concentrations of putrescine and cadaverine (Sigma–Aldrich), in RPMI-1640 medium (Sigma). The fungistatic activity was studied with the fluorescent probe FUN-1 and observation under epifluorescence microscopy and flow cytometry; MIC was also determined according the NCCLS protocol M 27-A. The fungicidal activity was assayed by MLC and viability counts (cfu). Membrane alterations induced in the yeast cells were evaluated by staining with propidium iodide (PI) followed by flow cytometry.
Results: MIC ranged from 15.6 to 62.5 μg/mL and MLC was 1–6-fold higher the corresponding MIC for cadaverine; MIC ranged from 62.5 to 250 μg/mL and MLC was 4–8-fold higher the MIC for putrescine. No correlation was

found between MIC and MLC to amines and the susceptibility pattern to antifungals. The concentrations associated to an extensive rate of killing induced an extensive permeation to PI, related to a direct damage of the cytoplasmic membrane. Lower concentrations induced a marked metabolic impairment.

Conclusions: Both putrescine and cadaverine, at concentrations commonly found in the vaginal fluid of women with BV, showed a marked antifungal effect, particularly fungistatic, on vaginal isolates of *Candida*. Our results firmly support the hypothesis that the presence of these and possibly other bacterial amines produced by anaerobes in the vaginal flora seen in BV may explain why candidosis is rarely seen in such instances.

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P541 *Candida* spp. and *Aspergillus* spp. emerging cause of nosocomial breakthrough fungemia in hospitalized patients

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Objectives: Invasive *Candida* spp. and *Aspergillus* spp. are the emerging causes of nosocomial infections in patients (pts) with cancer, bone marrow transplant recipients (BMT) and intensive care unit (ICU). The authors want to determine the causes of nosocomial mycosis infections and the efficacy of the therapy in patients with haematological disorders and risk device factors.
Methods: A retrospective study from 1998 to 2001 for the invasive *Candida* spp. and *Aspergillus* spp. was evaluated in 26 pts (14 males and 12 females), 62 ± 9 years old; 7 pts had Aspergillus pneumonia (AP) with LLC, NHD, HD, neutrophils $<500/cm^2$ (3 pts), corticosteroids and chemotherapy (4 pts) and airborne infection (1 pt). In 19 pts were isolated from blood culture *C. albicans* (13/19 pts), *C. sake* (1/19), *C. glabrata* (1/19), *C. tropicalis* (3/19), *C. parapsilosis* (1/19). Seventeen patients (17/19) were admitted in ICU and they had nosocomial disseminated mycosis caused by *Candida* spp. (12/17 *C. albicans*, *C. glabrata* 1/17, *C. tropicalis* 3/17, *C. parapsilosis* 1/17); in the urine there were different strains of *Candida* (*C. albicans* 4/17, *C. tropicalis* 1/17).
Results: The risk device factors (1 or 2 devices contemporary) were: central venous catheters (CVC) in 19 pts, pulmonary arterial catheters (2 pts), nutritional parentheral therapy (NPT, 2 pts), PEG (1 pt), urinary tract catheters (UTC, 3 pts). The treatment in AP was with amphotericin B (AMB, 5/7 pts) and itraconazole (2/7 pts), the exitus rate was 1/7 (14%). In patients with candidemia, the treatment was with fluconazole (7/17) or amphotericin B (8/17) and 2 pts (2/17) died before the therapy. The mortality rate for candidemia was 10/17 (58%) with less mortality in the AMB group.
Conclusions: Nosocomial infection in ICU and in patients with haematological disorders are very frequent, severe with a high mortality rate. High dose chemotherapy, BMT and airborne infection are the factors for emerging AP; the CVC is the most common risk device factor for disseminated *Candida* spp. in ICU and the treatment with AMB had better results than fluconazole.

P542 Prophylaxis and treatment of *Candida* infection in patients with infected pancreatic necrosis

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Objectives: The incidence of fungal infection has increased during recent decades. The purpose of this review was to determine the incidence of *Candida* infection in cases with infected pancreatic necrosis (IPN), to determine the most frequent risk factors, and to assess the best treatment approach for disseminated candidal infection and the role of prophylactic fluconazole medication.
Methods: Between 1986 and 1995, 30 (21%) of the 145 cases of IPN identified were infected with *Candida albicans*. Risk factors determined in patients with *Candida* infection included the presence of necrotic tissue, and the use of broad-spectrum antibiotics, intravascular catheters and parenteral nutrition. In the last 5 years prophylactic fluconazole medication was applied in all 50 patients with IPN.
Result: A total of 29 of the 30 *Candida*-infected cases involved mixed bacterial and fungal infections. A total of 16 of the 30 patients displayed fungal colonization, while the other 14 patients had disseminated fungal infection. There were two deaths in the disseminated group, but no mortality occurred in the colonization group. In both fatal cases, flucytosine was applied. When fluconozole was used against disseminated fungal infection, no mortality was noted. Following prophylactic fluconozole medication, *Candida albicans* colonization appeared in three patients, without any mortality, but one patient suffered fluconazole-resistant *Candida krusei* disseminated infection with a fatal outcome in spite of amphotericin-B medication. In 46 of the 50 patients with IPN, there was no evidence of fungal infection postoperatively.
Conclusion: The combination of adequate surgical treatment with effective antibiotics and early antifungal therapy is the ideal management approach for IPN with *Candida* infection, and fluconazole may be regarded as an efficient drug for the prophylaxis of candidiasis.

P543 Evaluation of Candida ID, a chromogenic medium for presumptive identification of *Candida* species

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Objective: Due to the increasing number of human severe infections caused by *Candida albicans* and other species of this genus, it is now mandatory to identify them correctly. Our purpose is to evaluate the ability of a chromogenic medium, Candida ID (bioMérieux, Spain) to differentiate *Candida albicans* from other species belonging to this genus.
Methods: We have studied 351 different clinical isolated from Hospital Universitario de Canarias, a 670-bedded tertiary setting. The samples included 113 urines, 52 vaginal swabs, 43 from blood cultures, 29 from nails, 25 from wounds, 20 catheters, 14 stools and 55 from miscellaneous origin. Distribution of the microorganisms were: 150 *Candida albicans*, 67 *C. tropicalis*, 61 *C. parapsilosis*, 37 *C. glabrata*, 5 *C. krusei* and 31 other species. All yeasts were isolated following standard proceedings using Sabouraud as primary medium. The isolates were stored in distilled water until the assay was performed. Then they were subcultures in blood plate 24 h at 35 °C and subsequently cultured in Candida ID where they were inspected after 24 and 48 h. Colony color was judged according manufacturer's instructions. Our reference identification method was API ID 32C (bioMérieux, Spain).
Results: Results are reported after the 48 h inspection. Plates were read by at least two of the authors independently. Of the 150 *C. albicans*, 138 showed blue colonies (sensitivity was 92%). Nine no-albicans yeasts also appeared blue, including three *C. tropicalis*, two *C. glabrata*, one *C. guilliermondii*, one *C. laurentii* and the only two *Trichosporon cutaneum* found in the study (specificity was 95.5%). The positive predictive value for presumptive identification of *C. albicans* was 93.9% and the negative predictive value was 94.1%. A total of 81 pink colonies were observed, mainly for *C. tropicalis* ($n = 58$), but also for other 23, including *C. famata* (five out of six found), *C. glabrata* ($n = 4$), *C. albicans* ($n = 3$). We found 123 white colonies: all isolates of *C. parapsilosis* ($n = 61$), 31 out of 37 *C. glabrata* and 31 from other species.
Conclusions: Candida ID is a simple and easy-to-read medium and is able to support the growth of all the yeast tested in this assay. We estimate adequate its sensitivity and specificity and appears to be as a valuable tool in accelerating the discrimination between *C. albicans* and *C. no-albicans* species.

P544 Effect of slime production of *Candida* species on in vitro antifungal susceptibility test of fluconazole

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We searched for the effect of slime production on in vitro fluconazole susceptibility of blood isolates of *Candida* spp. and if ever, changes in MIC and biofilm production of subsequent subcultures of these strains. Additionally, we looked for any effect of 8% glucose, which is suggested to induce biofilm formation on susceptibility test results.
Methods: Each *Candida* strain was studied at three occasions: i.e., after removal of blood cells, directly from blood culture bottles when Bactec 9240 system gave growth signal (denoted as "a") and two subsequent subcultures with 1 week intervals (denoted as "b" and "c")
(1) Fluconazole MIC determinations were performed on 'a', 'b', and 'c' of each strain by macrodilution method using RPMI-1640 medium according

to NCCLS M 27-A guidelines and modified form of this guideline by adding 8% glucose to the medium.
(2) Slime production of each strain was determined at three occasions (a, b, c) by using 8% glucose containing Sabouraud Dextrose Broth.
(3) Slime layer of each strain (a, b, c) was also investigated by means of transmission electron microscopy (TEM).
Results: Of the seven *Candida* isolates, two were *C. albicans*, three *C. parapsilosis*, and two *C. pelliculosa*. Non-slime producer two strains showed no polysaccharide projections on TEM; however, three strains with 2+ and 3+ slime production displayed markedly thick polysaccharide layer on TEM which showed gradual decline and, even vanished in subcultures.
Conclusions: Fluconazole MICs were completely similar indicating that thickness or presence of slime layer had no effect on susceptibility results of initial isolates and subcultures. Addition of 8% of glucose also did not cause any change in fluconazole MICs of either initial isolates or their subcultures with or without slime layer.

P545 The genotypic and proteolytic diversity of *Candida* spp. isolates from children with bone marrow transplantation and adult patients with haematological malignancies

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The purpose of this study was to determine the genotypes and proteolytic activity of *C. albicans* strains spread in the haematological units of two distinct hospitals of the same town. The strains (58 *C. albicans* isolates from 35 children with bone marrow transplantation and 32 *C. albicans* and 4 *C. dubliniensis* from 27 adult patients of haematology unit) were identified by the germ tube test, growth on Chromagar-Candida medium and by biochemical test ID-32C (bioMérieux, France). Genomic DNA was extracted from each isolate and PCRs were performed using CA-INT-L and CA-INT-R primers that detected the presence of the transposable intron in the 25S rDNA. Genotypes A, B, D resulted in a single PCR product (450, 840 and 1080 bp, respectively), and genotype C had two (450 and 840 bp). Activity of secretory proteinase was determined on the basis of clarification around the yeasts colony on agar medium containing 0.2% bovine serum albumin.

Genotype A was prevalent in children as well as in adult patients, but the percentage of genotype A isolates was higher in children (77.6%) than in adults (58.3%). Genotype B ranked second in prevalence (12 and 16.7% in children and adults, respectively) and genotype C ranked third (10.4 and 13.9%, respectively). Four strains isolated from adult patients were classified as genotype D identical to that of *C. dubliniensis*. We did not find significant differences in the percentage of genotype A, B, C, and D between the isolates from respiratory tract (69, 12.7, 14.6, and 3.7%, respectively) and the isolates from digestive tract (64, 16, 12, and 8%, respectively). In the genitourinary tract, we found only genotype A (85.7%) and B (14.3%). Multiple isolates from different body sites of the same patients revealed that most patients (16) were colonized by strains of the same genotype (A, C or D), and some patients (6) by two genotypes.

Examinations of the proteolytic activity of *C. albicans* and *C. dubliniensis* revealed no difference in the proteolytic activity of genotypes A, B and C, but *C. dubliniensis* showed a lower activity than *C. albicans*.

P546 The influence of antiretroviral therapy on oral mucosa candidiasis in HIV patients

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Cracow, PL

Objective: Oral mucosa candidiasis is the most common one among the numerous HIV infection manifestations. It may be the earliest sign of the disease. Long lasting observations give evidence that antiretroviral therapy is beneficial also in the cases of this opportunistic infection because it reduces both the number and severity of relapses, however, the prolongation of the patients' survival time creates the need of antifungal therapy prolongation, and thorough observation of its effectiveness and methods. We decided to analyze the efficacy of antifungal therapy on the development of oral mucosa candidiasis in the inpatients of the Jagiellonian University Medical College Hospital in Cracow.
Methods: The drug susceptibility was tested with nine antifungals. The susceptibility to 5-fluorocytosine, amphotericin B, nystatin. Miconazole and ketoconazole was tested by using ATB Fungus dilution test (bioMérieux) according to the procedures worked out by the manufacturer. The susceptibility to fluconazole, itraconazole, clotrimazole and tioconazole was evaluated by the disk-diffusion method (DHN Ltd., Poland). The study was carried out in 75 patients with confirmed HIV infection. The antiretroviral therapy was administered 34 patients (group I) and was not in 41 patients (group II). The set of antiretroviral drugs consisted of two inverse transcriptase inhibitors and one HIV protease inhibitor.
Results: Fungi were isolated from 69 out of 75 HIV patients. However, the signs of oral mycosis were found only in 50 patients, most often the pseudomembranaceous (21 patients) and atrophic (20 patients) types. The least common were the hypertrophic (seven patients) and angular (two patients) types. The signs of candidiasis were found in 23 out of 34 patients (67.3%) in the group treated with antiretroviral agents. In the untreated group, the signs were found in 27 persons (65.9%). We have shown a decrease in the number of fungi present in the oral cavity in patients under antiretroviral treatment as well as higher susceptibility to fluconazole.
Conclusions:
1 The susceptibility of fungal strains isolated from patients under antiretroviral therapy to fluconazole was higher than that of the strains from untreated patients.
2 The antiretroviral therapy of HIV patients did not influence the prevalence of oral mucosa mycosis manifestations, however, significantly reduced the number of fungi present in the oral cavity.

P547 Oral amphotericin B prophylaxis against candidemia in very low birth weight infants

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Objective: To evaluate the efficacy of oral amphotericin B solution for the prevention of CBSI in high-risk VLBW infants.
Methods: A prospective study with historic control comparing oral amphotericin B solution (OAMB) vs. no prophylaxis for the highest risk neonates in the neonatal intensive care unit (NICU) of a major children medical center in Israel. All VLBW infants who were born between January and December 2000 and had one of the following inclusion criteria: required mechanical ventilation, had central venous catheters (CVC), received total parenteral nutrition (TPN), required antibiotic or corticosteroid therapy were included in the study group and received OAMB as long as the above parameters persisted. The historic control group of patients included all VLBW infants who were born between January and December of 1998 and had the same inclusion criteria as above. Demographic and clinical data were retrieved from the patient's medical records retrospectively and prospectively for the control and the study group, respectively. All patients were evaluated for the development of CBSI and adverse side-effect of the treatment.
Results: Two hundred and seventy-eight patients were included, 151 patients in the study group and 127 patients in the control group. There were no significant statistical differences between the two groups regarding age, sex, gestational age, birth weight, mechanical ventilation, presence of CVCs, antibiotic and corticosteroid therapy, development of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD) and breast feeding. In the study group, 5 patients (3.3%) developed CBSI compared to 28 (22%) in the historic control group of patients ($P < 0.001$). The crude mortality was 15 and 18% in the study group and in the control group, respectively. The main *Candida* species were *C. glabrata*, *C. albicans* and *C. parapsilosis*. No serious adverse events occurred in the study group.
Conclusions: Oral amphotericin B solution is safe and may be effective in the prevention of CBSI in high-risk VLBW infants. Larger prospective randomized studies to evaluate the efficacy of OAMB solution on CBSI in VLBW are warranted before it can be recommended for routine use in this high-risk population.

P548 Antifungal drug susceptibility of *Candida* spp. in a neonatal intensive care unit (NICU)

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Objectives: Antifungal drug resistance has been demonstrated as an important medical problem among *Candida* spp. worldwide. *C.* non-*albicans* spp. have emerged as frequent causes of neonatal candidiasis with potential implications on antifungal management in the NICU.
Methods: A total of 91 isolates (21 obtained from blood in 1998–2000 and 70 obtained from mouth or perineum in 1999) were studied. Minimal inhibitory (MIC) and fungicidal (MFC) concentrations of amphotericin B (AMB), flucytocine (5FC), fluconazole (FLU) and itraconazole (ITRA) were evaluated by NCCLS micromethod. No antifungals are used in the NICU prophylactically. Dose-dependent susceptibility (S-DD) was considered as 16–32 for FLU or 0.25–0.5 mg/L for ITRA.
Results: MIC and MFC for the most frequent *Candida* spp. isolated are presented in Table 1 (R: resistant).

Table 1

Candida spp. (*n*)	AmB	5FC	FLU	ITRA
C. albicans (39)				
MIC	<0.06–0.5	<0.25–>128	<0.25–4	<0.06–<0.25
MFC	0.06–0.5	(1-R) 0.25–>128	4–>128	(2 S-DD) 4–>32
C. tropicalis (22)				
MIC	0.125–0.5	<0.25–2	0.25–>128	0.06–>32
MFC	0.25–1	<0.25–16	(4 S-DD, 1 R) 4–>128	(6 S-DD, 9 R) 32–>32
C. krusei (7)				
MIC	<0.06–0.5	<0.25–4	0.5–64	<0.06–16
MFC	0.125–0.5	0.5–128	(1 S-DD, 1 R) 4–>128	(2 R) 0.25–>32
C. parapsilosis (7)				
MIC	0.06–0.25	<0.25–0.5	0.25–2	<0.06–1
MFC	0.125–0.25	0.25–>128	4–64	(1 S-DD, 1 R) 0.25–32
C. glabrata (6)				
MIC	0.125–0.5	<0.125–0.125	<0.25–8	0.06–1
MFC	0.125–0.5	<0.125–0.5	128–>128	(2 S-DD, 2 R) 32–>32

Conclusions: NICU isolates of most frequent *Candida* spp. remain completely susceptible to AmB. *C. albicans* also remains highly susceptible to azoles. In contrast, non-albicans spp. exhibit decreased susceptibility especially to ITRA. Only 32% of *C. tropicalis*, 33% of *C. glabrata* and 57% of each *C. krusei* and *C. parapsilosis* were susceptible to ITRA. The decreased susceptibility of certain non-*albicans* spp. to azoles, particularly ITRA, needs continuous monitoring in the NICU and further studies of its causes.
Acknowledgement: Partially supported by PENED'99.

P549 Interaction of sertraline with *Candida albicans* selectively attenuates fungal virulence in vitro

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Objectives: Selective serotonin reuptake inhibitors (SSRIs) are used as first-line therapy for premenstrual syndrome and antidepressants. Recently we found, that sertraline (SSRI, Tresleen, Vienna, Austria) has in vivo and vitro antifungal activity against *Candida* spp. In this study, we investigated whether the interaction between sertraline and *Candida albicans* affects the virulence properties of this fungal pathogen in vitro.
Methods: The morphology of *C. albicans* was investigated by assessing hyphal elongation, and the extracellular phospholipase activity was measured by the egg yolk agar method. In addition, an assay for *Candida* secreted aspartyl proteinases (Saps) was investigated. The ability of macrophages to inhibit germination of blastoconida treated with and without sertraline was examined by a microbiological assay.
Results: Sertraline treatment of *C. albicans* significantly ($P < 0.01$) affected hyphal elongation, phospholipase activity, production of secreted proteinases and fungal viability. Monocyte derived macrophages (MDMs) treated with sertraline reduced inhibition of germination of blastoconidia in comparison to MDMs alone.
Conclusions: Our findings suggest that the interaction between sertraline and *C. albicans* diminish the virulence properties of this fungal pathogen.

P550 Susceptibility patterns of clinical isolates of *Candida krusei* isolated from hematological patients

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Objectives: Estimation of frequency of *C. krusei* isolation from specimens obtained from hematological patients and assessment of their susceptibility to antifungal agents.
Methods: Specimens sent for culture comprised: stool, sputum, blood, urine, as well as swabs of the throat, mouth, wound and peritoneum. The isolation and identification of cultured fungi was done according to standard mycological procedures and commercially available tests (bioMérieux, Sanofi Diagnostics Pasteur). Patterns of susceptibility to antifungal agents were assayed using Fungitest (Sanofi Diagnostics Pasteur).
Results: In a total of 342 specimens, 402 strains of yeast-like fungi and moulds were isolated. The most commonly isolated species were *C. albicans*, 172 strains (42.78%), *C. glabrata*, 93 strains (23.3%) and *C. krusei*, 34 strains (8.45%). Most fungal strains were isolated from stools, 235 (58.45%), sputum, 58 (14.42%) and blood, 24 (5.97%). Among 34 strains of *C. krusei*, 24 were cultured from stools, 3 from blood, 4 from sputum, 1 from urine and 2 from throat swabs. All isolates of *C. krusei* were resistant to 5-fluorocytosine, miconazole, and fluconazole. A total of 33 strains (97.05%) were resistant to ketoconazole and 28 strains (82.35%), to itraconazole. All were susceptible to amphotericin B.
Conclusion: 1. *C. krusei* comprises 8.45% of all fungal isolates cultured from hematological patients. 2. The strains are characterized by resistance to many commonly used antifungal agents, while remaining susceptible to amphotericin B. 3. Emergence of multidrug resistant *Candida* spp. warrants modification of antifungal therapy and prophylaxis, particularly in hematological patients.

P551 Error associated with the phenotypic identification of *Candida* species

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Objective: To assess the reliability of phenotypic methods in routine use for speciation of *Candida* isolates from blood.
Methods: Fifty-nine isolates of *Candida* spp. were obtained from the blood cultures of inpatients at Belfast City Hospital during 1994–2000. These were identified phenotypically at the time of isolation using the bioMérieux API 32C (R) scheme. Total genomic fungal DNA was extracted and PCR carried out initially using the small internal transcribed spacer (ITS) region primers, ITS3 and ITS4. Sequences obtained were compared with those stored in GenBank using BLAST (R) alignment software. When the phenotypic and genotypic findings differed a further PCR reaction was set up amplifying the large ITS region (ITS1-5.8S-ITS2) to corroborate the initial molecular identification.
Results: PCR sequence homology >98% was achieved in all cases. Five species differed when the genotype was compared to the phenotype of both *albicans* and non-*albicans* species; this represents an error rate of 8.5% in phenotypic identification. The errors correlated with both false-positive and false-negative results in the detection of acid production from galactose and saccharose in the API scheme. Two of the misidentifications were *C. dubliniensis*; these had both been phenotypically identified as *C. albicans*

and represent the first two reports of bloodstream infection (BSI) caused by this organism in northern Ireland. Interestingly, both occurred in patients with gynaecological malignancy who had none of the typical characteristics (HIV-seropositivity, neutropenia, transplantation or advanced liver disease) of *C. dubliniensis* BSI thus far described in the literature.

Conclusion: Given the importance of correct identification of organisms causing BSI for both epidemiological reporting and appropriate patient management there is evidence of need to improve current methods of candidal speciation. This might be achieved either by use of more discriminating phenotypic testing or by incorporating molecular methods into routine practice.

P552 Evaluation of the combined measurement of circulating *Candida* mannan and antimannan antibody levels by an enzyme immunoassay (EIA) in patients with proven invasive candidiasis

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Objectives: Because of the low sensitivity of cultures and the lack of specificity of clinical symptoms, diagnosis of systemic candidiasis remains difficult. In this study, we evaluated two recently developed EIAs for diagnosis of invasive candidiasis.

Methods: During a 10-month period, we prospectively collected 118 serum samples from 29 patients with proven candidemia. Patients were hospitalized in intensive care ($n=9$), surgical ($n=7$), haematological ($n=5$), and other units ($n=8$). For each patient, serial samples were taken within 3 weeks after *Candida* isolation from blood cultures. Forty-five control sera were included: 40 from 40 healthy blood donors and 5 from 5 hospitalized patients with *Candida* colonization without evidence of invasive candidiasis. *Candida* mannan and antimannan antibodies were determined in all 163 sera using a one-stage sandwich EIA for the detection of mannan (Platelia® *Candida* Ag, Sanofi Pasteur), and a two-stage EIA for the detection of antimannan antibodies (Platelia® *Candida* Ab, Sanofi Pasteur). A mannan threshold concentration of 0.5 ng/mL was used as the limit for antigenemia; a titer of 10 AU or more was considered to be a positive result by the antimannan antibody test.

Results: Sixty-six (56%) of the 118 sera had antibody titers that exceeded 10 AU and 60 (51%) displayed positive antigenemia values. Antigenemia was present in 38% of samples with and in 67% of samples without a positive antibody test. Only 25 specimens (21%) had both circulating mannan and significant antimannan antibodies. Positive antibody titers and positive antigenemia values were obtained in 7.5%, respectively, 5% of healthy blood donors and in one of five colonized patients. Sensitivity, specificity, and positive and negative predictive values, calculated per patient, for the antigen and antibody EIA and for the combined use of both EIAs are shown in Table 1. No difference in results could be demonstrated between *C. albicans* ($n=18$) and other *Candida* species (*C. glabrata*, $n=7$; *C. tropicalis*, $n=3$; *C. parapsilosis*, $n=1$).

Table 1 Sensitivity, specificity, and predictive values for the detection of antigen and anti-mannan antibodies

Statistics	Mannanemia EIA	Anti-mannan antibody EIA	Combination of EIAs
Sensitivity (%)	79	59	93
Specificity (%)	93	91	87
Positive predictive value (%)	92	87	87
Negative predictive value (%)	82	69	93

Conclusions: The combined use of EIA detection of mannanemia and antimannan antibodies allowed the detection of 93% of mycologically proven candidemias with a specificity of 87%, irrespective of species type, patient immune status, and hospital ward.

P553 In vivo efficacy of PLD-118 in mice models of systemic *Candida* infection

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Background: PLD-118 is a novel oral antifungal identified at Bayer AG as BAY 10-8888 and licensed to PLIVA. In vitro, PLD-118 has activity against various *Candida* species including azole-resistant strains.

Objective: To assess the in vivo efficacy of PLD-118 in mice with systemic infection caused by different *Candida* species.

Methods: Clinical isolates of *Candida* spp. were identified by standard methods and evaluated for pathogenicity in mice. Male CFW1 mice were used in *C. albicans* studies and DBA mice in experiments with *C. glabrata* or *C. krusei*. All animals were infected via lateral tail vein. *C. albicans* strains were fluconazole-susceptible (MIC < 4 mg/L, $n=27$) or fluconazole-resistant (MIC > 64 mg/L, $n=27$). All *C. albicans* strains caused lethal infection in mice. PLD-118 was given orally, twice daily for 4 days, starting 30 min after infection. Control groups received vehicle or fluconazole. Survival was monitored for 10 days. None of tested non-*albicans* strains caused lethal infection in naïve mice, and efficacy was assessed by cfu counts from kidneys. Animals received a single dose of PLD-118 or vehicle, 30 min after infection. Kidneys were removed and processed 1, 8, 24 and 48 h after infection.

Results: PLD-118 was highly effective in mice with lethal systemic *C. albicans* infection. Doses of 5 and 10 mg/kg bid resulted in survival rates of 80 and 100%, respectively. In contrast to fluconazole, PLD-118 was effective in vivo against fluconazole-resistant *C. albicans* strains. Animals infected with *C. glabrata* or *C. krusei* developed kidney infection that spontaneously cleared within following 2 days. In both infection models, PLD-118 caused a significant reduction of cfu as compared to controls.

Conclusion: PLD-118 is active in vivo against *Candida* species, including fluconazole-resistant and non-albicans strains, and warrants further development as an alternative treatment option for azoles and/or other antifungals for systemic use.

P554 Trends in antifungal use and susceptibility profile of nosocomial *Candida* spp. in a pediatric hospital, Italy

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Genoa, Verona, I

Objective: Surveillance on antifungal agents use and emergence of nosocomial resistant *Candida* (Ca) spp. strains is useful tool to guide local antimicrobial therapy. Therefore, we studied antifungal consumption, Ca spp. occurrence and antifungal susceptibility profile of strains isolated from 01/01/1995 to 31/12/2000 in a pediatric hospital.

Methods: We analyzed data of 234 Ca spp.: 182 (77%) *albicans* and 52 non-albicans (including 24 *C. glabrata*, 16 *C. parapsilosis*, 4 *C. lusitaniae*, 4 *C. tropicalis*). Strains were isolated with conventional methods from various specimens (mucocutaneous 32%, urine 30%, sputum 22%, blood 16%) collected in different wards (NICU and PICU 23%, surgery 24%, infections disease 23%, pulmonology 19% and pediatrics 11%). Species identification was obtained by API 20 C *Candida* (bioMérieux) system. Antifungal susceptibility to amphotericin B (AM), flucytosine (5FC), fluconazole (FZ), itraconazole (IZ), and ketoconazole (KZ) was determined with *E*-test (AB Biodisks) performed according to the manufacture, NCCLS proposed breakpoint (M27.A) were applied and ATCC *C. krusei* and *C. parapsilosis* QC strains were used. The use of antifungal agents was calculated (from 1996) as defined daily doses/1000 pts-days (DDDs).

Results: The rate of susceptible strains was: 91% AM, 98% 5FC, 47% FZ, 24% IZ and 66% KZ for *C. albicans* and 65% AM, 88% 5FC, 48% FZ, 27% IZ and 90% KZ for Ca non-*albicans*. Rates of susceptibility to azoles among isolates from HIV-infected patients were significantly lower (FZ 22%, IZ 12%, KZ 59%). Comparing 1995–2000, we found (i) among *C. albicans* an increase rate of susceptible strains to FZ from 32% (14/44) to 80% (8/10) ($P=0.0019$); (ii) an increase rate of Ca non-*albicans* from 9.8% (5/51 total strains) to 67% (20/30 strains) ($P=0.0018$). The most prescribed drugs (DDDs) were AM 8.2 and FZ 8.1 compared with 5FC 1.06 and IZ 4.4. The use of AM, FZ and IZ remained constant over the years while the use of 5FC decreased from 0.24 in 1996 to 0.125 in 2000.

Conclusions: 5FC was the most active agent against our series of isolates; FZ and IZ resistance in of *C. albicans* and non-albicans isolates is exceedingly high. The increased rate of non-*albicans* found is worrisome and requires strict monitoring and better control of antifungal agents use. New active antifungal compound are needed.

P555 The role of 1,25-dihidroxycalciferol on the candidacidal of human macrophage

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Objectives: To investigated the candidacidal activation of macrophage induced by 1,25-dihidroxycalciferol (calcitriol).
Methods: Seven days cultures of human macrophages which isolates from tonsil donors of tonsilectomy patients were growth on medium content of 1,25-dihidroxycalciferol at gradual concentration and medium free of 1,25-dihidroxycalciferol. All of the culture then induced by *Candida albicans*. The activation of macrophages measured by potency of phagocytosis, content of *Candida intracell*, count of viable *Candida* culture on Sabouroud agar medium and nitric oxid (NO) secretion.
Results: The results showed that the highest of percentage phagocytosis achieved by macrophages population treated by 10–7 1,25-dihidroxycalciferol. The count of *Candida* reduced which isolated from macrophages was got on treat with calcitriol 10–7. While the NO secretion among all of group of macrophages population were almost the same value.
Conclusion: The result clearly that 1,25-dihidroxycalciferol gave an effect on increased of phagocytosis and killing effect to *Candida*, but not gave an effect on NO secretion.

P556 Disk diffusion method for determining susceptibility of *Candida* spp. to voriconazole

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Seville, E

Objective: The purpose was to evaluate the disk-diffusion test for determining susceptibility of *Candida* spp. to voriconazole(V) in comparison with the reference method (NCCLS, M27A document).
Material and methods: We studied 203 *Candida* spp. (168 *C. albicans*, 126 *C. glabrata*, 9 *C. tropicalis*) isolated from clinical specimens. Susceptibility test was carried out by the agar diffusion method using disk with 1 μg of V (Beckton Dickinson) on Mueller–Hinton agar with 2% glucose and 0.5 μg/mL of methylene blue. The test was read after 24 and 48 h of incubation at 35 °C. The MICs (mg/L) obtained by the reference broth microdilution method (NCCLS, M27A document) (MDB) were defined as the lowest concentration of V that inhibited the 50% of growth, they were read at 24 and 48 h of incubation at 35 °C.
Results: At 24 h, 197 of the 203 strains (97%) showed and inhibition zone diameter of major or equal to 18 mm^2 for V and 194 of these (98%) showed MICs values minor or equal to 2 μg/mL for V by MDB read at 24 h. All the strains with the inhibition zone diameter <18 mm^2 had also MIC values of V less than or equal 2 μg/mL being resistant to fluconazole. At 48 h, 181 of the 203 strains (89%) showed an inhibition zone diameter of greater than or equal to 18 mm^2 for V and 178 of these (98%) showed MIC values less than or equal to 2 μg/mL for V by MDB read at 48 h. Nineteen of the 22 strains (86%) with inhibition zone diameter <18 mm^2 had MICs values to V at 48 h less than or equal to 2 μg/mL and only 3 (14%) had MICs values > 2 μg/mL. Sixteen of the 22 strains were resistant to fluconazole.
Conclusions:
1 Better correlation between disk diffusion and the reference broth microdilution method at 24 h/24 h than 48 h/48 h.
2 Good correlation was observed between inhibition zone greater than or equal 18 mm^2 and the MICs values less than or equal to 2 μg/mL.
3 Most of the strains with inhibition zone diameter <18 mm^2 were resistant to fluconazole.
4 Reading of the inhibition zone diameter became more difficult at 48 h than at 24 h.

P557 Candidaemia in a tertiary care hospital: in vitro susceptibility of *Candida* isolates to five antifungal agents

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Background: *Nosocomial fungemia* occurs with increasing rates in patients with severe underlying disease or immunosuppression. *Candida* species are the main cause of fungal bloodstream infections.
Objective: To determine the in vitro susceptibility of five antifungal agents against yeast strains isolated from blood cultures in the University Hospital of Crete, Greece.
Methods: A total of 82 yeast strains isolated from blood cultures were included in the study. The isolates were identified by API 20 C AUX system (bioMérieux) and conventional methods. The susceptibility to antifungals was performed by *E*-test (AB Biodisk) for amphotericin-B (AP), 5-fluorocytosine (FC), ketoconazole (KE), fluconazole (FL), and itraconazole (IT).
Results: *Candida albicans* was the most common species isolated (34 strains; 41.5%), followed by *C. tropicalis* (20 strains; 24.4%), *C. parapsilosis* (11 strains; 13.4%), *C. glabrata* (8 strains; 9.8%), *C. lusitaniae* (5 strains; 6.1%), *C. famata*, *C. humicola*, *C. incospicua*, and *C. norvegiensis* (1 strain each; 1.2% each). The MIC_{90} (and MIC range, mg/L) determined for each antifungal agent against *C. albicans* were: AP 0.25 (0.012–1), FC > 32 (0.032–>32), KE 0.125 (0.004–16), FL 8 (0.019–32), and IT 1 (0.016–1.5), while against *Candida* non-*albicans* were: AP 0.75 (0.016–12), FC 0.5 (0.004–>32), KE 1 (0.004–>32), FL 48 (0.19–>256), and IT>32 (0.016–>32). Of *C. albicans* isolates 11.8% showed resistance to FC and IT, and only 2.9% to AP and KE. All *C. albicans* isolates were sensitive to FL. Of non-albicans isolates resistance rates to IT, AP, KE, FL and FC were 37.5, 20.8, 12.5, 10.4 and 6.25%, respectively.
Conclusions: *C. albicans* predominates among *Candida* species isolated from bloodstream fungal infections in our hospital, with quiet low resistance rates to antifungals. However, susceptibility to antifungal agents can help to determine optimum therapy.

P558 In vitro susceptibility of 65 *Candida* spp. oral isolates to fluconazole

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Objective: To assess for the fluconazole susceptibility among *Candida* spp. oral isolates recovered from Romanian individuals included in a study between June 2000 and September 2001.
Methods: A total of 65 *Candida* spp. isolates were collected from 53 oropharyngeal samples. For identification, the colony appearance on chromagar-Candida medium, chlamidospores production and substrate assimilation profiles with API 20 C AUX system were used. The fluconazole-susceptibility assessment was conducted using *E*-test method.
Results: From 65 *Candida* spp. isolates 63% (41) were identified as *Candida albicans* and 13.8% (9) were *C. krusei*. Other *Candida* spp. represented less than 25% of the isolates (4 *C. kefyr*, 3 *C. glabrata*, 2 *C. dubliniensis*, 2 *C. norvegensis*, 1 *C. inconspicua*, 1 *C. lusitaniae*, 1 *C. magnoliae*, 1 *C. tropicalis*). A total of 40 from 41 *C. albicans* isolates were susceptible (MIC < 8 mg/L) and 1 *C. albicans* isolates was susceptible dose-dependent S-DD (MIC = 16 mg/L) to fluconazole. Concerning the fluconazole susceptibility of the 24 non-albicans isolates, the results are: 4 *C. krusei* isolates were resistant (MIC = 64 mg/L); 4 *C. krusei*, 2 *C. norvegensis* and 1 *C. glabrata* isolates were S-DD to fluconazole. The other *Candida* spp. isolates were susceptible to fluconazole (MIC < 8 mg/L).
Conclusions:
1. The majority (97.5%) of *C. albicans* isolates were susceptible to fluconazole and no resistant isolate was recovered in this study.
2 Despite the fact that *C. krusei* is considered an intrinsic resistant species to fluconazole, in this study, four isolates were S-DD and one isolate was susceptible to fluconazole
3 The two *C. dubliniensis* isolates were susceptible to fluconazole.

P559 Typing and antifungal susceptibility testing of *Candida* strains isolated from the blood cultures of candidemia patients

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Objectives: Fungal infections have dramatically increased in recent years along with the occurrence of drug-resistant isolates especially in immunocompromised patients. These infections cause high morbidity, mortality and the antifungal drug choices are limited. Some *Candida* species are intrinsically resistant to some antifungal drugs and this makes the treatment choices more difficult. The typing procedures and antifungal susceptibility testing of *Candida* species are not routinely performed in many microbiology laboratories. The aim of this study is to type and determine the antifungal susceptibilities of *Candida* strains isolated from blood cultures of candidemic patients.
Methods: A total of 50 *Candida* strains isolated from candidemic patients were typed by the help of classical mycologic methods and API 20C AUX identification kits. The germ tube test positive strains were typed as *C. albicans* and the germ test tube negative strains were identified by inoculating onto the corn meal agar and API 20C AUX system. Antifungal susceptibility testing was performed according to the standard NCCLS M27A procedure by microdilution. The four drugs studied were amphotericin B, fluconazole, ketoconazole, flucytosine.
Results: Thirty of the isolates (60%) were *C. albicans*, 9 (18%) were *C. parapsilosis*, 5 (10%) were *C. tropicalis*, 2 (4%) were *C. krusei*, 1 (2%) was *C. kefyr*, 1 (2%) was *C. glabrata*, 1 (2%) was *C. lusitaniae*, 1 (2%) was *C. kefyr* and 1 (2%) was *C. famata*. In recent years, an increasing incidence of non-albicans candidal infections has been reported and this study shows similar results; 40% were non-*albicans* strains. None of the *Candida* isolates were resistant to fluconazole besides the two *C. krusei* strains which are known to be intrinsically resistant. All the strains were found to be susceptible to amphotericin B, ketoconazole, flucytosine.
Conclusion: Typing and determining antifungal susceptibility patterns of the *Candida* strains are helpful in the management of candidemic patients.

P560 Evaluation of the in vitro activity of caspofungin against invasive *Candida* from cancer patients: comparison of *E*-test and NCCLS methods

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Background: Emerging non-*albicans Candida* infections and azoles resistance have changed the pattern of invasive fungal infections in cancer patients.
Objective: To compare the in vitro activity of caspofungin (Cas), fluconazole (F), itraconazole (I) and amphotericin B (AMB) against 178 fungemic yeasts recovered between 1997 and 2001 from cancer patients using two different susceptibility testing methods.
Methods: Susceptibility testings were performed using the *E*-test and the broth microdilution reference NCCLS M27-A method.
Results: The comparative MIC_{90} (mg/L) were given in Table 1.

The percentages of agreement within two dilutions between NCCLS reference method and *E*-test for I, F, CAS and AMB were 81, 82, 84, and 97%, respectively.
Conclusions: Overall caspofungin is very active against invasive *Candida* in cancer patients. The *E*-test results correlated well with reference MICs.

Table 1 Comparative MIC_{90} (mg/L)

Species (Nb)	NCCLS				E-test			
	CAS	F	I	AMB	CAS	F	I	AMB
All organisms (178)	1	32	1	1	1	16	2	1
C. albicans (92)	0.25	4	0.25	0.5	0.25	1	0.25	0.5
C. glabrata (26)	0.5	16	2	1	0.19	16	64	1
C. tropicalis (22)	0.25	8	1	0.5	0.5	1	0.5	1
C. parapsilosis (20)	2	2	0.25	0.5	2	0.7	0.06	0.7
F-resistant (21)	0.5	512	16	1	1	512	64	1.5
I-resistant (21)	1	512	16	1	0.5	512	64	1

P561 Expression of an alternative respiratory pathway in *Candida* spp.

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Introduction: The alternative respiratory pathway (cyanine-resistant) of mitochondrial respiration occurs in all higher plants, many fungi and some protozoa. Such a pathway uses electrons from the ubiquinone pool to reduce oxygen to water, bypassing the complex III and the cytochrome oxidase, two sites of energy conservation in the main respiratory chain. An alternative oxidase, sensitive to salicylhydroxamic acid (SHAM), is responsible for this alternative pathway (1). The cyanide-resistant respiration has been found in a few yeast of the genera *Candida*, such as *Candida krusei* (2) and *C. parapsilosis* (3). The effect of sodium azide was overided by the expression of an alternative respiratory pathway (4).
Objective: To evaluate the presence of an alternative pathway to citochrome oxidase, resistant to sodium azide but susceptible to salicylhydroxamic acid.
Materials and methods: Thirty-four clinical isolates of *Candida* were studied to determine the presence of an alternative respiratory pathway including 14 *C. krusei*, 3 *C. parapsilosis*, 6 *C. albicans*, 5 *C. glabrata*, 2 *C. lusitaniae*, 1 *C. rugosa*. We used as control strains *Debaryomyces hansenii* PYCC 2968 (known to express an alternative pathway) and *Saccharomyces cerevisiae* PYCC 4072 as a negative control. The oxygen consumption was measured with a Clark-type electrode in the presence of respiratory inhibitors (3.2 mM of sodium azide and SHAM), after addition of glucose.
Results: Following the inhibition of cytochrome oxidase pathway (with sodium azide), the consumption of O_2 stopped in all strains except in the 14 strains of *C. krusei*, one strain of *C. parapsilosis* and *D. hansenii*.
Conclusions: *C. krusei*, a pathogen with wide resistance to antifungals, invariably possesses an alternative metabolic pathway that could justify its promoted pathogenicity and resistance to adverse conditions. Interestingly, such a pathway was expressed in only one strain of *C. parapsilosis*

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Pathogenesis of infection

P562 Virulence characteristic of *Escherichia coli* strains causing asymptomatic bacteriuria

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Objectives: Urine colonization often occurs in the absence of clinical symptoms and is called asymptomatic bacteriuria (ABU). Physicians have little knowledge of the potential for urovirulence of the ABU-associated bacterial strain. Usually, the strain is allowed to persist until that, or another invading strain, produces symptomatic urinary tract infection (UTI). A better understanding of the virulence potential of the ABU-associated organism would reduce the uncertainty in the treatment decisions. In the present study, we compared the prevalence of different virulence characteristic among *Escherichia coli* strains isolated from a group of women in whom ABU was detected at the follow-up, and a group of children in whom ABU was detected during the screening.
Methods: *Escherichia coli* isolates were collected from 19 women and 17 children in whom ABU was detected. All isolates were serotyped using 17 different O-antisera. The microtiter hemolysin assay was used to quantitate

the hemolytic activity of bacteria. Serum-sensitivity testing was performed according to a modification of the method described by Schiller and Hatch. The expression of adhesins was defined by hemagglutination (HA) and inhibition of HA in microtiter plates. Buffalo green monkey kidney (BGMK) cell line was used for the adhesion assay.

Results: There were no significant differences in the prevalence of most virulence characteristics between the strains detected in the two groups. The strains were equally often non-typeable, expressed low amounts of capsular polysaccharide, and were mostly sensitive to the bactericidal action of the serum. In addition, P-fimbriated strains were not detected, and most strains were non-adherent or adhered very poorly to the BGMK cell line. The prevalence of hemoloysin production was 23 and 26% among strains isolated from patients with and without a history of recurrent UTIs, respectively. The only statistically significant difference observed was a more common HA ability detected among the strains isolated from children without a history of UTI than among the strains isolated from women with recurrent UTIs ($P < 0.01$).

Conclusions: More virulent *E. coli* strains were detected in the group of children without a history of previous UTI in whom ABU was detected during the screening. As ABU is associated with an increased risk of symptomatic UTI a prospective study is needed to evaluate whether the HA ability is the predictor of subsequent symptomatic UTI.

P563 Adherence ability to cellular and inert substrata and other virulence factors in opportunistic enterobacteria with different origins

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The versatility of opportunistic bacteria is owing to the fact that different strains have acquired different sets of virulence genes, without species barrier. Attachment to epithelial cells is the first step of bacterial pathogenicity. Most of pathogenic and opportunistic bacteria possess adhesins, which mediate their adhesion to susceptible host cells and promote several outcomes, including colonization, cell damage, internalization, disturbances of regulatory cell mechanisms and intracellular proliferation/persistence.

The aim of the present study was to investigate the adherence capacity to different substrata and other virulence features as invasion and cytotoxicity of some opportunistic enterobacteria strains with different sources of isolation. A set of 120 strains of opportunistic enterobacteria were preliminarily screened for their adhesion capacity to HEp-2 cells using Cravioto's qualitative method. We selected 16 strains which were tested for adhesion + invasion to the intestinal Caco-2 cell line by the Cravioto's adapted method and FAS method (using DAPI and FITC fluorochromes). We also studied the cytotoxic effect on Caco-2 cells after releasing the cytotoxins using polymixin B and a rapid colorimetric assay of MTT salts reduction as a measure of cellular viability. The strains exhibiting intensive adhesion and invasion features were tested by TEM.

One strain of *Enterobacter cloacae* exhibited high adherence and invasion potential. This strain was also tested for its adhesion and biofilm-forming ability to an inert substratum – an urinary catheter fragment inserted in a bacterial culture flow system using a peristaltic pump. Internal catether surface was examined by SEM. The presence of adherent single cells as well as cells embedded in a biofilm matrix was observed. This strain proved also the highest cytotoxic effect. The TEM examination showed the presence of A/E lesions. In our knowledge, this last aspect, signaled for EPEC, some *Citrobacter* sp. and *Hafnia* sp. strains, has not yet been mentioned for *Enterobacter genus*. Our results showed that some opportunistic bacteria could exhibit multiple virulence factors: adherence to inert substratum, adherence + invasion to the cellular substratum, cytotoxic effect and also the capacity to induce actin rearrangements and A/E lesions. Our results are proving that the virulence features determine the pathogenicity of opportunistic enterobacteria, not their species and genus designation.

P564 Bacterial translocation following acute edematous pancreatitis: any correlation to the advent of an infection?

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Objective: To define whether advent of fever in acute edematous pancreatitis is attributed to subsequent endotoxemia or infection.

Methods: Thirty-three patients with signs of acute edematous pancreatitis diagnosed with an upper abdomen CT scan of less than 12 h before admission were enrolled. Eighteen were male and 15 female aged 61.5 ± 17.3 years with APACHE II score 5.82 ± 4.21. Blood was sampled by venipuncture before insertion of a nasogastric tube, past three and six hours and on the second and third days for determination of endotoxins (LPS) and of C-reactive protein (CRP) and for culture. LPS were measured by the QCL-1000 LAL assay and CRP by nephelometry. Results were correlated to the presence or absence of fever ($T > 38\,^{\circ}C$) and were expressed as mean ± SE.

Results: Twenty-one patients presented afebrile with LPS 2.11 ± 0.88 EU/mL and CRP 65.2 ± 23.8 mg/L. Concentrations of LPS past 3 and 6 h were 4.01 ± 1.36 and 2.42 ± 0.95 EU/mL, respectively. Fourteen of them (66.7%) remained afebrile on the second day with LPS 1.72 ± 0.57 EU/mL and CRP 16.3 ± 3.4 mg/L. Ten of these patients remained also afebrile on the third day with LPS 0.44 ± 0.01 EU/mL and CRP 10.3 ± 2.3 mg/L whereas four became febrile on the 3rd day with LPS 1.31 ± 0.91 EU/mL and CRP 208.5 ± 11.5 mg/L. From the patient admitted afebrile, seven (33.3%) became febrile on the 2nd day with LPS 3.70 ± 1.56 EU/mL and CRP 56.9 ± 24.1 mg/L. Fever also persisted on the third day with LPS 3.96 ± 2.39 EU/mL and CRP 183.7 ± 20.3 mg/L. From the total number of enrolled patients 12 were admitted febrile and remained febrile on both the 2nd and 3rd days. Concentrations of LPS of these patients on admission, past 3 and 6 h and on the 2nd and 3rd days were 3.54 ± 1.25, 3.03 ± 1.14, 5.84 ± 2.28, 2.98 ± 0.88 and 2.94 ± 1.88 EU/mL, respectively. Respective CRP values of these patients on admission and on the second and third days were 80.0 ± 40.1, 80.6 ± 37.2 and 105.8 ± 96.8 mg/L. Blood cultures of all patients were sterile.

Conclusions: Fever in the field of acute edematous pancreatitis is accompanied by systemic endotoxemia and subsequent increase of CRP in the absence of bacteremia. As a consequence the need for the administration of antimicrobials in acute edematous pancreatitis is doubtful.

P565 Production of extracellular enzymes by strains of *Stenotrophomonas maltophilia* of both clinical and environmental origin

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Background: Virulence factors of the nosocomial pathogen *Stenotrophomonas maltophilia* (Sm) are ill understood. *Ecthyma gangrenosum* (EG) is a recognized complication of Sm bacteremia. In EG associated with *P. aeruginosa* septicemia, enzymes such as protease and elastase are deemed important virulence factors. It is possible that exoenzymes may also play a role in pathogenesis of EG and other manifestations of Sm infection. Earlier studies of Sm exoenzymes examined only very small numbers of strains. We investigated a much larger number of isolates of clinical and environmental origin. As susceptibility of Sm to some antimicrobials is temperature-dependent, we also studied the influence of temperature on enzyme production.

Methods: A total of 130 isolates of Sm from clinical (61) and environmental (69) sources were studied. Agar diffusion assays were used for DNAse, RNAse, esterases, elastase, mucinase, gelatinase, protease, lecithinase, lipase, chondroitinase, hyaluronidase and haemolysin. Elastase was detected with a Congo Red assay. Assays were at 30 and 37 °C.

Results: All strains produced DNAse, RNAse, esterases and gelatinase at either temperature. Lipase and lecithinase were produced by significantly more isolates at 30 °C compared with 37 °C ($P < 0.05$). Ninety-two percentage of strains were hemolytic on horse blood agar (HBA) at both temperatures. Fewer strains were hemolytic on human blood agar irrespective of temperature compared with HBA, but blood culture isolates were significantly more likely to be hemolytic at 37 °C than other clinical or environmental isolates. Chondroitinase and hyaluronidase were produced by most strains with the exception of cystic fibrosis isolates which, when grown at

either temperature, were statistically significantly less likely to elaborate these enzymes. Clinical isolates were significantly more likely ($P < 0.05$) to produce protease and mucinase at 37 °C compared with environmental strains. All strains produced elastase at either temperature, but quantitative differences in production were apparent.
Conclusions: This is the largest study to date of exoenzyme activity of Sm and is the first report of chondroitinase production by Sm. Although a range of enzymes were produced by most strains, clinical isolates were more likely to produce particular enzymes when grown at 37 °C, suggesting that these may be important in the pathogenesis of Sm infection.

P566 Prosthetic valve endocarditis due to polyclonal coagulase-negative *Staphylococcus* infection

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Objectives: Coagulase-negative *Staphylococcus* (CNS) *endocarditis* is generally believed to be monoclonal. Research in this field always started from blood cultures yielding CNS. Only a handful of researchers have investigated the hypothesis of polyclonality in foreign-device related CNS infection. In our laboratory, we have cultured the valve of a man who was diagnosed with prosthetic valve endocarditis peri-operatively. The aim of our study was to investigate the possible polyclonality on the infected valve.
Methods: After surgical valvular repair, the prosthetic valve was cultured in our laboratory. After 24 h of incubation at 37 °C at least four different types of colonies could be distinguished visually on chocolate agar. Different colonies were subsequently isolated on blood and mannitol salt agar (MSA). Species identification was performed by the API Staph Ident System (API) and susceptibility testing by ATB anti-biogramme (bioMérieux, Marcy l'Etoile, France). At the university hospital of Leuven, genomic macrorestriction was performed using *Sma* I digestion of chromosomal DNA (Gibco BRL, Gaithesburg, MD) and separation by pulsed-field gel electrophoresis (PFGE) (CHEF Mapper system, Bio-Rad, Hercules, CA).
Results: On chocolate agar, four visually different types were identified: strains A, B, C and D. Strain B showed no growth on MSA and was not hemolytic. Strains A, C, D were mannitol negative and faintly hemolytic. All types were catalase positive and coagulase negative. Identification with API gave *Staphylococcus epidermidis* for all strains. Strains B, C and D matched with the 5505113 analytical profile, strain A with 5305113. Strains B, C and D were susceptible to all antibiotics tested except to oxacillin. Strain A was resistant to erythromycin and susceptible to the other antibiotics tested. PFGE revealed that only strain A was genetically different.
Conclusions: Traditionally, foreign device related bacteremia and in particular infective endocarditis were thought to be monoclonal. These findings suggest that positive blood cultures yielding different types of CNS can be the result of a true infection. These findings also remind us of the fact that susceptibility to antibiotics can be different for different strains. Theoretically, the most resistant strain should guide clinical therapy.

P567 Identification of membrane receptors for hydroxsamate-type siderophores in *Staphylococcus aureus*

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Objectives: *Staphylococcus aureus* strain B47 has produced an endogenous siderophore called staphylobactin which belongs to hydroxamic class chelators. This strain utilize five egzogenous chelators as Fe(III) sources: schizokinen, 2,3-dihydroxybenzoylglycine, 2,3-dihydroxybenzoylserine, aerobactin, and acinetoferrin. 2,3-Dihydroxybenzoic acid, rhodotorulic acid and desferrioxamine B were not utilized Fe(III) carriers by this strain. Little is known about membrane protein receptors for endo- and egzogenous siderophores utilized by staphylococci. The aim of this studies was to establish of membrane protein, which serve as siderophore receptors and correlation between particular siderophores and detected proteins.
Methods: The strain was cultured in synthetic Fe(III)-deficient medium ($1 \times 10{-}6$ M) and in medium containing excess of Fe(III) iron ($1 \times 10{-}4$ M). After lysis of protoplasts and differential centrifugation, the membrane protein fraction was obtained. The photoreactive *p*-azidobenzoil analog of ^{59}Fe-labeled ferrioxamine B, [^{59}Fe]rhodotorulic acid and [^{59}Fe]acinetoferrin was used to photolabeling of membrane proteins. After photolysis, membrane fraction was subjected to gel electrophoresis (SDS–PAGE). For the ^{59}Fe-labeled chelators detection gels were subjected to autoradiography.
Results: Six iron-regulated proteins have been identified in the cytoplasmic membranes of strain B47 cells harvested from synthetic Fe(III)-deficient medium. They occurred in the 96–14 kDa region. No new proteins were found in cell membranes harvested from medium with excess of iron. One protein (14 kDa) has been linked to ^{59}Fe-labeled acinetoferrin. Using ^{59}Fe-labeled ferrioxamine B it was demonstrated that this chelator has bound to the 43 kDa membrane protein. Ferrioxamine B has not stimulated growth of strain B47. The complex of ^{59}Fe-siderophor was not transported into the cell, because it was not detected in cytoplasm fraction. No radioactive bands occurred on autoradiograms of membranes with ^{59}Fe-labeled rhodotorulic acid.
Conclusion: *Staphylococcus aureus* strain B47 has produced six iron-regulated proteins witch may bind not only the currently synthesized siderophores. In the condition of limited Fe(III) availability this provides the possibility of interchange of siderophores among various microorganisms, and of utilization of siderophore–Fe(III) complexes from other bacteria present in the environment.

Cytokines and immune resistance

P568 Antibody response elicited by *Escherichia coli* bladder infection in primates

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Introduction: Symptomatic urinary tract infections (UTI) are one of the most common infectious diseases in clinical practice and uropathogenic *Escherichia coli* is the major causative agent. Clinical observations suggest that cystitis induces a protective immune response but the mechanisms are still unclear.
Objectives: To study antibody response following experimental *E. coli* bladder infections in primates, the influence of the PapG class II adhesin, and to correlate the systemic and local immune response to the protective effect.
Methods: Cystitis were established in primates through inoculation of a bacterial suspension of *E. coli* (1 mL, 107 cfu/mL) via an urethral catheter. Blood samples and suprapubic bladder urine aspirations were performed twice a week until two negative urine cultures had been obtained. The monkeys were then challenged with a second intravesical inoculation. Both a wild-type PapG class II P-fimbriated *E. coli* strain and two non PapG class II *E. coli* mutants were used as in the experimental model. Levels of IgA, secretory IgA and IgG against LPS derived from *E. coli* were detected in urine and serum samples from the monkeys by an indirect ELISA.
Results: The wild type *E. coli* strain and the two isogenic mutants induced primary bladder infections equally well. Protective immunity was induced by the wild type *E. coli* strain but not by the non PapG class II mutants. The protective effect was correlated to local secretory IgA response against the surface lipopolysaccharide in urine.
Conclusions: The *E. coli* PapG class II adhesin is required to induce protection against subsequent bacterial challenge. Protection against urinary tract infections is correlated to the production of secretory IgA and infections in the urinary tract of monkeys may or may not induce a protective immune response, depending upon properties of the infecting strain.

P569 Macrophage Inflammation Protein-3α (Mip-3α) in cerebrospinal fluid of patients with meningitis

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Objectives: The inflammatory response observed in infections of the subarachnoid space is mediated by chemokines. Among those interleukin-8 (IL-8) is known to play a pivotal role. Macrophage inflammatory protein-3α (MIP-3α) is a recently discovered CC chemokine involved in the regulation of humoral immunity and leukocyte homeostasis. The objective of this study was to evaluate the cerebrospinal fluid (CSF) MIP-3α concentrations in patients with meningitis and the relationship between MIP-3α and CSF inflammatory parameters.
Methods: We studied 54 CSF samples obtained from 21 patients affected by pyogenic meningitis (PM) ($n=14$: five cases *S. pneumoniae*, three *N. meningitidis*, one *P. aeruginosa*, five of unknown etiology) or aseptic meningitis (AM) ($n=7$: *three tuberculous*, four viral), and eight CSF samples of non-inflammatory CNS diseases as the controls. MIP-3α was measured by an ELISA test developed with specific antibodies purchased from R&D Systems (Minneapolis, USA) with a detection limit of 10 pg/mL. IL-8 levels were determined by means of a commercial kit (R&D Systems, Minneapolis, USA). Differences in chemokine levels between the two groups of patients were calculated with Mann–Whitney test, while correlations of MIP-3α with CSF cells count, protein level and CSF/blood glucose ratio were evaluated by means of Pearson test.
Results: At admission, median MIP-3α was 1595 pg/mL (range 117–4500) in PM patients and 39 pg/mL (0–192) in AM ($P=0.000$). Median IL-8 was 3036 pg/mL (1217–8000) in the formers and 468 in the latters ($P=0.014$). MIP-3α and IL-8 were undetectable in all noninflammatory CSF samples. A correlation between MIP-3α levels and neutrophil counts was observed when considering the 21 admission measures ($r=0.66$, $P=0.01$) and all the 54 CSF samples ($r=0.68$, $P=0.01$). MIP-3α did not show any correlation with CSF protein level and CSF blood/glucose ratio.
Conclusion: This is the first report showing the presence of MIP-3α in CSF of patients affected by meningitis. MIP-3α was found to be lower than IL-8 levels. However, MIP-3α levels were significantly higher in PM rather than in AM and correlated with CSF neutrophil counts. These data suggest a possible role of MIP-3α as a chemotactic factor in pyogenic meningitis. Further studies are needed to elucidate the precise role of this chemokine in pathogenesis of PM and the potential application in the differential diagnosis of meningitis.

P570 Macrophage migration inhibitory factor (MIF) is elevated in cerebrospinal fluid (CSF) from patients with septic meningitis

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Objectives: MIF has an essential role in the pathogenesis of outcome induced by sepsis (Calandra, T. *Nature Med.* 2000; 6: 164–70). However, its role in meningitis remains to be defined.
Methods: MIF was determined by ELISA in CSF samples taken on admission from 101 patients suspected of having meningitis. Based on clinical, microbiological and biochemical characterization the patients were divided into four groups: 43 with septic meningitis (I), 42 with aseptic meningitis (II), four with encephalitis (III), and 12 without evidence of meningitis (IV).
Results: Data are shown as medians (min–max). Significant differences in CSF MIF levels (pg/mL) were detected among groups (I: 5006 (457–12 746), II: 1832 (0–12 746), III: 6433 (2037–8353), and IV: 2365 (0–6991), Kruskal–Wallis, $P<0.005$), with significantly higher CSF MIF levels in patients with septic meningitis than in patients with aseptic meningitis (Mann–Whitney with Bonferonis correction, $P=0.01$). CSF MIF levels were associated with severity of disease (number of days of hospitalization, Spearman, $P<0.001$). CSF MIF levels correlated significantly with CSF protein levels, CSF/blood glucose ratio, CSF IL-8 levels, and with number of leukocytes (lymphocytes, monocytes, and neutrophils) in the CSF, as well as with number of leukocytes (neutrophils) in blood ($P<0.05$).
Conclusions: MIF may have an important role in the pathogenesis/pathophysiology of septic meningitis.

P571 Detection of opsonic antibodies in patient sera after staphylococcal infections

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Objectives: Staphylococcal infections are among the leading causes of mortality in hospitalized neonates and infants. To better understand the immune response associated with these infections, we studied 16 sera from neonates and older children with *Staphylococcus aureus* infection and 10 normal sera.
Methods: Specific antibodies against native and deacetylated extracellular staphylococcal polysaccharide (PNAG) were studied. In addition, the PNAG-specific opsonic killing ability of these sera was measured to determine if a potential protective immune response was generated.
Results: The difference between titers against native and deacetylated antigen in patients was not statistically significant. The titers in normal sera against both antigens were higher than in patients sera but again the difference was not statistically significant. However, complement-mediated, PNAG-specific killing in patients sera was considerably higher (between 11.6 and 80.2%) as compared to normal human sera wherein no significant killing was observed. Opsonic killing thus showed a statistically significant difference. Patients as well as healthy controls possess specific antibodies against PNAG, although these antibodies in healthy controls are not opsonic. Patient sera showed mean killing levels of about 50% while there was no opsonic activity in the control sera.
Conclusions: Patients as well as healthy controls possess antibodies specific for PNAG and these antibodies are directed against the native and deacetylated form of PNAG. Although anti-PNAG antibodies are present in healthy controls, these antibodies are not opsonic.

P572 The influence of methicillin-resistant *Staphyloccocal septicemia* on the sizes of T lymphocyte subpopulations and CD25+ cells in full-term eutrophic neonates

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Objectives: Severe bacterial septicemia (BS) may change natural immunity system in neonates. Variety of results of those investigations was the cause to undertake research aimed to evaluation how methicillin-resistant staphyllococcal septicemia influence the sizes of T lymphocytes subpopulations and CD25+ cells in full-term eutrophic neonates.
Methods: The analysis comprised 46 neonates aged from 2 to 10 days of life with birth weight from 3100 to 4500 g, with gestational age from 39 to 41 weeks, among them 24 (14 boys, 10 girls) septic and 22 (12 boys, 10 girls) healthy, born vaginally, with Apgar score >9 points, without perinatal risk factors (control group). In 67% (16 cases), early onset and in 33% (eight cases) late-onset BS were stated. Most frequently (79%) *S. epidermidis* was isolated from the blood, both in early (13 cases) and late-onset (six cases) infections. Other staphylococcal strains (*S. sciuri, varneri, hominis*) in five cases were found. Two early death of septic neonates were associated with severe birth asphyxia and multiorgan insufficiency. Flow cytometric immunophenotyping using lysed whole vein blood and monoclonal antibodies anti CD3+, CD4+, CD8+, HLA-DR + and CD25+ (Becton Dickinson) was performed to determine these cells.
Results: In septic neonates, the mean relative size of CD8+ ($18.9 \pm 4.5\%$) was significantly ($P<0.008$) lower than in control ($23.6 \pm 6.6\%$). Significant ($P<0.03$) increase of the mean relative size of CD25+ cells in septic neonates ($8.1 \pm 2.3\%$) in comparison to the control ($6.6 \pm 1.3\%$) was noted. The mean relative sizes of CD3+ ($67.2 \pm 5.9\%$), CD4+ ($49.9 \pm 10–0.7\%$) and HLA-DR + ($1.8 \pm 0.9\%$) did not differ from the control. The mean absolute sizes of CD3+ (3.642 ± 1.299 G/L), CD4+ (2.743 ± 1.199), CD8+ (1.005 ± 0.298), HLA-DR + (0.091 ± 0.042) and CD25+ (0.446 ± 0.169) were similar to the mean values in control one. CD4+/CD8+ ratio ranged from 1.5 to 4.9 (mean 2.94 ± 1.38) in septic neonates and did not differ significantly from the control group (2.29 ± 1.29, 1.2–6.3).
Conclusion: Methicillin-resistant staphylococcal septicemia in full-term neonates may lead to decrease of mean relative size of CD8+ T lymphocytes and to increase of CD25+ cells.

P573 Expression of Fc receptors for IgG on peripheral blood phagocytic cells in bacteriemic patients

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Background: Early markers of bacteriemia are useful for prognosis and, in decision making for i.v. antibiotic therapy.
Objectives: To assess the diagnostic power of the surface expression of Fc receptors for IgG (FcgRs) for the prediction of bacteriemia in febrile patients.
Methods: We performed a prospective case–control study on 75 consecutive patients (pts) with an episode of bacteriemia as compared to 83 randomly selected concurrent febrile pts with negative blood cultures (control). Demographic and clinical data were collected by chart review and/or questioning their attending physicians. Plasma levels of C-reactive protein (CRP), TNFα, IL-1β, IL-6, IL-8 and IL-10 were determined. The surface expression of Fc receptors for IgG (FcgRs): FcgRI, FcgRII and FcgRIII on peripheral blood monocytes (M) and granulocytes (G) was assessed by flow cytometry. These studies were done concomitantly with blood cultures.
Results: Both groups were not different for age, sex, previous administration of immunosupressants or antibiotics, clinical severity index or comorbid conditions. In univariate analysis, cases had significantly higher levels of CRP ($P<0.001$), TNFα ($P<0.001$), IL-1β ($P<0.001$) and IL-6 ($P<0.01$) than controls. The expression of FcgRIII on M (PBM-FcgRIII) and FcgRI on G (G-FcgRI) was significantly enhanced ($P<0.001$) in bacteriemic pts as compared to culture-negative febrile pts; while the expression of the other FcgR-type, FcgRII by either M or G was significantly decreased ($P<0.03$). Setting a cut-off value $\geq 25\%$ of the mean fluorescence intensity over controls for M-FcgRII, M-FcgRIII, G-FcgRI or G-FcgRII surface expression and, assuming a prevalence of bacteriemia of 5–10% among hospitalized pts undergoing blood cultures, results in a sensitivity, specificity, positive and, negative predictive values of: 77, 95, 74, and 98%, respectively, for M-FcgRII, 73, 96, 74 and 97%, respectively, for M-FcgRIII, 58, 91, 49 and 96%, respectively, for G-FcgRI and, 72, 93, 57 and 93%, respectively, for G-FcgRII.
Conclusions: Our results suggest that the surface expression of Fc receptors for IgG on peripheral blood monocytes and granulocytes may help clinicians to rule out bacteriemia in febrile patients.

P574 *Haemophilus influenzae* porin contributes to signalling of the inflammatory cascade in rat brain

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In the present study we observed that the *Haemophilus influenzae* type b (Hib) porin, among the different surface bacterial components, is involved in the pathophysiology of bacterial meningitis. Hib porin (or P2 or protein BLC) is the most dominant band in SDS-PAGE outer membrane protein (OMP) preparations according to Nurminem method; its molecular mass varies between 36 and 42 kDa. This protein exists as a trimer and function as a porin. Hib porin has also been reported to be an important immunoprotection target. TNF-α and IL-1 were shown to play an important role in the host's response to bacteria and their products. Astrocytes and microglial cells produce IL-1 and TNF-α within the central nervous system (CNS). The evolution of the inflammatory process in the CNS depends on the set of cytokines released during the first steps of the infection. We analyzed by PCR the pattern of cytokines release after stimulation with Hib porin to establish the exact pathogenic mechanisms by which Hib contributes to signalling of the inflammatory cascade. This study demonstrates that inoculation of Hib porin into the fourth cerebral ventricle causes the simultaneous expression of interleukin-1α (IL-1α), tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein 2 (MIP-2) at 6 h after inoculation. At 24 h time-point the MIP-2 expression decreases, while expression of IL-1α and TNF-α increases. The mRNA expression of IL-1α, TNF-α and MIP-2 is correlated with the brain blood barrier injury as demonstrated by the appearance of serum proteins and leukocytes in CSF and by the increase in brain water content. In conclusion, the interplay between bacterial components and host inflammatory cells is likely to be a major determinant of the experimental presentation and outcome of bacterial brain edema.

P575 Effects of *Pseudomonas aeruginosa* experimental endocarditis in rabbits on granulocyte and monocyte phagocytosis and respiratory burst

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Introduction: *Pseudomonas aeruginosa* (PA) left-sided endocarditis has a low medical cure rate. Poor penetration of antibiotics in the vegetations, β-lactamase production, resistance to aminoglycosides, lack of postantibiotic effect of β-lactams and alginate production are the main known reasons. Vegetations are also sites of impaired host resistance where the infiltration of phagocytic cells is decreased. In addition, microbe cell-associated surface structures protect the organism and extracellular products further impair host defense.
Objective: To evaluate the influence of PA endocarditis on the phagocytic activity and the respiratory burst of granulocytes and monocytes in the model of experimental endocarditis in rabbits.
Materials and methods: Fifteen New Zealand male rabbits were used. One day after catheterization, 108 cfu of a PA mucoid strain were injected. Two days after bacterial challenge, animals were randomized to treatment (ceftazidime plus tobramycin, $n=10$), or no treatment ($n=5$) groups. Eight animals from the treatment group and only one from the no treatment survived and all where sacrificed on day 8. Leucocyte phagocytic activity and respiratory burst determination was performed twice (before catheterization and just before sacrifice). Commercial test kits PHAGO-test and PHAGO-BURST-test of Orpegen Pharma were used. Cells were analyzed using the flow cytometer FACS Calibur (Becton Dickinson).
Results: (1) The results concerning the rabbits surviving from the treatment group are: (Phagocytosis (% monocytes): before treatment: 60.1 ± 19.4; after treatment: 40.8 ± 6.4, $P=0.043$. Phagocytosis (% granulocytes): before treatment: 75.0 ± 8.4; after treatment: 56.0 ± 6.8, $P=0.018$. Burst (% monocytes): before treatment: 18.4 ± 9.0; after treatment: 4.5 ± 2.8, $P=0.018$. Burst (% granulocytes): before treatment: 83.1 ± 6.7; after treatment: 37.4 ± 9.2, $P=0.018$. Wilcoxon Signed ranks test for related samples was used for the statistical analysis. (2) The one survived control without treatment showed similar values.
Conclusions: Leucocyte phagocytic ability and oxidative burst are attenuated during the course of PA experimental endocarditis in rabbits. This decreased function of monocytes and granulocytes may be a contributing factor in the high rate of refractoriness to antibiotic therapy of left-sided PA endocarditis in humans.

P576 Effect of TNF-α on uropathogenic *E. coli* adherence to human endothelial cells

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Objectives: The polyfunctional effect of tumor necrosis factor (TNF-α) on endothelial cells has already been well documented. Present study aimed at analysing effects of TNF-α on adherence of uropathogenic *E. coli* to human endothelial cells.
Methods: The studies were performed using *E. coli* strains, which were isolated from urine of adult patients. The strains were identified as belonging to *E. coli* species using API ID 32 GN strip and the ATB system (bioMerieux). All the strains were assayed by agglutination using the latex reagent for detection of P fimbriae (P-fimbriae particle agglutination test). Adherence of bacterial cells to human umbilical vein endothelial cells (HUVEC) was studied after labeling of the bacteria with BrdU. Bacterial adherence was detected by immunoenzymatic assay with peroxidase-labeled monoclonal anti-BrdU antibodies. HUVEC were preincubated for 30 min in PBS containing r(h)TNF-α (Amersham) at 5, 10, 25, 50, 75 and 100 pg/mL or containing no r(h)TNF-α (control).
Results: Mean value of absorbance for the *E. coli* strains was 0.42 ± 0.05. As compared to the control values, bacterial adherence to HUVEC significantly increased following preincubation with r(h)TNF-α at 25 pg/mL ($P<0.05$). Higher doses of r(h)TNF-α did not significantly alter the bacterial adherence to host cells.
Conclusion: TNF-α may promote bacterial adherence to endothelial cells, which requires, however, specific concentration of the cytokine.

P577 Ex-vivo activation of autologous BKV-specific cytotoxic T cells for the treatment of BKV-associated interstitial nephritis after kidney transplantation

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Polyoma BK virus (BKV)-associated interstitial nephritis is a recently described complication that affects with increased frequency renal allograft recipients. The infection leads to graft failure in as many as 45% of the affected patients. Currently, no established antiviral treatment is available, and control of viral replication is tentatively obtained by means of reduction of immunosuppression. However, most patients with nephropathy due to BKV slip into a disheartening cycle, alternating between viral interstitial nephritis and rejection, precipitated by lowering immunosuppressive drug dosage. Thus, there is a need to develop alternative therapeutic tools able to control the infection. Adoptive cellular immunotherapy has proven to be a successful approach for prevention and/or treatment of Epstein–Barr virus- and cytomegalovirus-related complications in the immunocompromised host. To assess the feasibility of translating this strategy to the prevention of BKV-associated interstitial nephritis, we established a system for generating and expanding BKV-specific cytotoxic T cells (CTLs) from peripheral blood lymphocytes (PBL) of renal transplant recipients with evidence of active virus replication. In detail, we optimized a procedure for the in vitro reactivation of virus-specific CTLs using dendritic cells as BKV antigen-presenting cells. Immunophenotyping showed that the CTLs thus obtained were >90% CD3+, >50% CD8+ , 20–30% CD4+ , and contained 5–10% cells of NK phenotype, 10–20% CD3+/CD8+/CD56+ cells and variable numbers of gamma/delta + CTLs. BKV specificity of the lines was indicated by an efficient lysis of BKV-infected targets, with little or no reactivity observed against noninfected autologous or HLA-mismatched targets. The CTLs successfully generated with our method could be expanded in vitro to reach sufficient numbers for infusion requirements. Overall, our preliminary data hold promise for defining a CTL-based immunotherapy approach to BKV-related complications in kidney transplant recipients.

P578 Plasmid pVM of *Yersinia pseudotuberculosis* has considerable immunosuppressive properties

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We investigated the role of PVM (82 MDa) plasmid in pseudotuberculosis pathogenesis. In this study, two different strains of *Y. pseudotuberculosis* (Yp.) (both pYV + (47 MDa) and pVM + virulence plasmids strain and pYV +- strain) and pYV + *Y. enterocolitica* (Ye.) were studied. Each strain was incubated for 24 h with the whole blood of healthy volunteers at a ratio of 1 million microbial cells per 1 mL of blood. Blood without strain was used as control and with lipopolysaccharide of Yp. (LPS) and superantigen of Yp. (YPMa) as positives controls. Concentrations of interferon-α (IFN-α), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6) were measured by ELISA. Also we investigated the levels of the same cytokines in patients with pseudotuberculosis induced by pVM + pYV + and pYV + strains. In experiment, YPMa induced higher levels of IFN-α, TNF-α, IL-1 and IL-6 that LPS and much more that any strain. Yp. pVM + pYV + induced significantly lower IFN-α, TNF-α, IL-1 and IL-6 levels that pYV + Yp. and pYV + Ye. (Fig. 1). Levels of IL-2 were unchanged. In the patients clinical course of pseudotuberculosis caused by pVM + pYV + Yp. were more severe than that pYV + Yp. Levels of cytokines production in the patients with pVM + pYV + Yp. were less insignificant than that provoked by pYV + Yp. The first week of disease levels of all cytokines were significantly diminished comparing with their levels in the healthy volunteers. Levels of IFN-α, IL-1 and IL-2 rose on the third week of disease but the level of TNF-α remained unchanged and levels of IL-6 continued to decrease. These data show that Yp. has significant immunosuppressive properties and diminishes the cytokine production both in vitro and in vivo. Plasmid 82 Ìda exacerbates immunosuppression.

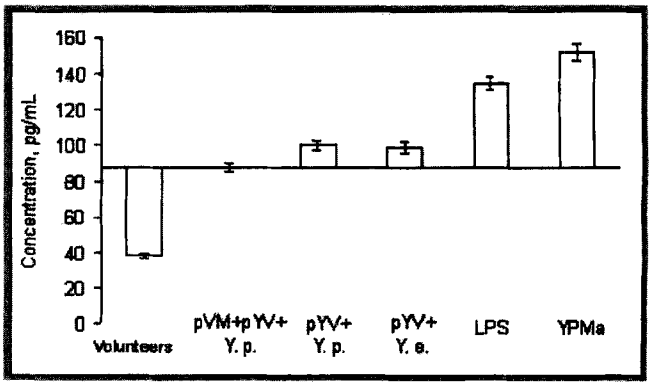

Figure 1 TNF-α production in vitro.

Infections in transplant patients

P579 Do the positive donor blood cultures influence the success of organ transplantation?

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Objectives: Organs transplanted from bacteremic donors do not transmit bacterial infections. The aim of our study was to evaluate the impact of positive donor blood culture on the 30-day graft and patient survival after organ transplantation.
Methods: Periferic blood samples were taken for blood cultures in BACTEC PLUS aerob/anaerob bottles. Bottles were incubated in BACTEC 9120 systems. (Becton Dickinson). Positive samples were cultured in blood, chocolate and EMB agar produced by NOVAMED LTD. (Jerusalem, Israel), bacteria were identified using VITEC systems (bioMérieux).
Results: Between 1998 and 2001, 132 kidney and 25 liver organ donors were examined. Total 40% of the kidney organ donors as well as 48% of liver organ donors were found to be bacteremic. The authors assessed the distribution of bacteria in donor blood cultures. The coagulase-negative *Staphylococcus* had the largest distribution ratio in 79% kidney and 66% liver donors. Gram-negative bacteria and *Candida* sp. were found to be only 1%. UTI was observed to be 10.7% in kidney recipients. Wound infections occured in 3.9% of kidney and in 16.6% of liver recipients. There was no death and no graft lost from infectious complications in recipients that received organs from bacteremic donors.
Conclusions: From the results of these examinations, it has been assumed that organs transplanted from bacteremic donors do not result in poor outcomes.

P580 Invasive *Aspergillosis* in solid-organ transplantation: report of eight cases

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Objective: We retrospectively evaluated the clinical and laboratory findings in eight patients who were solid-organ transplant recipients and treated for invasive aspergillosis (IA) at our center. Between 1998 and 2001, 8 (4 liver and 4 kidney recipients) of 207 transplant recipients (27 liver and 180 kidney) were diagnosed with IA. All the affected patients were male, and their mean age was 38 years (range: 22–55 years). The recipients' immunosuppressive protocols included various combinations of prednisolone, cyclosporine, mycophenolate-mofetil, azathioprine, and tacrolimus. The mean time from transplantation to IA diagnosis was 24 days (range: 15–34 days). The risk factors for the development of the disease were acute rejection ($n=4$), long-term antibiotic therapy ($n=4$), OKT3 treatment ($n=3$), thrombocytopenia ($n=3$), and re-transplantation ($n=1$). The clinical symptoms in the group were fever in 6 (75%) cases, productive cough with sputum and dyspnea in 5 (62.5%) cases, and unexplained diarrhea in 1 case (12.5%). All the patients' chest X-rays showed pneumonic infiltration or nodular involvement. Thoracic computed tomography (CT) revealed bronchopneumonia, nodular, and nodular and cavitation patterns. Definitive diagnosis was based on isolation of *Aspergillus fumigatus* from cultures of bronchoalveolar lavage fluid and sputum in two patients, bronchial biopsy in one patient, and autopsy findings in five cases. The four patients who were diagnosed with aspergillosis premortem were treated with liposomal amphotericin B (5 mg/kg/day). Two patients with clinical and laboratory findings that were highly suggestive of IA were empirically treated with amphotericin B (3 mg/kg/day), and the diagnosis was confirmed postmortem. Two of the five patients who were diagnosed at autopsy did not receive premortem antifungal therapy. Six (75%) of the eight patients died. One of the remaining two patients was discharged after 21 days of amphotericin B and 14 days of itraconazole therapy. The other individual received 21 days of amphotericin B therapy only.
Conclusion: The high mortality rate in patients with IA underlines the importance of prompt diagnosis and treatment. This disease should always be considered a potential threat in high-risk transplant recipients, and therapy should be initiated as soon as specimens have been obtained for microbiological testing.

P581 Cryptosporidiosis in renal transplanted and hemodialysis patients in Imam Hospital, Urmia

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Background and Objectives: Some intestinal parasitic infections caused by parasites such as *Cryptosporidium* spp. are frequently seen in renal transplant and hemodialysis patients.
Methods: Our study of infection by *Cryptosporidium* spp. were determined in three groups. First group consisted of 87 renal-transplanted patients and the second group of 103 hemodialysis and other control cases. For every patient and control case, at least two fecal specimens were examined.
Results: The frequencies of *Cryptosporidium* spp. infection in renal-transplanted, hemodialysis patients and control groups were 11.5 and 3.88%, respectively. All control groups were negative for *Cryptosporidium* spp. Statistical analysis showed significant differences among the renal-transplanted and control group ($P<0.0239$). But significant differences were not found among the hemodialysis patients and control group ($P<0.0418$).
Conclusion: The frequencies of *Cryptosporidium* spp. infecton in renal-transplanted patients was higher in comparison to hemodialysis patients. It is concluded that renal-transplanted patients are at high risk for infections of *Cryptosporidium* spp. because of their immunocompromised state.

P582 Infectious agents causing urinary tract infections in renal transplant recipients and their antimicrobial susceptibilities

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Introduction: Urinary tract infections (UTI) are the most common bacterial infections affecting renal transplant recipients. The most common pathogens causing UTI in this group of patient include *Enterobactericeae, Enterococci, Staphylococci* and *Pseudomonas* spp.
Objectives: To determine the bacterial agents causing UTI and their antibiotic susceptibilities in renal transplant recipients.
Methods: Baskent University Hospital is a 300-bed, tertiary care hospital at which approximately 80 kidney transplantations are performed annually. Total 1050 renal transplant recipients are included in follow-up program in our hospital. Thirty-one UTI episodes in 25 of these 1050 renal transplant recipients were evaluated. Inclustion criteria of UTI: fever ($\geq$38 °C), positive mid-stream urine culture ($\geq$ 100.000 cfu/mL), pyuria ($\geq$ 25/μL). Antibiotic susceptibility testing was performed by Kirby–Bauer disk diffusion method.
Results: The incidence of UTI was 0.3%. Twelve (38.7%) of the 31 isolates were *Escherichia coli*, six (19.3%) were *Klebsiella pneumoniae*, three (9.6%) were *Enterobacter aerogenes*, three (9.6%) were *Acinetobacter baumanii*, two (6.4%) were methicillin resistant *Staphylococcus aureus* and one (3.2%) methicillin sensitive *Staphylococcus epidermidis*. Seven (30.4%) of 23 Enterobacteriaceae were resistant to ciprofloxacin and 17 (73.9%) were resistant to trimethoprim-sulfomethoxazole. Five of the episodes have occured in the first month after transplantation, four in the period from the second through the sixth month and 22 beyond the sixth month.
Conclusion: The incidence of UTI in renal transplant recipients who are included in follow-up program in our hospital is found to be low (31 episodes in 1050 recipients). Enterobacteriaceae are still the most frequently isolated agents in UTI among renal transplant recipients. Ciprofloxacin resistance should be considered when empirical therapy will be initiated.

P583 Tuberculosis in renal transplantation recipients

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Background: Immunosuppressed patients after renal transplantation (RT) are at increased risk of developing tuberculosis (TB). It is a serious infectious problem, especially for the patients living in the countries where this infection is endemic. In the present study, we retrospectively evaluated RT patients with tuberculosis of different organs with respect to clinical outcome, diagnostic procedure and response to antituberculosis therapy.
Methods: We evaluated the side of infection, clinical manifestations, diagnostic procedures, and response to the antituberculosis therapy in eight RT patients who developed TB after transplantation during a 5-year period.
Results: The mean age of patients was 38. A total of 62% of them received the graft from a living donor. The infection was limited to the thoracic cavity in 37.5% of the cases, a single extrapulmonary site in 37.5%, and it was disseminated in 25% of the patients. TB developed from 6 months to 90 months (a median of 28 months) after transplantation. Fever was the most common symptom (observed in seven patients). In four cases (one thorasic and three extrathorasic), the disease appeared as fever of undetermined etiology. None of the patients responded to PPD skin test. *M. tuberculosis* culture were positive in five cases and microscopy revealed AFB in five patients. The diagnosis was performed with only histopathological evaluation in one patient. All of the patients were treated with four drug regiment (INH + ethambutol + rifampin + pyrazinamide). One allograft rejection episode occurred during therapy. Hepatotoxicity developed in two patients during treatment. Seven patient completed 12 months therapy. One patient who was under antituberculosis therapy died following the diagnosis of disseminated TB.
Conclusion: In RT patients, we have found that:
- extrapulmoner involvement was more frequent;
- TB might be considered seriously in patients who have prolonged fever of undetermined etiology;
- most of the cases were encountered later than the first transplantation year;
- rifampin-containing antituberculosis therapy was effective and did not cause graft failure.

P584 Disseminated *Nocardia asteroides* and coinfection with *Trichophyton rubrum* in a renal transplant recipient

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A 45-year-old male patient who had undergone renal transplantation 16 years ago was admitted to hospital with a mass in his left groin and blurred vision and orbital pain in the left eye. At the time of admission, the patient was taking tacrolimus, prednisolone, and cyclosporine-A. Pathological examination of the lesion revealed invasive fungal infection. A fundoscopic examination showed plaques suggestive of fungal infection, and *Trichophyton rubrum* was isolated from material obtained from the left groin. On the basis of these findings, immunosuppressive therapy was stopped, the patient was given an intravitreal injection of 0.1 mg amphotericin B and was started on antifungal treatment with amphotericin B (3 mg/kg/day). On the third day of hospitalization, the patient became acutely febrile. A chest X-ray showed nodular infiltration in the left lower lung lobe. Computed tomography (CT) demonstrated a solid, lobulated mass in the lateral lower basal segment of the left lung. Since the patient developed right-sided weakness, magnetic resonance imaging (MRI) of the brain was also performed and showed multiple abscesses. A vitreal specimen was obtained by fine-needle aspiration and a positive growth signal was noted on the fourth day of blood culture incubation. Modified acid-fast staining of both the vitreal specimen and the growth on the blood cultures showed acid-fast, delicate, branching filaments, which are characteristic of *Nocardia* spp. Further investigations revealed the bacteria as *Nocardia asteroides*. Due to the patient's deterioration and the diagnosis of nocardial sepsis, the treatment was changed to amphotericin B (3 mg/kg/day), TMP-SMX (2 × 160/800 mg/day), amikacin (1 × 1 g/day) and cefotaxime (3 × 1 g/day). At the end of the first week on this new regimen, the patient's fever subsided and overall condition improved dramatically. Two weeks later, the patient was discharged from hospital. He continued treatment with amikacin (1 × 1 g/day), TMP-SMX (2 × 2 ds tablets/day), and imipenem (4 × 500 mg/day) for 28 days. Subsequently, ototoxicity developed and γ-glutamyl transferase levels elevated. The treatment was changed to meropenem 3 × 1 g/day. After 6 months of therapy, cranial MRI demonstrated almost complete resolution of the abscesses. We plan to stop the meropenem treatment at the end of 12 months.

P585 Necrotizing fasciitis in a renal transplant recipient

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Invasive group A Streptococcal infection (GAS) is characterized by signs of shock, multiorgan failure and destructive soft tissue infection like necrotizing fasciitis. Necrotizing fasciitis is rarely seen after renal transplantation, with a few cases cited in literature, and survival is infrequent. A 51-year-old male renal transplantation recipient who had diabetes mellitus for 5 years was admitted to hospital with fever, sore throat and localized pain erythema and edema without crepitation over his right elbow. His nasopharynx was hiperemic with hypertrophic tonsils. Since the patient was in shock, a presumptive diagnosis of necrotizing fasciitis was made. Therapy consists of penicillin G 24 million units/day plus clindamicin 1800 mg/day was initiated and urgent debridement was carried out. Gram-positive cocci were seen in Gram stain of tissue specimen. GAS grew in throat, tissue, and blood cultures. Anaerobic cultures yielded no bacteria. Histopathologic examinations revealed subcutaneous and muscle necrosis. Multiorgan failure, DIC and superinfections with methicillin-resistant *S. aureus* and *Acinetobacter* spp. developed in postoperative period despite intensive and appropriate antimicrobial and surgical intervention. Following progressive worsening, patient died on the 22nd day of admission. No postmortem biopsy was obtained because of the disagreement of his family. In conclusion, for renal transplantation patients, necrotizing fasciitis is a life-threatening disorder that requires early recognition and aggressive treatment, including surgical intervention. In this case report, isolation of GAS in throat culture was an interesting finding.

Corynebacteria

P586 The European Commission DG RTD INCO Copernicus program: an example of successful collaboration between countries of Eastern and Western Europe

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The European Commission DG RTD INCO Copernicus programme IC15.CT.98.0302 commenced in September 1998 with the following objectives: to improve microbiological surveillance of potentially toxigenic corynebacteria, to organize a network of Reference Laboratories within Eastern Europe (EE) and to stimulate information and technology exchange between countries of Eastern and Western Europe (WE). Eleven partners from nine countries of EE and WE participated in this program, including the UK, France, Armenia, Belarus, Kazakhstan, Latvia, Romania, Russian Federation and the Ukraine. The total duration of the project was 40 months. Methodologies were focused upon the organization of an effectively functioning network, harmonization of methods for laboratory diagnosis of diphtheria, development of rapid techniques for determination of toxigenicity of corynebacteria and the implementation of a surveillance database with regular data submission. An international quality assurance program was also developed to allow improvements in microbiological surveillance and standardization of laboratory methodologies. In addition, specific research activities, involving the training of partners in laboratories situated within WE (UK and France), were essential for successful collaboration of partners. New National Diphtheria Reference Centres were organized in Armenia, Belarus, Kazakhstan, Latvia and the Ukraine. Microbiological surveillance was established successfully by the implementation of specialized diagnostic methods and also the development and introduction of new, rapid methods for determination of toxigenicity (e.g. EIA and immunochromatographic strip test) used in several field evaluations. A surveillance database was designed using Microsoft® Access, which currently includes data on 1649 strains. This project can be considered as an example of successful collaboration between countries of EE and WE. It has not only led to improvements in laboratory diagnosis by harmonization of methodologies, and implementation of new diagnostic methods, but has also helped in strengthening surveillance and establishment of scientific collaboration, information exchange and transfer of technology (via visiting scientists program) between participating countries. In addition, available funds have allowed countries to acquire necessary reagents and equipment which overall stimulated the establishment of a reliable surveillance system for diphtheria.

P587 Epidemiological surveillance of *Corynebacterium diphtheriae* and *C. ulcerans* infections in Europe

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Objective: An epidemiological database for the surveillance of *Corynebacterium diphtheriae* and *C. ulcerans* infections has been established within the remit of the European Commission BioMed2 BMH4.CT.98.3793 Programme to monitor all laboratory-confirmed diphtheria cases in Europe.
Methods: A database (DipEpi) for epidemiological surveillance of *C. diphtheriae* and *C. ulcerans* isolates was established at the Resource Centre, PHLS, London. Clinical and epidemiological data accompanying, where possible,

each laboratory-confirmed case of diphtheria was collected. Each partner (Finland, France, Greece and Italy) submitted retrospective data on the isolates received and characterized by their laboratory. From 1998, data were submitted every 6 months for entry onto the database. UK data held on the Oracle database system was integrated within the DipEpi database for analysis.

Results: The database contains 1945 entries, >95% of these are represented by UK data and the remainder are data submitted by partners. This includes information on 107 toxigenic strains, 1754 nontoxigenic strains and 84 others. Among the toxigenic strains, there is a predominance of *C. ulcerans* (43), and a significant number is represented by *C. diphtheriae* var *mitis*; of the nontoxigenic strains, *C. diphtheriae* var *gravis* predominate, whilst *C. diphtheriae* var *mitis* and *C. pseudodiphtheriticum* are also significant. Other species include *C. diphtheriae* var *belfanti*, *C. ulcerans*, *C. striatum* and *C. urealyticum*.

Conclusion: Preliminary analysis highlights the prevalence of nontoxigenic *C. diphtheriae*, the significance of 'toxigenic *C. ulcerans* associated diphtheria' and the successful centralization of epidemiological data of laboratory-confirmed diphtheria cases in Western Europe. The global incidence of infections caused by nontoxigenic *C. diphtheriae* and *C. ulcerans* is unknown and their monitoring within the surveillance framework for diphtheria is warranted. Continued monitoring of laboratory-confirmed cases of diphtheria in Europe is one of the objectives of the new DGSANCO European Diphtheria Surveillance Network programme, providing the basis for effective investigation and control.

P588 Nontoxigenic *Corynebacterium diphtheriae* in England and Wales: genetic and biological characterization studies

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Objectives: Since 1990, there has been a marked increase in the number of nontoxigenic *C. diphtheriae* isolates referred to the PHLS/WHO Streptococcus and Diphtheria Reference Unit (SDRU). SDRU and the Communicable Disease Surveillance Centre (CDSC) enhanced surveillance of all nontoxigenic *C. diphtheriae* infections in England and Wales during 1995 and 1996. Since then, the number of referrals have continued to increase, from 51 in 1993 to >300 in 2000. It has been proposed that nontoxigenic *C. diphtheriae* could become toxigenic by acquiring the *tox* gene if their chromosomal gene (diphtheria toxin repressor gene, *dtxR*) is functional. Hence, naturally occurring nontoxigenic strains could constitute a potential reservoir for toxigenicity by phage conversion. Ribotyping has revealed that several genotypes are circulating in the UK and a predominant genotype (provisionally designated as 'A') had disseminated widely within England and Wales. This has highlighted the importance of performing studies to determine whether the strains possessed functional *dtxR* genes and their distribution amongst clinical isolates. Sequence analysis of the amplified product was also undertaken, and to determine whether or not these were functional genes, production of siderophores in varying concentrations of iron was also demonstrated.

Methods: A total of 140 *C. diphtheriae* isolates were ribotyped. PCR amplification of the *dtxR* gene was carried out on a selection of 40 isolates and sequencing of the PCR products was performed by dye terminator cycle sequencing using a CEQ 2000 capillary sequencer. Siderophore production was determined using a modified Chrome Azurol S assay.

Results: Results obtained have shown that these strains possessed the *dtxR* gene and they produced siderophores when grown in an iron-limiting environment. Sequence data detected 45 point mutations amongst the UK isolates with three amino acid substitutions.

Conclusions: PCR amplification showed that all strains possessed the *dtxR* gene and sequence data detected considerable heterogeneity within the ORF of the *dtxR* gene. However, none of the amino acid substitutions appeared to have inactivated the *dtxR* gene. Detection of siderophore production showed that the *dtxR* gene is fully functional.

P589 Prevalence of *Corynebacterium urealyticum* in adult urinary tract infections in 4-year period (1998–2001)

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Objectives: To study (a) the prevalence of *Corynebacterium urealyticum* in adult urinary tract infections in a period of 4 years (1998–2001), (b) their susceptibility to antibiotics.

Methods: Urine samples were plated in blood agar and incubated in ambient air, 35 °C for 48–72 h. API Coryne System (BioMerieux) was used for identification. Sensitivities to antibiotics were tested by microdilution method, according to NCCLS guidelines.

Results: *C. urealyticum* was isolated in a total of 24/19 482 (0.12%) patients (13 men, 11 women; mean age 66 years) during the study period. The underlying diseases of these patients were: urologic manipulation 9/24; previous urologic diseases 5/24; indwelling catheter 6/24; diabetes melitus 2/24; hematologic malignacy 1/24; lung cancer 1/24. The majority (18/24) presented urologic symptoms (hematuria, dysuria frequency). The urine were alkaline (pH > 8) in 80%. Hematuria (microscopic/gross) and pyuria were found in 75 and 70%, respectively. The resistence to antibiotics was: penicillin 100%, gentamicin 85%, erythromycin, clindamycin, cotrimoxazole 80% each, quinolones 72%, tetracycline 35%, rifampin 30%, since all the isolates were sensitive to Glycopeptides.

Conclusions: (1) The prevalence of *C. urealyticum* in urinary tract infections is 0.12% mainly in patients suffering from urologic diseases. (2) *C. urealyticum* isolates were highly resistant to many antimicrobial agents including quinolones and cotrimoxazole. (3) *C. urealyticum* isolates appeared highly sensitive to glycopeptides. (4) rifampin may be an alternative antimicrobial agent for *C. urealyticum* urinary tract infections.

P590 Corynebacterium mucifaciens bacteriaemia: two case reports

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C. mucifaciens sp. nov. is an unusual species recently described, isolated from human clinical specimens. The morphologies of colonies are slightly yellowish, glistering and markedly mucoid. We describe the isolation of *C. mucifaciens* from blood in two patients.

Case 1: A 36-year-old man infected with HIV-1 diagnosed 3 years ago. He came at emergency ward of our hospital complaining of fever, thoracic pain and malaise of 2 days duration. He had clinical records of recurrent pneumonia during the last year. Chest radiograph was normal. He was diagnosed of upper respiratory tract infection, and treated with azithromycine. Blood and sputum cultures were performed before antimicrobial therapy, but no pathogen was recovered. Seven days later, he remained febrile but the respiratory symptoms were resolved. A new blood culture was performed and one of the three bottles yielded growth of a Gram-positive, coryneformed bacteria identified as *C. mucifaciens*.

Case 2: A 52-year-old woman was admitted at our hospital with an acute episode of high fever, dyspnoea and malaise. She had a history of arterial hypertension and a record of pigeon contact. A chest radiograph revealed diffuse bilateral infiltrates suggestive of atypical pneumonia. She was treated with macrolides and discharged. After 48 h, she had another episode of similar characteristics. The treatment of macrolides and prednisone was started again, with complete improvement. *C. mucifaciens* grew in one of the three blood cultures performed. In both the cases, the isolates presented in sheep blood agar a colonies of 1–1.5 mm diameter, circular, convex, glistering, slightly yellowish and markedly mucoid. Gram-stain revealed typical diphteroids Gram-positive cells in clusters with a mucous sheath around them.

Conclusions: Recently, a new species named *C. mucifaciens* obtained from human clinical specimens has been described. Although at present, blood culture is the most common source where this bacterium has been recovered, there is no data about its clinical significance. In our patients, the isolation was in bloodstream too, but it was not clearly associated to his clinical symptoms and could be a colonizer of skin or contaminant of sterile body sites like other corynebacteria. However, a potential pathogenic role cannot be rejected and more reports are necessary.

Paediatric infection and prevention

P591 Prospective study of bacteremia in children during a 7-year period

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Objectives: To analyze the etiology of pediatric bacteremia in a tertiary medical center and the relationship with age, source, risk factors, antibiotic susceptibility and mortality.
Patients and methods: We conducted a 7-year prospective study of bacteremia episodes with clinical significance in children. A single blood culture was obtained and inoculated into aerobic bottle. Anaerobic bottle was inoculated only in clinical settings associated with anaerobic infection.
Results: A total of 213 episodes of bacteremia were studied for 7 years. After the neonatal period, 169 episodes were diagnosed. Among this group of children, community-acquired accounted for 85%, and 53% of the patients were younger than 2 years old. The most frequent isolates were *Streptococcus pneumoniae* (18%), *Neisseria meningitidis* (16%), *Staphylococcus aureus* (12%), *Escherichia coli* (11%) and coagulase-negative *Staphylococcus* (10%). The main sources of bacteremia were pneumonia (14%), indwelling vascular catheters (14%), gastrointestinal and abdominal infections (10%), urinary tract infections (7%) and bone and joint infections (7%). In 35%, the source of bacteremia could not be established (occult bacteremia accounted for 10%). Fifty episodes were diagnosed in children with underlying diseases (50% malignant neoplasms). In the neonatal period, 44 episodes were studied. The main etiologic agent in early neonatal sepsis was *Streptococcus agalactiae* (55%) and in late onset sepsis was *E. coli* (31%). Some degree of penicillin resistance was observed in 45% of pneumococcal strains (MIC > 0.06 μg/mL) and in 46% of the meningococcal strains (MIC > 0.12 μg/mL). Of the pneumococcal isolates, 19% were also nonsusceptible to third generation cephalosporins (MIC > 0.5 μg/mL). Four deaths attributed directly to the bacteremia were observed all in newborn infants.
Conclusions: After the neonatal period, bacteremias were mainly community-acquired episodes in patients younger than 2-year-old and the most frequent causative agent was *S. pneumoniae*. No deaths attributed to bacteremia occurred in children older than 1 month. Although our study is hospital based, it represents more closely a community-based study and may be applicable to most of the pediatric emergency departments.

P592 Acute mastoiditis in children – *Pseudomonas aeruginosa* as a leading pathogen in older patients

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Objectives: To assess the clinical features, pathogens, management, and outcome of acute mastoiditis (AM) in children in northern Israel.
Methods: A systematic review of medical records of all children who were admitted with acute mastoiditis between January 1990 and December 2000 to the Pediatric Wards at Ha'Emek Medical Center, Afula, Israel.
Results: During the study period, 57 children with acute mastoiditis were admitted. The median age was 34.5 months (range: 4 months to 13 years). In 26 (45.6%) patients, acute mastoiditis complicated a first episode of acute otitis media. Twenty-five (44%) children received antibiotic treatment before admission. Frequent symptoms on admission included erythema over the mastoid area (94.5%), proptosis of the auricle (91.2%), fever (75.8%). Middle ear, subperiostal, and mastoid aspirates yielded growth of pathogen in 35 (78%) of the 45 children. The most frequent pathogens were: *Pseudomonas aeruginosa* (26.6%), *Streptococcus pneumoniae* (17.7%) and group A *Streptococcus* (11.1%). A higher incidence of *S. pneumoniae* on culture was found in children who did not suffer from AOM as compared to those who had AOM during the last week before admission (35% vs. 5%) and in children younger than 2 years. Seventeen children underwent computed tomography of the mastoid for suspected surgical complication. Bone destruction of the mastoid was demonstrated in five children, subperiostal abscess was found in eight children. Fifty-two (91%) children were treated by antibiotics alone. Simple mastoidectomy was successfully performed in five children. The median duration of hospitalization was 7 days (range 1–28 days).
Conclusions: The diagnosis of AM could be made on clinical basis and CT is needed only in those children in which surgical complication is suspected. Half of the children admitted with AM have no previous history of recurrent AOM. In those children, *S. pneumoniae* was the leading pathogen, while *P. aeruginosa* was more prevalent in children with recurrent AOM. The great majority of the patients recovered with medical therapy, and did not require surgical intervention.

P593 Nontyphoidal *Salmonella* bacteremia in childhood

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Objectives: To study the incidence, clinical and bacteriologic characteristics and outcome of nontyphoidal *Salmonella* (NTS) bacteremia in children.
Methods (population): All the children with culture-proven NTS infection hospitalized in the Department of Paediatrics and Neonatal Intensive Care Unit, University Hospital of Crete during the 9-year period from January 1993 to September 2001 were included. The medical and microbiologic records of the children were retrospectively reviewed, and demographic, clinical and laboratory characteristics, serotype and susceptibility of the strains, treatment and outcome were analyzed.
Results: During the study period, the total number of children with NTS infection was 337 (mean annual number = 37.2). NTS infection was the cause of 0.015% of admissions. Among the 337 children with NTS infection, bacteremia was found in 13 children (0.04%). Most of the bacteremic cases presented between July and September (9/13). The mean age of the children was 1.8 years (range: 5 months to 7.5 years) and 9/13 were infants of <1 year. All children but one presented with fever >39 °C, one with febrile seizures, two with a mascular rash and two with extraintestinal focal infections (prevertebral and rectal abscess). None of the children had meningitis or urinary tract infection. Two of the cases presented without gastroenteritis. None of the children had a predisposing underlying disease, such as sickle cell disease. The mean blood white cell count was 11 300/mm^3 (range: 7800–15 700/mm^3) and none of the children had leucopenia. *S. enteritidis* was the most common serotype isolated (6/13), followed by *S. virchow* (4/13), *S. agona*, *S. newport*, and *S. typhimurium* (one case each). All strains were sensitive to all β-lactamic antibiotics and to cotrimoxazole. Repeat blood cultures were obtained in all patients and were all sterile. The median duration of hospitalization was 11 days and all children received antibiotics intravenously for a minimum of 7 days (range: 7–30 days). Fever subsided after a mean time of 3 days after initiation of antibiotics. All the children had a full recovery and no NTS infection was manifested during a mean follow-up period of 3.45 years (range: 2 months to 9 years).
Conclusion: NTS bacteremia, although currently uncommon, may still cause considerable morbidity in healthy children, especially in the very young. The risk of focal infection is small and the outcome is favorable with appropriate antibiotic treatment.

P594 Efficacy of 5-day cefprozil versus 10-day amoxicillin/clavulanate for acute otitis media in children

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Objective: To compare the efficacy and safety of 5-day cefprozil (CEFP) versus 10-day amoxicillin/clavulanate (AMX/CL) suspensions for acute otitis media (AOM) in children.
Methods: In this prospective, open-label, multicenter study children aged 6 months to 12 years with AOM (either first AOM or recurrent AOM) were randomized to receive CEFP (30 mg/kg/day BID) for 5 days or AMX/CL (50 mg/kg/day of AMX TID) for 10 days. Follow-up otoscopic and tympanometric controls were scheduled at the end of treatment and at day 28–32. Outcome was defined as satisfactory (cure, complete resolution of signs and symptoms and absence of effusion + improvement, partial resolution of signs and symptoms and persistence of effusion) or unsatisfactory (failure by the end of therapy; recurrence by day 28–32).
Results: Out of the 140 enrolled children, 73 (44 M, mean age 4.3 years) received AMX/CL and 67 (42 M, mean age 4.1 years) CEFP. By the end of therapy, a satisfactory outcome was observed in 67/67 (100%) children treated with CEFP and in 67/73 (90.4%) children treated with AMX/CL ($P = 0.02$)

(cure: CEFP 37.3% vs. AMX/CL 42.4%; improvement: CEFP 62.7% vs. AMX/CL 48.0%). By day 28–32, a satisfactory outcome was observed in 61/64 (95.3%) children treated with CEFP and in 56/61 (91.8%) children treated with AMX/CL ($P=0.48$) (cure: CEFP 64.1% vs. AMX/CL 43.9%; improvement: CEFP 26.9% vs. AMX/CL 40.9%). Mild drug-related adverse events (mainly gastrointestinal) were observed in 8/67 (11.9%) children treated with CEFP compared with 17/73 (23.3%) children treated with AMX/CL ($P=0.12$).

Conclusions: Shortened, effective, antibiotic therapies for community-acquired upper respiratory infections are desirable especially in children. Our data suggest that treatment with CEFP given for 5 days is as effective and well tolerated as the conventional treatment with AMX/CL for 10 days and may offer a valid alternative regimen with potential for better compliance.

P595 Epidemiology of invasive *Streptococcus pneumoniae* infections in children in an area of Barcelona, Spain

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Background: *Streptococcus pneumoniae* remains a leading cause of serious community-acquired infections among young children. With the advent of conjugate vaccines, it is becoming possible to prevent disease caused by this organism.

Objectives: To determine the incidence and the clinical and microbiologic characteristics of this disease in children resident in Sabadell, an industrial area with 371 663 inhabitants in the province of Barcelona, Spain.

Methods: From January 1990 to December 2000, case records of children with pneumococcal invasive disease at Sabadell Hospital were retrospectively (1990–1996) reviewed and prospectively (1997–2000) collected.

Results: A total of 112 children, 54% less than 24 months of age and 93% less than 6 years of age with invasive pneumococcal disease were diagnosed in a period of 11 years. The incidence was 76 per 100 000 for children aged 0–24 months, 45 for children aged 0–48 months, and 16.6 for children aged 0–14 years old. Occult bacteremia was the most common manifestation observed (66 cases), pneumonia was the second form (34 cases) and meningitis (10 cases) and arthritis (2 cases) were the other clinical manifestations. Of the 105 strains tested, 8.6% were highly penicillin-resistant, 37.1% were intermediately penicillin-resistant, 16.2% were intermediately cefotaxime-resistant and 32.4% were erythromycin-resistant. Pneumococci of serogroups 6, 14, 18, 19, 1, 5, 4, 9, 23 and 33 were isolated most frequently (92%) but only 6, 9, 14, 19 and 23 were resistant to penicillin, cefotaxime or erythromycin.

Conclusions: The pneumococcus is the second cause of invasive bacterial infection in children in Sabadell, behind the meningococcus infection. Our incidence is greater than the incidence reported previously in Spain and Europe, perhaps due to the number of occult bacteremia cases in our study. Penicillin-resistance levels in our area are very high, but the trend towards increasing penicillin resistance may have ended over the last few years. The currently licensed seven-valent pneumococcal conjugate vaccine representing serogroups 4, 6, 9, 14, 18, 19 and 23 would cover 78% of cases of invasive pneumococcal in children aged 0–14 years, 80% in children aged 0–24 months and 100% of cases of invasive pneumococcal disease penicillin or cefotaxime-resistant in children resident in Sabadell.

P596 Phenotype and genotype diversity of the HMW adhesins in nonencapsulated *Haemophilus influenzae* isolates from invasive disease

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Among surface antigens of nonencapsulated *Haemophilus influenzae*, the HMW1 and HMW2 proteins are the major adhesins responsible for attachment to human epithelial cells. These high molecular weight proteins were originally identified as the predominant target of the host immune response during host otitis media and were proposed as vaccine components. In *H. influenzae* strain 12, both the HMW1 and HMW2 proteins are present; the *hmw1A* and the *hmw2A* genes, which encode for the HMW1 and HMW2 structural proteins, are identical in the sequence of the first 1259 bp, but thereafter partially diverge (overall identity 80%). In the present study, 62 nonencapsulated *H. influenzae* strains isolated from invasive disease were studied. The presence of the *hmwA* genes was determined by PCR using primers recognizing a sequence in the conserved region of the *hmw1A* and the *hmw2A* genes from the strain 12. Western blotting with polyclonal rabbit antiserum and with HMW-specific monoclonal antibodies (Mabs) was performed to assess the presence of HMW1 and HMW2 proteins. To further characterize the HMW type expressed by the different strains, a PCR–RFLP analysis was performed. A 2115-bp sequence, located in the divergent region of *hmw1A* and *hmw2A* in strain 12, was amplified by PCR and sequentially digested with specific restriction enzymes. PCR demonstrated the presence of *hmwA* genes in 38 out of the 62 (61%) strains studied. Western blotting with polyclonal rabbit antiserum showed the presence of HMW proteins in 30 out of the 38 *hmwA*-positive strains (78.9%). Contrary to the prototype *H. influenzae* strain 12, the majority of the strains exhibited only one HMW protein band. A large variability in the molecular mass of the proteins was observed (ranging from 110 to 160 kDa). Using the HMW-specific Mabs, six different patterns were found. The expected PCR product of 2115 bp was amplified in all the 38 strains tested. Several different RFLP profiles were obtained with each restriction enzyme. Our results demonstrate that the HMW adhesins are expressed by the large majority of nonencapsulated *H. influenzae* isolates from invasive disease. A large heterogenicity was found among the different strains studied, suggesting the presence of several variants of the HMW1 and HMW2 proteins compared to those detected in the prototype strain 12.

P597 Immunogenicity of anti-*Haemophilus influenzae* type B CRM197 conjugate following mucosal vaccination with oligodeoxynucleotide containing immunostimulatory sequences as adjuvant

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Objectives: Most vaccines are delivered by injection. Mucosal vaccination would increase compliance and decrease the risk of spread of infectious diseases due to a reduction of mucosal colonization and of contaminated syringes. However, most vaccines are unable to induce immune responses when administered mucosally, and require the use of strong adjuvant or effective delivery systems. Synthetic oligodeoxynucleotides containing CpG immunostimulatory sequences (ISS) have been shown to act as potent adjuvants of type-1 immune responses also when mucosally coadministered with protein or peptide vaccines. We have shown that ISS can increase the antipolysaccharide (CHO) antibody titers and antidiphtheria toxin-neutralizing antibody, if used as adjuvant of anti-*Haemophilus influenzae* type b (HIB) CHO vaccine conjugated with cross-reacting material (CRM) of diphtheria toxin in mice.

Methods: BALB/c mice were immunized by intranasal (i.n.) inhalation of 2.5 μg CHO/mouse of HIB-CRM alone or in combination with 0.5 μg/mouse of CT, 10–20 μg/mouse of ISS or M-ODN in a total volume of 66 μL (33 μL/naris). HIB-CRM conjugate vaccine was administered in a three-dose schedule (0, 10 and 20 days). Adjuvants were administered in the first inoculum only, or in three inocula of vaccine. The antibody response to HIB-CHO in sera was determined by enzyme-linked immunosorbent assay (ELISA). Diphtheria toxin-neutralizing activity of anti-CRM antibodies in sera of mice immunized with HIB-CRM alone or in combination with one or three doses of ISS (10 μg/mouse) was determined in vitro according to the Miyamura assay with Vero cell line.

Results: Here we show that ISS have the potential to increase host local and systemic antibody response against both the CHO and the protein component of a conjugated vaccine when mucosally administered in mice. Mucosal administration of HIB-CRM vaccine induced anti-CHO and neutralizing antidiphtheria toxin antibodies of all the IgG subclasses, with a predominance of type-1 immune response-associated IgG2a and IgG3. At odds with systemic administration, the mucosal delivery of HIB-CRM induced anti-CHO and antidiphtheria toxin mucosal IgA.

Conclusions: Our data envisage the feasibility of a mucosal vaccination with an already licensed HIB-CRM vaccine and encourage the investigation to identify strategies of vaccination with schedules aimed at the valuation of protein carriers as protective immunogens.

P598 The introduction of HIB vaccination in the Czech Republic

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Objectives: Active surveillance was introduced in the Czech Republic to estimate the incidence and case fatality rate of invasive disease caused by *Haemophilus influenzae* b (HIB) and to assess the impact of routine HIB vaccination of infants.
Methods: Active surveillance was introduced nation-wide in January 1999 and case definition of invasive HIB disease included meningitis, epiglotitis, bacteremia and/or sepsis and pneumonia. According to the laboratory results, HIB cases were defined as confirmed, probable or suspected. Routine HIB vaccination of infants was introduced in July 2001 using tetravalent DiTePe + HIB vaccine.
Results: The results of active surveillance of HIB invasive disease showed a total incidence of 1.0/100 000 population in 1999 and 1.1/100 000 in 2000. The highest incidence, in both years, was in age group 0–11 months (17.1/100 000 and 15.6/100 000, respectively) and 1–4 years (17.4/100 000 and 20.9/100 000, respectively). The most frequent clinical form, in both years, was meningitis (53.5 and 59%, respectively) followed by epiglotitis (35.6 and 27.3%, respectively). Total case fatality rate was 1% in 1999 and 2.6% in 2000. The data of active surveillance for 2001 indicate the decrease of HIB invasive disease after the introduction of routine HIB vaccination. The number of HIB invasive cases in the period after the introduction of routine vaccination (July–October 2001) decreased to 59.2% of the cases found by active surveillance in the same months in the previous 2 years (27 cases in July–October 1999, 27 cases in July–October 2000 and 16 cases in July–October 2001). The introduction of active surveillance of HIB invasive disease 2 years before the start of routine HIB vaccination of infants gives a possibility to assess the efficacy of this mass vaccination and to follow eventual vaccine failure according to the rules of the EU project.
Conclusion: Routine HIB vaccination of infants was introduced in the Czech Republic in July 2001. The results of active surveillance indicate decrease of HIB invasive disease under the influence of this mass vaccination.
Acknowledgments: This study was supported by research grant NI/6803-3 of the Internal Grant Agency of the Ministry of Health of the Czech Republic. We thank Dr. K. Jolley (University of Oxford, UK) for kind editing of the text.

P599 Distribution of *Burkholderia cepacia* complex isolates from patients with cystic fibrosis

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Objectives: To confirm all isolates obtained from patients with cystic fibrosis (CF) as *Burkholderia cepacia* (Bc) Complex and to identify the genomovar of Bc isolates.
Methods: Sixty-nine isolates, identified by Api 20NE as Bc, were subjected to a PCR-based identification by amplification of the 16S rDNA gene and by amplification of the *RecA* gene, and the profile obtained from RFLP analysis of these genes were analyzed.
Results: Sixty isolates (86.9%) were identified as Bc Complex. Four (6.7%) isolates were found to be Bc genomovar I, 27 (45%) isolates to be Bc genomovar III-A, 28 (46.7%) isolates to be Bc genomovar III-B and 1 (1.6%) isolate to be Bc genomovar V. Nine (13%) isolates, misidentified by biochemical tests were found to be *Pseudomonas aeruginosa* (three isolates, 33.3%), *Alcaligenes xilosoxidans* (three isolates, 33.3%), *Stenotrophomonas maltophilia* (two isolates, 22.2%) and *Ralstonia pickettii* or *Commomonas acidovorans* (one isolate, 11.1%).
Conclusion: All isolates obtained from patients CF, identified by commercial test, are to be further confirmed by using genotyping methods.

P600 Characteristics of bacterial pathogens isolated from cystic fibrosis patients. Typing of *Staphylococcus aureus* isolates by use of PFGE method

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Objectives: The aim of the study was the determination of the frequency of isolation of various bacterial species from respiratory tract of cystic fibrosis (CF) patients treated in the Children's Memorial Health Institute in Warsaw, Poland.
Methods: A total of 49 children with cystic fibrosis hospitalized between January 1999 and November 2001 were investigated. Clinical specimens used for bacteriological examination were: sputum, deep throat swabs and epipharynx swabs.
Results: Total 446 samples were taken from 49 children. *Staphylococcus aureus* was the most frequently isolated pathogen (167 isolates). Fifteen *S. aureus* strains were resistant to methicillin. The second most frequent pathogen was *Pseudomonas aeruginosa* (61 isolates). Other isolated bacterial species were: *Haemophilus influenzae* (24), *Moraxella catarrhalis* (21), Gram-negative rods of Enterobacteriaceae family (19), *Streptococcus pneumoniae* (12), *Stenotrophomonas maltophilia* (5), *Streptococcus pyogenes* (5), β-hemolytic *Streptococcus* non-A (4), and other nonfermenting rods (3). It was ascertained, that in spite of antibiotic treatment and regression of clinical symptoms, the cultures were still positive for *S. aureus* and *P. aeruginosa*. Phenotype of *S. aureus* was based on antibiotic resistance profile. PFGE was used for clonal characteristics of isolates.
Conclusions: CF children hospitalized in our Institute were colonized/infected mainly by *S. aureus* and *P. aeruginosa*. Persistence of these pathogens was observed in spite of antibiotic therapy.

Neo-natal infection

P601 Nosocomial enterococcal infection in neonates

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Definitions: *Enterococcus* spp. is the third commonest cause of nosocomial infection in adults and the fourth in children. Therefore, we decided to perform retrospective surveillance of enterococcal infections in neonates in tertiary neonatal center in Bratislava, from January 1999 to December 2000.
Methods: We investigated all enterococcal infections within 2 years, among 246 neonates hospitalized for infections in the neonatal intensive care unit. We used univariate analysis to assess risk factor for neonatal infections caused by *Enterococcus* spp. (99) versus those neonates caused by other organisms (147).
Results: Within 246 neonates, in 99 cases *Enterococcus* spp. was isolated from various samples, in 21 cases from blood cultures (bacteremia) ($P < 0.0016$), in 29 cases from urine culture ($P < 0.0063$), in 39 cases from umbilical swabs (omphalitis), and in 28 cases from gastric aspiration. The only risk factors for enterococcal infection was isolated from colonization (a positive skin swab $P < 0.01$). Early markers of enterococcal infections in comparison to other infection were: higher procalcitonin level (PCT) ($P < 0.0037$), positive septic score ($P < 0.018$) and leucopenia defined as <1000 ($P < 0.0013$). Bacteremia ($P < 0.0055$) was significantly more frequently observed among neonates with enterococcal infections than among neonates with other infections. *Klebsiella/Enterobacter* (58.59% vs. 36.05%, $P < 0.0008$) and *Candida* spp. (25.25% vs. 13.61%, $P < 0.031$) as copathogenes were significantly more frequently observed in neonatal infections caused by *Enterococcus* spp. Outcome (both attributable and overall mortality) was similar to other infections. However,

neurological sequel such as intraventricular hemorrhage (III/IV) ($P < 0.018$) was significantly more frequently observed in cases with enterococcal infections than in neonates with other infections.
Conclusion: Overall and attributable mortalities were (both) very low (2.02%) which is in contrast to adults, where mortality on enterococcal infections exceeds 15%. This is probably because early, empiric, anti-infectis therapy in neonates with fever contained in our center anti-enterococcal antibiotics, such as ampicillin/sulbactam, or ampicillin (55.56%) plus gentamicin (45.45% vs. 23.81%, $P < 0.00063$). Such therapy may be a protective factor against inferior outcome of enterococcal infections in neonates, where mortality (2%) was much lower than reported from bacteremia studies in adults.

P602 Nosocomial infections in pediatric intensive care units

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Objectives: To determine the incidence, the site and bacterial epidemiology of nosocomial infections among patients admitted to pediatric and neonatal intensive care units of an university hospital (650 beds).
Methods: A prospective study as a part of hospital infection control program was performed for 4-month with periodic chart review during hospitalization using an uniform prospective questionnaire in each unit. Infants admitted at the pediatric intensive care units (PICU) or the neonatal intensive care units (NICU) at least 24 h were included in the study.
Results: During the 4-month period, 121 patients were studied (31 in PICU and 90 in NICU). In these units, mechanical ventilators were used in 32% PICU patients and in 30% NICU patients, urinary catheters in 65% PICU patients and in 20% NICU patients and central intravascular catheter in 42% PICU patients and in 36% NICU patients. This study showed a devices utilization ratios comparable with that of NNIS data. The incidence of nosocomial infection was 6.45% in PICU and 22.22% in NICU. In both, the most frequent nosocomial infection was bacteremia (PICU-100% and NICU-51.28%). In NICU, the highest nosocomial infection incidence was observed for very low birth weight infants. In PICU, the microorganism isolated was *S. epidermidis*. In NICU, the most common pathogens were *E. cloacae* (29.6%), *C. albicans* (18.5%) and *S. epidermidis* (14.8%), but there was a outbreak of *E. cloacae* in this unit in October.
Conclusions: There is a high prevalence of invasive devices and procedures in these settings and improving infection control practices when these devices are used should be an important measure to reduce infections. PICU and NICU (specially NICU) provide care for patient populations at the greatest risk for nosocomial infection, for that, surveillance of these units should be included in the infection control program all the year, because it is fundamental to develop intervention strategies for the prevention of nosocomial infections in this population. Also, observe the evolution of nosocomial infection in the same unit should be observed to have epidemiological validity that can be used to evaluate the efficacy of prophylactic measures.

P603 Nosocomial infections (NIs) in a neonatal and pediatric intensive care unites (ICU): a prospective analysis

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Genoa, I

Objectives: The assessment of NIs incidence and of related risk factors (RFs) in an individual institution is an effective tool to design local guidelines.
Methods: We performed a prospective study in the period January 15, to July 15, 2000 in a 25-bed intensive care unit (ICU). Eighty-one newborns (N) and 144 children (C) were studied. The RFs chosen were: birth weight, hospitalization days, invasive procedures, surgery and antibiotics use. Data were collected on standardized forms and statistically analyzed.
Results: The incidence of NIs was 20.9% (47 infections): 33.3% in N and 13.8% in C. bloodstream infections (BSIs) were the most frequent infections (55% in N and 60% in C) followed by pneumonias (PNE) (22% in N and 40% in C). Pathogens involved in NIs were Coagulase-negative Staphylococci (CNS) (48%), Gram-negative (26.4%), *S. aureus* (13%) and *Candida* spp. (7.5%). The isolation rate (%) in BSIs and PNE was, respectively: CNS 69 and 25, Gram-negatives 20 and 43, *S. aureus* 11 and 18, *Candida* 0 and 14. The oxacillin resistance rate was: 94.7 in CNS and 7.6 in *S. aureus*; 66% of *Klebsiella* were resistant to third generation cephalosporins. All *Candida* PNE were caused by non-*albicans* strains. For BSIs the most significant RF was prolonged total parenteral nutrition (TPN) ($P < 0.0001$) and for PNE were prolonged mechanical ventilation, arterial catheter and TPN ($P < 0.0001$). RFs related to CNS infections were CVC, TPN, arterial catheter ($P = 0.0012$); to *Klebsiella* infections were low birth weight, hospitalization days, CVC/days, NPT, arterial and bladder catheter ($P = 0.0001$). Antibiotic therapy was prescribed in 211/225 (93.7%) patients; 97 patients were empirically treated with a first-line therapy aminoglycoside + glycopeptide. The mortality rate, only due to BSIs, was 1.7 (3.7 in N and 0.7 in C).
Conclusions: Our results show a high incidence of BSIs caused by resistant CNS, the related RFs stress the need to improve educational measures on management of invasive procedures, particularly CVC. A careful antimicrobial policy is mandatory concerning the use of glycopeptides and third generation cephalosporins as first-line therapy.

P604 Therapeutic effect of imipenem/cilastatin against severe neonatal infections

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Objectives: Severe bacterial infections remain the most important cause of mortality and cost for neonates hospitalized in neonatal intensive care unite (NICU). The clinical efficacy of imipenem/cilastatin has been assessed in the management of severe early onset and nosocomial infections in neonates.
Methods: A total of 42 (23 boys and 19 girls) neonates with birth weight 830–3900 g (31 prematures and 11 full-term) admitted to the NICU in a university hospital from January 1, 2000 to September 30, 2001 because of acute respiratory disorders.
Results: Septicemia was documented in 33 babies (early onset in 21, nosocomial in 12) by clinical findings (pneumonia, NEC, shock), hematological disorders (thrombocytopenia, anaemia, DIC) and the presence of *Klebsiella pneumoniae* (15), *Pseudomonas aeruginosa* (5), *Acinetobacter baumanii* (2), *Serratia marcescens* (1), *E. coli* (1), *S. haemolyticus* (4), *S. epidermidis* (4) and *S. hominis* (1) in blood cultures. All Gram-negative strains were sensitive to imipenem. Pyuria (*Klebsiella* 10.5 in urine culture) in one full-term neonates with congenital defect of urinary tract, bilateral purulent coxitis in one baby and severe pneumonia in seven prematures (early onset in three and ventilator-associated in four cases) were diagnosed. In five pneumonic mechanically ventilated neonates Gram-negative bacteria (*Klebsiella*: 3, *Pseudomonas*: 2) susceptible to imipenem in tracheal aspirates were noted. Twelve neonates obtained imipenem as alone antibiotic therapy from the beginning of infection, 28 babies were treated earlier by aminoglicosides, cephalosporins or amoxicillin/clavulanic acid, one by vancomycine and one by meropenem without satisfactory results. Imipenem was administered in dose 50–60 mg/kg every 6 h during 10–16 days (in case of coxitis: 28 days): in 16 neonates as alone antibiotic, in 25 with vancomycine and in one with ceftazidim. This therapy produced satisfactory clinical response in 34 (81%) patients (complete in 42%, partial in 39%). The bacteremia, Gram-positive (2) and Gram-negative (2) persisted despite of imipenem administration in four prematures. The pneumonic prematures died: one because of massive intracranial hemorrhage and one of bronchopulmonary dysplasia.
Conclusion: Clinical efficacy of imipenem/cilastatin confirms its usefulness in the treatment of severe neonatal infections in the intensive care unit.

P605 An outbreak of neonatal sepsis caused by *Klebsiella pneumoniae* ESBL

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Objectives: *Klebsiella pneumoniae* is an unusual cause of sepsis in healthy newborns. In our hospital, there had been no infections caused by *K. pneumoniae* ESBL until January 2001 when five neonates had clinical and laboratory confirmed sepsis. We describe the results of our investigation as well as infection control measures that stopped the outbreak.
Methods: All neonates were of low birth weight and low gestational age. They had been given breast milk by tube plus parenteral feeding (multidose vials). Mothers stayed long in the hospital, they were treated by cefalosporins

perinatally and became colonized by nosocomial pathogenes. Strains were identified by conventional biochemical tests. Antibiotic susceptibitity was evaluated by disk-diffusion test according to NCCLS. ESBL production was detected by the double-disk synergy test and ESBL E-test (AB biodisk).
Results: Shortly after birth all affected neonates had been given cefuroxime + gentamicin. Between the 10th and 15th day of life (few days after therapy had finished) they developed sepsis. *K. pneumoniae* ESBL was isolated from blood culture in five neonates and from stool of two more neonates (colonization).All strains had the same susceptibility pattern (resistant to all cephalosporins, gentamicin, and susceptible to amikacin, imipenem and ciprofloxacin).The result of additional cultures: HCW-hands — nurseA *K. pneumoniae* ESBL, nurseB *Enterococcus*; breast milk — motherA *K. pneumoniae* ESBL, motherB *Serratia marcescens*, *Pseudomonas aeruginosa*; Salviamin (multidose vial): *Serratia marcescens*.
Conclusion: Excessive usage of antibiotics especially cephalosporins selected resistant *K. pneumoniae*. One of the possible ways of colonization was obviously contaminated breast milk that was given to newborns by tube. Poor compliance regarding infection control procedures as well as overcrowding and understaffing led to the outbreak. The outbreak was terminated after all affected neonates were cohorted and requirement for infection control procedures was stressed: increase hand antisepsis and reinforcement of single dose vials. In order to restrict the resistance, modification of early empiric antibiotic regimen (exclusion of cefalosporins) was also recommended. Surveillance cultures 10 months after the outbreak showed susceptible *Escherichia coli* as predominate bacteria in NICU. In that period, neither case of neonatal sepsis nor isolation of resistant *K. pneumoniae* was registered in this unit.

P606 Reliability of polymorphonuclear elastase for the diagnosis of neonatal sepsis

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Infections are a major problem in neonatal intensive care units throughout the world, and early diagnosis and therapy would certainly reduce associated morbidity and mortality as well as decrease unnecessary antibiotic treatment.
Objectives: To evaluate the usefulness of polymorphonuclear elastase (PMN-E) serum concentration as an early indicator of neonatal sepsis in comparison with routinely used infection markers.
Methods: PMN-E was measured in 76 newborns with suspicion of systemic bacterial infection treated in a tertiary intensive care unit. The measurements were performed with commercially available enzyme immunoassay (Milenia).
Results: Mean PMN-E-value in noninfected newborns was 38.85 ng/mL, and for infected 184.12 ng/mL ($P < 0.05$) (Student's *t*-test). The sensitivity to the diagnosis of culture-proven bacterial systemic infection was 68% for PMN-E, 60% for C-reactive protein, 59% for the immature to total neutrophil ratio and 56% for the total white blood cell count. The corresponding specificity amounted to 92, 86, 100 and 80%, respectively.
Conclusion: Serum PMN-E level determination yields diagnostic advantages in comparison with infection markers routinely used in sepsis screen and may serve as a valuable early indicator of neonatal systemic bacterial infection.
Acknowledgements: This work was carried out as a part of the research project no. 6 P05E 049 21 sponsored by the Polish State Committee for Scientific Research.

Enteropathogenic *E. coli*

P607 Diarrhea in Danish children under 5 years of age: a case–control study

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Objectives: To clarify the most important etiologies of bacterial diarrhea in Danish children under 5 years of age.
Methods: Stools from 219 cases with diarrhea from all over the country and 416 healthy controls were examined for *Salmonella*, *Campylobacter*, *Yersinia*, *Shigella* and *Vibrio* spp. using standard methods. Stools were also examined for verocytotoxin-producing *Escherichia coli* (VTEC), attaching and effacing *E. coli* (A/EEC) including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAggEC) by colony hybridization of virulence genes and serotyping.
Results: Overall, a bacterial cause was found in 18% of the cases. The following pathogens were associated with disease ($P < 0.05$ when comparing isolation rates between patients and controls, Fisher's exact test): *Campylobacter coli/jejuni*: 11/219 (5%) patients versus 3/416 (<1%) controls; *Salmonella*: 8/219 (4%) versus 0/416 (0%); EPEC: 7/219 (3%) versus 2/416 (<1%); VTEC: 6/219 (3%) versus 2/416 (<1%); and *Yersinia*: 6/219 (3%) versus 0/416 (0%). EAggEC, A/EEC (eae positive nonclassical EPEC serotypes) and ETEC hybridizing with the EAST1 gene-probe were found with equal frequencies in patients and controls. No *Shigella*, *Vibrio*, EIEC or ETEC hybridizing with gene probes for heat-labile or heat-stable enterotoxin (LT and ST) were isolated.
Conclusions: *E. coli* (VTEC and EPEC) seems to be a major cause (6%) of diarrhea in children under 5 years of age. We recommend that routine examinations of stool cultures from children of this age group should include tests for VTEC and EPEC.

P608 Antimicrobial resistance of coliform bacteria from hospital sewage and the environment in Bangladesh

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Objectives: The extensive use of antibiotics and other chemotherapeutics in hospitals as well as in animal production and fish farming has resulted in drug-resistant bacteria in the environment all over the world, particularly in developing countries. The object of the study is to investigate the spread of antibiotic-resistant coliform bacteria from hospital to the environment in Bangladesh.
Methods: The sampling site was a model system of duckweed-aquaculture-based hospital sewage treatment plant. Total 132 samples were collected from various stages of a treatment plant, from feces of hospitalized diarrheal patients, from fish culture pond and from cultured fish as well as from a control treatment plant. Coliform bacterial isolates were biochemically typed by the PhP system and one representative from each PhP type (strain) were tested for susceptibility to eight different antibiotics at four concentrations by minimal inhibitory concentration method (MIC).
Results: A total of 1009 coliform bacteria were typed by the PhP-RS system and 125 common PhP types were found altogether. Within the common types, 29 major types were identified, which were found at several sites and on several occasions. The overall antimicrobial resistance among the 125 common strains was as follows: sulphamethoxazole (98%), ampicillin (74%), tetracycline (37%), erythromycine (42%), chloramphenicol (43%), nalidixic acid (78%), cephalothin (56%), streptomycin (38%).
Conclusions: Our data indicate that the percentage of antibiotic-resistant bacteria in the hospital sewage, in most cases, are higher than from other sources. Our results suggest that the discharge/use of hospital sewage in community/environment might constitute a public health hazard through the spread of antibiotic resistance. The pathogenic properties of these (resistant) isolates are presently being characterized.

P609 Antibacterial activity of Thai medicinal plants against enterohemorrhagic *Escherichia coli* O157: H7

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Objectives: The stimulating effect of subinhibitory concentrations of antibiotics on the production of verocytotoxin by enterohemorrhagic *Escherichia coli* O157: H7 has been claimed. The purpose of this study was to find an alternative, but effective medicinal plant for the treatment of this organism.
Methods: Fifty-five preparations of the extracts of 38 kinds of Thai herbs were tested for their antibacterial activity against different strains of *E. coli*, including three strains of *E. coli* O157: H7, *E. coli* O26: H11, *E. coli* O111: NM, *E. coli* O22, five strains of *E. coli* isolated from bovine and *E. coli* ATCC 25922.

Inhibition of growth was tested by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by agar microdilution method and agar dilution method in petridishes with millipore filter.

Results: Seventeen extracts showed some antibacterial activity against *E. coli* O157: H7 and most strains. *Quercus infectoria* Olivier, *Punica grantum* Linn, *Peltophorum dasyrachis* (Miq) Kurz ex Baker, *Walsura orbusta* Roxb showed the greatest inhibition zones, ranging from 12.30 to 7.05 mm. *P. granatum* and *P. dasyrachis* were demonstrated to be highly effective against *E. coli* O157: H7 with the MIC values of 0.39 and 0.78 mg/mL and the MBC values of 1.56 and 3.12 mg/mL, respectively.

Conclusions: As both *P. granatum* and *P. dasyrachis* were proved to be active against *E. coli* O157: H7, they should be further examined for their effect on verocytotoxin production in order to provide an alternative treatment of *E. coli* O157: H7 infection.

P610 Detection of a possible adhesion of 67 kDa from diarrheagenic enteropathogenic *Escherichia coli* (EPEC)

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EPEC strains are a worldwide cause of severe infantile diarrhea. Essential for pathogenicity is their ability to adhere to the small intestinal mucosa and to produce a characteristic attaching and effacing (A/E) lesion in the enterocyte brush border membrane. Very little information is available about the nature of the specific bacterial surface appendages or surface components (adhesins) of EPEC responsible for the epithelial cell attachment. The aim of the present study is to identify the adhesins responsible for the adherence of EPEC to the intestinal mucosa. In this study, we have detected a putative adhesin of 67 kDa from an EPEC strain. To obtain the adhesin fraction, EPEC strain (E2348/69) was disrupted by brief sonication and the cell extract containing surface proteins and fimbriae were obtained by ultracentrifugation. The extract was partially dissociated into fimbriae and an adhesin-enriched fraction by heating to 70 °C. The adhesins released from the fimbriae were collected in the supernatant after centrifugation. Partially purified adhesin was able to bind to human intestinal mucosa and tissue-cultured HEp-2 cells. A specific rabbit antiserum raised against the 67 kDa protein inhibited EPEC adhesions to HEp-2 cells by 80%. Immunoprecipitation assay showed that 94–96 kDa and the 67 kDa establish a fimbriae–adhesins complex. Expression of the 67 kDa protein was diminished significantly when the bacteria was grown at 25 °C in broth medium. Growing the bacteria at this temperature also diminished the bacterial binding to HEp-2 cells. This protein was detected in other adherent EPEC strains in contrast to nonadherent *E. coli* isolates that did not produce this putative adhesin. These results suggest that the 67 kDa protein might be an adhesin that participates in the initial stages of EPEC adhesion. The adhesins detected in the present study may be useful in the development of future vaccines.

P611 Mucosal immunization of young rabbits against enterotoxigenic *Escherichia coli* cells by CFA/I antigen entrapped in liposomes associated with *Lactobacillus casei*

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In this work, a mucosal vaccine containing colonization factor antigen (CFA/I) entrapped in liposomes associated with *Lactobacillus casei* has been tested for safety and immunogenicity in young rabbits. Six animal groups were used. Two hours after cimetidine administration, the young rabbits (500–800 g) were given three oral doses (7–10 days apart): CFA/I antigen (5 mg/dose) entrapped in liposomes (batch A); soluble CFA/I antigen (batch B); CFA/I antigen entrapped in liposomes (CFA/I-L) associated with *L. casei* (1×109 cells/dose) (batch C) and control batches: *L. casei* (batch D); free liposomes only (batch E) and Hank's balanced salt solution (HBSS) (batch F). Experimental parameters (a) serological and (b) lymphocyte proliferation assays; (c) adhesion of 3H-leucine-labeled *Escherichia coli* (H10407 strain) to the intestinal mucosa; (d) histopathological and (e) transmission electron microscopy (TEM) evaluation. The results showed that three mucosal doses of liposomes containind CFA/I, and *L. casei* were protective against oral challenge with 5×10^9 *E. coli* cells (H10407 strain). The animals from batch C exhibited increased titers of specific antibodies to CFA/I antigen in 15 of the 18 (83.3%) animals and had a higher rate of antigen-specific response than the animals receiving soluble CFA/I antigen and controls. Specific Peyer's patches (PP), lymph nodes (LN) and spleen (SPL) lymphocyte proliferation response rose to the highest value by the six postimmunization week. However, lower PP, LN and SPL proliferation was observed in young rabbits immunized with soluble CFA/I antigen, free liposomes and *L. casei* cells. The results concerning the 3H-leucine-labeled *E. coli* H10407 cells adhesion to the intestinal mucosa showed: (i) protection of rabbit intestinal mucosa agaisnt virulent *E. coli* H10407 strain; (ii) inhibition of ETEC bacteria adhesion to intestinal mucosa, especially in the animals from batch C; and (iii) significant faster release of 3H-leucine-labeled *E. coli* H10407 strain from the intestinal tract of animals immunized with CFA/I antigen entrapped in liposomes combined with *L. casei* cells. The histopathological and electron microscopy findings confirm the above results. Summing up, the results of this study point out the possibility of providing an efficient protection against infection with *E. coli* strains (ETEC) after mucosal immunization with CFA/I antigen entrapped in liposomes associated with *L. casei* cells.

P612 Epidemiology of antibiotic resistance in commensal *Escherichia coli* of cattle

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Wellcome Trust International Partnership Research Award in Veterinary Epidemiology

Objective: To examine the genetic diversity of commensal *Escherichia coli* isolates in a cohort of newborn calves and follow changes in *E. coli* populations resistant to ampicillin, apramycin and nalidixic acid with age.

Methods: Fecal samples were collected weekly for 3 months from a cohort of 46 beef suckler calves on a Scottish farm. Fecal samples were screened for resistant *E. coli* by plating onto selective agar containing 16 mg/L ampicillin, 16 mg/L apramycin or 8 mg/L nalidixic acid. The genetic diversity of *E. coli* across the cohort and within individual fecal samples was examined by pulsed-field gel electrophoresis (PFGE). The proportion of resistant *E. coli* in fecal samples from a selection of the cohort was examined over time using a viable count method.

Results: To date 27 calves have been sampled weekly from birth for 4 weeks. *E. coli* resistant to ampicillin, apramycin or nalidixic acid were detected in 96, 11 and 56% of 1-week-old calves and in 81, 7 and 33% of calves by 1 month of age. Resistance to two or more antibiotics was found in 59 and 37% of animals at 1 week and 1 month of age, respectively. Ampicillin-, apramycin- and nalidixic acid-resistant bacteria were isolated from 16, 4 and 4% of dams sampled post-partum, respectively. Considerable variation in PFGE types were found in *E. coli* isolated from nonselective media both within a single fecal sample and between calves. Diversity of nalidixic acid-resistant *E. coli* isolates was low and only two PFGE types have been distinguished to date across the cohort. The total *E. coli* counts ranged from 105 to 108 cfu/g feces. Resistant *E. coli* constituted a minority of the total population.

Conclusion: The cohort studied carried a genetically diverse population of commensal *E. coli*. Calves were colonized within days of birth by resistant bacteria, and carriage of resistant bacteria by calves was greater than that by their dams. This suggests that calves acquired antibiotic-resistant bacteria either from the environment or directly from other cohort animals. Preliminary PFGE results provide evidence for the clonal spread of nalidixic acid-resistant *E. coli* through the cohort.

P613 Hemolytic uremic syndrome associated with consumption of unpasteurized cow's milk

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Objectives: Acute hemolytic uremic syndrome (HUS) is often associated with enterohemorrhagic *Escherichia coli* (EHEC) infections. In addition to EHEC O157, particular non-O157 EHEC serogroups, especially O26, have emerged as significant causes of HUS. The source and the vehicle of contamination with EHEC O26 are not often identified. We report two Austrian cases of HUS due to *E. coli* O26:H- affecting an 11-month-old boy and a 28-month-old girl in which transmission through unpasteurized cow's milk was positively identified.

Methods and results: The isolates were indistinguishable from each other using automated ribotyping and yielded the virulence genes *stx2*, *eae*, and *hly*.

An epidemiological investigation revealed that the children had stayed in the same hotel. Both patients had consumed unpasteurized cow's milk from the breakfast buffet. Fecal samples were taken from the cows of the farm producing the incriminating milk and in one of three cattle EHEC O26:H-isolates had a PFGE pattern indistinguishable from the patients' strains. Austrian law does not allow delivery of raw cow's milk to hotels; for the few exceptions allowing delivery of untreated milk, labeling with 'raw milk, boil before consumption' is mandatory. The manager of the hotel catering to babies and young children justified the use of untreated milk with a perceived 'general trend towards biological products.'

Conclusions: These two cases illustrate the hazards associated with the consumption of raw milk and underline the importance of microbiological diagnostic approaches able to detect sorbitol fermenting, non-O157 EHEC. Sorbitol fermenting EHEC, like EHEC O26, would be missed by diagnostic methods purely relying on Sorbitol–MacConkey agar plates. Stool specimens from patients with HUS should at least be tested for the presence of Shiga toxin.

P614 *Escherichia enterotoxins* genes are detectable among clinical isolates of *Klebsiella* spp. and *Proteus* spp.

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Objectives: We studied the prevalence of virulence-associated genes by examining total DNA of clinical isolates *Klebsiella* spp. (59 strains) and *Proteus* spp. (42 strains), which were isolated from different clinical searches.

Methods: The three virulence determinants examined were heat-stable enterotoxin a (STa), heat-stable enterotoxin b (STb) and heat-labile enterotoxin h (LTh) *Escherichia coli*. Three sets of primers for amplification of sequences of the *sta, stb, lth* genes in polymerase chain reaction (PCR) were used to screen strains for the presence of these virulence determinants.

Results: The PCR products specific for the sequences of the *STa, STb* and *LTh* genes were found in 12 out of the 101 tested strains (11.9%), *Klebsiella* spp. were positive in 15.3%. As a result of investigation, four strains of *Klebsiella* spp. sta+ (6.8%), three strains *Klebsiella pneumoniae* stb+ (5.1%), one strain of *K. pneumoniae* lth+ (1.7%) and one isolate of *Klebsiella* spp. stb+, lth+ (1%). Among tested strains of *Proteus* spp., only three (7.1%) were lth-positive in PCR, two *Proteus vulgaris* (11%) and one *P. mirabilis* (4.2%).

Conclusions: Our experiments indicate that ST- and LT-enterotoxins are necessary for *Klebsiella* and *P. virulence*, and suggest that a similar mechanism is responsible for the dissemination and acquisition of 'pathogenicity islands' in Enterobacteriaceae.

P615 The frequency of *E. coli* O157:H7 in the Zagreb area, Croatia

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Objectives: To determine the prevalence of *E. coli* O157:H7 in Zagreb, Croatia.

Methods: In our laboratory, EHEC detection was introduced in 1995. Since 1996, it has been used routinely. During 1996, we investigated 10 588 stool specimens from outpatients with acute diarrhea. All samples were inoculated on Sorbitol–McConkey agar at 37 °C. Sorbitol-negative colonies were agglutinated with *E. coli* O157:H7 antiserum. EHEC was not found in any of the samples. We also tested 82 food samples (veal) and all were negative. In 1997, we analyzed 11 449 samples with negative outcome. In 1998, seven stools tested positive out of 11 578 samples examined. In 1999, only one stool tested positive out of 12 832 samples. In 2000, we examined 13 612 stools and only two were positive. In 2001, we found no EHEC-positive stools in 13 512 samples. All nine cases with proven EHEC were children aged 7 months to 7 years. They all had diarrhea, one of them also vomited. Five of the children had fever and blood in the stool. There were no clinical sequelae.

Conclusions: Incidence of *E. coli* O156:H7 in Zagreb area was very low.

P616 Virulence markers of entero-aggregated *Escherichia coli* isolated from children and adult diarrhea

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Enteroaggregative *Escherichia coli* (EAEC) have been associated with cases of acute and persistent diarrhea. The mechanisms by which these organisms cause diarrhea are not well understood, however, several virulence-related markers have been described, such as the pAA plasmid which encodes for the aggregative adherence fimbrias AAF/I and AAF/II and also the enterotoxins EAST1 and Pet. A serine protease (Pic), encoded by a chromosomal gene, has also been associated with EAEC strains.

Objectives: This study examined the presence of pAA plasmid in *E. coli* strains isolated from sporadic cases of acute diarrhea in children and adults, and asymptomatic children controls. The strains hosting the pAA plasmid were further analyzed for the presence of sequences homologous to virulence markers associated with EAEC pathotype and extraintestinal *E. coli* virulence factors.

Methods and results: Initially, 586 strains isolated from 143 children with diarrhea, 173 strains isolated from 37 asymptomatic controls and 435 strains isolated from 145 adults with diarrhea were investigated by colony hybridization to detect the presence of pAA. Positive strains were found in, respectively, 19.6, 10.8 and 11.7% of the cases of children diarrhea, asymptomatic controls and adults diarrhea. The incidence of pAA+ strains was not significantly different in the groups studied. The pAA+ strains were analyzed for the presence of *AAF/I, AAF/II, EAST1, Pet* and *Pic* using PCR assay. The frequency of *EAST1* and *Pic* genes were significantly higher in strains isolated from children diarrhea than from adults or asymptomatic controls. Only 34.1% of the pAA+ strains showed *AAF/I* or *AAF/II*. The frequency of extraintestinal markers AFA and SFA were significantly higher in strains isolated from adults than from children diarrhea. More than one extraintestinal virulence marker was found in 58% of the strains from adults and in only 7% of the strains from children diarrhea.

Conclusions: Our results indicate that *E. coli* strains harboring pAA plasmid isolated from children diarrhea, adults diarrhea or asymptomatic controls present distintive profiles of virulence markers.

P617 Infant diarrhea caused by enteropathogenic *Escherichia coli*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is a widespread bacteria and was associated for the first time with gastrointestinal infection in 1940. After this period, small outbreaks or isolated cases were reported in all the continents. This pathogen is described as the major etiologic agent of infant diarrhea in developing countries. In Brazil they are responsible for almost 30% of infant bacteria-related diarrhea.

Objective: Verify the prevalence of diarrhea caused by EPEC and the main serogroups in children under 4 years old.

Material and methods: From January 1998 to April 2001, a total of 5276 stool samples from children of >4 years with suspicion of gastrointestinal infection were analyzed. The samples were collected in Cary–Blair transport media, plated in MacConkey agar and incubated at 35–37 °C for 18–24 h. All strains of *E. coli* with biochemical differences were submitted to agglutination (PROBAC) against the following serogroups: 026, 055, 0111, 0119, 0114, 0125, 0142, 0158, 086, 0126, 0127 and 0128.

Results: Out of the total of 5276 samples, 4727 (89.6%) cultures were negative for enteropathogens and 549 (10.4%) were positive. Of these, 215 (39.1%) were positive for EPEC. The serogroups found and their prevalence were: 026, 44 (20.5%) cases; 0125, 32 (14.9%); 0126, 23 (10.7%); 0128, 21 (9.8%); 055, 18 (8.45); 0111, 16 (7.4%); 0142, 14 (6.5%); 0199, 13 (6.0%); 0127, 11 (5.1%); 086, 8 (3.7%); 0158, 5 (2.3%). There were 12 (2.2%) cases of infection of EPEC associated with other organisms. Other etiologic agents isolated were 142 (25.9%) *Campylobacter* spp., 138 (25.1%) *Salmonella* spp., 34 (6.2%) *Shigella* spp., 5 (0.9%) enteroinvasive *E. coli*, 2 (0.4%) simultaneous infection of *Salmonella* spp. and *Campylobacter* spp., and 1 (0.2%) *Aeromonas* spp. The prevalence of EPEC with age distribution was: 0–1 year: 33.5%; >1–2 years: 41.9%; >2–3 years: 19%; and >3–4 years: 5.6%.

Conclusion: The major gastrointestinal infections during the first 2 years of life were caused by EPEC. O26 serogroup was the more prevalent among the EPEC analyzed.

Salmonella, Shigella, Vibrio and other enteropathogens

P618 Antibiotic resistance of *Shigella* and *Salmonella* strains isolated from stool cultures over a 12-month period in Bucharest

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Objective: To define the resistance rates to commonly used antibiotics (AB) of all strains of *Salmonella* and *Shigella* spp. isolated from stool specimens between December 2000 and November 2001 from patients admitted at our institute with a diagnosis of diarrhea.
Methods: Review of the results of antibiograms of *Salmonella* and *Shigella* spp. obtained from stools. The identification of the species was done by conventional microbiological methods; the antibiograms were performed by the disk diffusion method with respect to 1999 NCCLS standards with results expressed as: resistant (R), intermediate (I), and sensitive (S). The AB taken in to study were: tetracycline (T), cloramphenycol (CL), co-trimoxazole (CTX), ampicillin (A), nalidixic acid (NA), ciprofloxacin (CP), colimycin (CO), 3rd generation cephalosporins (3GC).
Results: From 1253 stool cultures, 900 (72%) were positive for *Shigella* spp. (97% *S. flexneri*, 3% *S. sonnei*) and 77 (6%) positive for *Salmonella* spp. (94.8% group BO, 5.2% group CO and DO). The AB-resistance study of *S. flexneri* showed practically the absence of resistance to CP and 3GC, low rates of R to CO (7%) and NA (4%), moderate rates to CTX (22%) and high rates to CL (77.3%), T (78%), A (84.7%). The four strains of *S. sonnei* were also 100% sensitive to CP and 3GC. For *Salmonella* group, BO strains were recorded the following R rates: no resistance to CP, 4.8% to 3GC, 7% to CO, 10% to NA, 32.8% to CTX, 58.2% to T, 69% to CL and 70% to A. For the few strains of group CO and DO there was no AB resistance noted, except one intermediate resistance to 3GC.
Conclusions: In our geographic region, the most frequent pathogen recovered from stools belong to *Shigella* spp. They showed a very good sensitivity to ciprofloxacin, third generation cephalosporins, nalidixic acid and colimycin. The *Salmonella* group BO, seldom isolated, showed higher resistance rates to all AB tested, but still preserved a very good sensitivity to ciprofloxacin and third generation cephalosporins that remains the antibiotics of choice in cases of diarrhea necessitating treatment

P619 Increase in antibiotic resistance in *Salmonella enteritidis* strains from humans in a 3-year period in Bosnia and Herzegovina

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Objective: *Salmonella enteritidis* was the predominant *Salmonella* serotype in Zenica–Doboj Canton, Bosnia and Herzegovina, in the period 1998–2000, and accounted 79.6% of all *Salmonella* spp. isolates. It provided an opportunity to carry out its antibiotic resistance pattern.
Methods: A total number of 238 *Salmonella enteritidis* (SE) strains were isolated from human stools during 1998–2000, 164 of which were sporadic and 74 isolated from 27 outbreaks. The strains identified and serogrouped by conventional methods. Antimicrobial susceptibility tests were performed by disk-diffusion method, according NCCLS recommendations to 16 antimicrobials: ampicillin, ampicillin–clavulanic acid, cefalothin, cefuroxime, cefotaxime, azithromycin, gentamicin, tetracycline, doxycycline, trimethoprim–sulfamethoxasole (SXT), chloramphenicol, nalidixic acid, pefloxacin, ofloxacin, ciprofloxacin and nitrofurane.
Results: The overall resistance rate of the *S. enteritidis* strains in the 3-year period was as follows: 34.5, 39.7, and 50.9%, respectively. The resistance rate to only one antimicrobial in this period was 53.8, and 46.2% to more than one, respectively. The occurrence of ampicillin resistance increased from 10.6 to 17.6%. Nalidixic acid resistance rate was 6.7% in the 3-year period. Stel, all of the SE isolates were susceptible to pefloxacin, ofloxacin and ciprofloxacin during this period. Doxycicline showed the highest resistance rate, 28.8%. Increasing trend, but stel relatively low resistance rate showed SXT and chloramphenicol. There no were differences in the rates of the overall antimicrobial resistance between sporadic and outbreak isolates as well as between in- and out-patients isolates.
Conclusion: Generally, the resistance to antomicrobial drugs in SE is rare in many European countries. The percentage of resistance in this region has shown increasing trend and higher values even compared in some Mediterranean countries. Although the resistance rate to nalidixic acid is still low, its increase in resistance draws our attention to the possible appearance of the resistance to the fluoroquinolones in the near future. Monitoring of *S. enteritidis* strains for antimicrobial resistance is also important from an individual perspective, because the outcome is worse in persons with resistant *Salmonella* infections.

P620 Infectious diarrhea: review of etiology, and antimicrobial resistance in patients at a university hospital in South-eastern United States

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Objectives: Infectious diarrhea is the leading cause of mortality in the underdeveloped world. Patients in western countries may suffer serious morbidity owing to infectious enterocolitis. This study was undertaken to determine the microbiologic profile of bacteria associated with diarrheal illness in patients at a large community teaching hospital in the South-eastern United States.
Methods: Review of microorganisms from patients with diarrhea from January 01, 1997 to December 31, 2000 at Richland Memorial Hospital was performed retrospectively.
Results: During 36 months, 131 patients presented with diarrheal illness. Among 31 adults (15 male, single HIV+) and 100 pediatric (50 males) patients' median age was 37 ± 16.4 and 3 ± 12 years, respectively. *Shigella sonnei* 'group D' (50%) was the most common pathogen (66 cases, 51 in children and 15 in adults), followed by *Salmonella typhimurium* 'group B' (19%) (25 cases, 17 in children and 8 in adults). *Salmonella meunchen* 'group C' accounted for a single case in adults, whereas 11 pediatric patients developed diarrheal illness. Among adults, a single case of *Yersinia enterocolitica*, *Salmonella* 'group C2', *Campylobacter jejuni*, *Shigella flexneri* (group B), *Plesiomonas shigelloides* was noticed. In children, four each had *E. coli* O157:H7, and *P. shigelloides* isolated; five *Y. enterocolitica*, three *C. jejuni*, and two each had *Edwardsiella tarda*, and *Salmonella* 'group D' infection. The in vitro antimicrobial susceptibility (MIC_{50} & MIC_{90} μg/mL) of *Shigella sonnei* to ciprofloxacin (<0.5 and <0.5), trimethoprim-sulfamethoxazole (TMP–SMX) (<10 and <10) was within susceptible range, whereas for ampicillin (2.0 and >32.0) an increased resistance was observed. Similarly, among *Salmonella typhimurium* isolates no resistance was noticed for ciprofloxacin (<0.5 and <0.5) and TMP–SMX (<10 and <10), whereas a high level resistance for ampicillin (>32 and >32) in US was concerning.
Conclusions: During last 3 years, 'group D' *Shigella sonnei* was the most common cause of infectious diarrhea among both adults and pediatric population in our region. Infections due to *E. coli* O157:H7, *V. cholerae*, *C. jejuni*, and *P. shigelloides* were dominant pathogens in younger patients. A high level of ampicillin resistance among *S. typhimurium* was consistent with reports from South-east Asia. Cirpofloxacin and TMP–SMX appears to have retained in vitro effect against most bacterial pathogens in this setting.

P621 What can a national telephone help line for health advice tell us about the epidemiology of gastrointestinal infections in the community?

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Objectives: To determine the level of community-based gastrointestinal infection symptoms reported to NHS Direct, in order to establish national, regional and subregional baselines. To describe the outcomes of these calls in terms of the advice or further care recommended. NHS Direct is a free nurse led national telephone help-line for health advice in the UK.

Methods: NHS Direct is open 24 h, 365 days of the year. The service is organized into 23 separate sites (call centers) and answers over 5 million calls a year. Call data presented within this paper have been collected from three NHS Direct call centers over a 12-month period. On receipt of a call, a nurse uses his/her judgement to select the most appropriate computerized clinical algorithm. The algorithm, along with the nurse's clinical knowledge, is used to advise the caller or refer them to appropriate medical care (call outcome). The algorithms chosen to describe NHS Direct calls about gastrointestinal infection symptoms are vomiting, diarrhea and food poisoning. Calls are also categorized by the call outcome (e.g. self-care, routine doctors appointment, further immediate care).
Results: Symptoms of gastrointestinal infection are common reasons for telephoning NHS Direct, comprising 10% of all calls. The rate of gastrointestinal infection symptoms reported is particularly high for children (25.3 calls per 100 000/week compared to 3.3 for adults, ratio children : - adults = 7 : 1). The proportion of calls made about children with gastrointestinal infection symptoms, that are referred to further immediate care is twice that of adults (0–4 year-olds = 42% (95% CI 41–43), 5–14 = 40% (37–44); all adults = 22% (20–23)). There is a seasonal rise in February–April in all gastrointestinal infection symptoms, which is most marked for calls about vomiting and for all gastrointestinal infection symptoms in 1–4 year-olds. This may be owing to the viral rather than bacterial pathogens.
Conclusions: NHS Direct data offer new opportunities to explore the epidemiology of gastrointestinal infection within the community and to detect cases with symptoms of gastrointestinal infection that are not ascertained by existing surveillance systems. Examining the age distribution and outcome of calls provides insight into how the age-specific distribution of gastrointestinal pathogens detected by laboratories may be influenced by patient and doctor behavior.

P622 Antimicrobial resistance in 743 patients with acute gastroenteritis: prevalence and clinical implications

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Owing to the inappropriate use of antibiotics in the clinical practice as well as their extensive use in agriculture, the antibiotic resistance of the most common etiological agents of acute gastroenteritis is increasing. However, in developed countries the prevalence and the clinical relevance of antibiotic resistance in patients with acute gastroenteritis have not extensively been studied yet.
Objectives: To evaluate the prevalence and the clinical impact of antibiotic resistance in the setting of acute gastroenteritis in adults patients.
Materials and methods: Clinical, microbiological and therapeutic data of 743 cases with acute gastroenteritis hospitalized between 1995 and 2000 were analyzed.
Results: The diagnosis of infective gastroenteritis was performed in 259 (34.8%) cases: *Salmonella* spp. 129 (49.8%); *Campylobacter* spp. 58 (22.3%); others 72 (27.7%). Out of the 129 strains of *Salmonella* spp., 31% were resistant to one or more antibiotics, as follows: ampicillin 35%; amoxicillin 25%; cotrimoxazole 7.5%. Interestingly, only one strain of *Salmonella* spp. isolated was found to be resistant to fluorchinolones, whereas 6.2% strains displayed intermediate sensitivity. Among *Salmonella* spp., the rate of antibiotic resistance was higher for *S. typhimurium* (62%) compared either to *S. enteritidis* (10.6%) or other subtypes (33%). The antibiotic resistance of *Salmonella* spp. increased from 28% in 1995 to 39% in 2000. Seventy-nine percent of *Campylobacter* spp. isolated had one ore more antibiotic resistance (*C. laridis* 100%; *C. jejuni* 69%). The rate of antibiotic resistance was 50, 23.9, 48.7 (22/46), and 3.9% to ampicillin, fluoquinolones, cotrimoxazole and macrolides, respectively. The rate of fluoroquinolones resistance increased from 26.5% in 1995 to 85.7% in 2000. This behaviour was not observed for other antibiotics tested. Despite the non negligible increase of antibiotic resistance, no significant variations were found in term of duration of symptoms (6.2 ± 3.3 days vs. 5.8 ± 2.6 days), duration of hospitalization (6.9 ± 4.9 days vs. 5.7 ± 2.5 days) and the clinical course of the gastroenteritis.
Conclusions: Our data confirm a progressive increase of antibiotic resistance of *Salmonella* and *Camylobacter* spp. The antibiotic resistance appears to be very important for *Campylobacter* spp. which is particularly resistant to fluoroquinolones and aminopenicillin derivatives. However, at the moment, antimicrobial resistance has not a clinical impact.

P623 Efficacy of live recombinant *Salmonella* therapeutic vaccine against *Helicobacter pylori* colonization in mice is influenced by the immune responses induced by the attenuated carrier

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Objectives: *Helicobacter pylori* is a human pathogen that persistently colonizes the gastric mucosa. Although *H. pylori* elicits local and systemic antibody responses, it still evades elimination by the host immune responses partly due to its surface antigens mimicking those of the host. Therefore, a cellular immunity might be needed for effective protection against *H. pylori* carrier state. In this study, we aimed to compare the immunogenicity and therapeutic efficacy of two attenuated live *S. typhimurium* carriers, delta-phoPQ and delta-phoPQ overproducing PhoP global regulator in *H. pylori* mouse model.
Methods: *S. typhimurium* phoP was expressed from the trc promoter on a p15A-based plasmid that was introduced into a delta-phoPQ strain. This strain overproduced PhoP response regulator without the cognate histidine kinase and was referred to as PhoPOVER. *H. pylori* urease A and B subunits were cloned under the lambda promoter right on a pBR322-based plasmid that was introduced into both delta-phoPQ and PhoPOVER backgrounds. Mice were infected with *H. pylori* prior to intranasal vaccination with the delta-phoPQ and PhoPOVER vaccine constructs. The efficacy of the vaccination was assessed by viable count of *H. pylori* recovered from the gastric tissues. The humoral and cellular immune responses were measured.
Results: The recombinant delta-phoPQ strain reduced the *H. pylori* colonization only by 1 log as compared to challenged but untreated mice. However, the PhoPOVER was able to reduce *H. pylori* colonization in the gastric tissues by 2.5 logs. Both delta-phoPQ and PhoPOVER carriers elicited a balanced Th-1/Th-2 systemic antibody responses against *Salmonella* total membrane. However, cellular immune responses against both *Salmonella* and *H. pylori* were dominated by a Th-1 response in PhoPOVER strain and undetectable in the delta-phoPQ carrier, correlating with the degree of protection.
Conclusions: The efficacy of *H. pylori* urease A and B subunits in protecting against mouse-adapted *H. pylori* strains has only been assessed in the context of prophylactic vaccination. Our findings suggest that high efficacy against *H. pylori* colonization in the gastric mucosa can be achieved in therapeutic vaccination using live attenuated *Salmonella*-vectored vaccine. The success of such a vaccination is dependent upon the nature of the *H. pylori* antigen and the ability of the carrier to induce cellular immune responses in the host.

P624 A study of *Salmonella* spp. isolates from 1990 to 2000, Lakonia, Greece

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Objectives: To study and determine the *Salmonella* spp. strains during 1990–2000 and their susceptibility to antibiotics.
Methods: During an 11-year period, 109 *Salmonella* spp. were isolated from 1145 stool and blood cultures (16.5% positive tests) in our hospital. Their antibiotic resistance was assessed with the Kirby–Bauer disk diffusion test, and their serotype was determined by the National School of Public Health in the University of Athens.
Results: The serotype results were: 66% *S. enteritidis*, 6% *S. typhi*, 3.6% *S. blokley*, 3.6% *S. agona*. Various others strains (*Bovismor-bificans*, *Virchow*, *Newport*, *Oranienbury*, *Typhimurium* and others in two or less isolates). A complete year and season incidence is described as well as antigen serotyping of the strains. Antibiotic resistance was found to be 23.8% for ampicillin, 11.9% for amoxicillin + clavulanic acid, 3.6% for cotrimoxazole whereas the strains were fully susceptible to ciprofloxacin and third generation cephalosporins.
Conclusions: We recorded an increasing incidence of *Salmonella* infections during this 11-year period. *Salmonella enteritidis* is still the most common serotype isolated, though there is a growing appearance of various other isolates, probably owing to changes in dietary habits and population exchange. Antibiotic resistance seems to make the use of ampicillin inapropriate, whereas susceptibility to ciprofloxacin and third generation cephalosporins is still complete.

P625 Serotype distribution of *Salmonella enterica* subspecies *enterica* isolated from humans in Turkey between 1987 and 2000

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Objectives: To determine the serotype distribution of *Salmonella* strains from humans in Turkey.
Methods: Total of 1334 cultures (1167 stool, 100 blood, 29 urine, 22 abscess, 13 wound, 1 bone marrow, 1 bile, 1 peritoneal fluid) were performed by standard methods. All *Salmonella* spp. isolates were serotyped by using O and H antisera according to Kauffmann–White scheme.
Results: Serotype distribution of *Salmonella enterica* subsp. *enterica* isolated from humans in Turkey between 1987 and 2000 is summarized in the Table 1.

Table 1 Serotype distribution of *S. e.* subsp. *enterica*

	Years			
Serotype	1987–1989 *n*=360 (%)	1992–1994 *n*=353 (%)	1995–1997 *n*=339 (%)	1998–2000 *n*=282 (%)
Typhimurium	68.1	62.3	48.1	23.0
Enteritidis	14.7	22.9	41.0	64.9
	4.4	6.8	1.8	2.1
Typhi	2.2	1.1	3.2	2.5
Paratyphi B	2.5*	–	–	–
Haifa	–	1.4**	–	–
Corvallis	–	–	–	2.1
Hadar				
Other serotypes	8.1	5.4	6.0	5.4

*All strains isolated from an outbreak in 1987.
**All strains isolated from an outbreak in 1994.

Conclusion: The total of Typhimurium and Enteritidis account for about 85% of *Salmonella* isolates. Incidence of Typhimurium has declined consistently from 1987 to 2000. In contrast, a linear increase in the incidence of Enteritides has observed in the same period. These changes are statistically significant by Chi-square test, and are similar to many European countries and USA.

P626 *Salmonella enterica* subspecies *houtenae* as a rare cause of meningitis

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A 2.5-month-old male baby was hospitalized with high fever. He was pale, irritable, groaning and refused to eat. His medical history was unremarkable but his parents had iguanas as pets. On physical examination some petechiae were noted and bulging of the fontanel. A clinical diagnosis of bacterial meningitis was made, blood cultures were obtained, a lumbar punction was performed and ceftriaxone and ampicillin were started. A *Salmonella* species, susceptible to ceftriaxone and ampicillin (MIC by *E*-test 0.064 and 1 mg/L, respectively) was isolated from the cerebrospinal fluid. Blood cultures (Bact/Alert 3D, Organon Teknika Corp.) remained negative. The baby recovered and was discharged after 4 weeks of intravenous antibiotic therapy with ceftriaxone. Two weeks later the baby presented a relapse and a *Salmonella* species was again cultured from the cerebrospinal fluid. Intravenous antibiotic therapy was restarted for 6 weeks. The baby was last seen 4 months later and was doing fine. The Belgian reference laboratory for *Salmonella* and *Shigella* serotyped the *Salmonella* strain. It was identified as *Salmonella enterica* subspecies *houtenae* (S IV 44: z4z23), a species of cold-blooded animals. Feces of the iguanas were cultured on selective media direct and after enrichment in broth containing selenite. No pathogens were grown, but one animal died shortly after the baby became ill. The isolation of this species of *Salmonella* suggests the iguanas as source of the baby's infection. Since the end of the 1990s, there have been several publications demonstrating an increase in the number of salmonellosis cases associated with pet ownership of reptiles (iguanas, turtles, snakes, ...), especially in young infants and immunocompromised patients. We believe inquiring into pet ownership of exotic animals to be appropriate if *Salmonella* is cultured in CSF or blood cultures from patients with meningitis. In cases of *Salmonella meningitis* relapse or recrudescence of disease are often observed, as was the case in this patient. Close follow up after appropriate antibiotic therapy and discharge from hospital are therefore needed. Last but not the least, owners of exotic pets should be informed about handling and infection risks.

P627 Surveillance data of *Salmonella* infection during 1996–2001 in Bari, Italy

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Objective: The *Salmonellae* constitute a threat to our health and welfare. The prevalence of Salmonellosis in the world is steadily increased. In Europe is activated a Surveillance Program of *Salmonella* infections. This Program involve 19 European country and is under the control of Communicable Disease Surveillance Centre of the PHLS London. We report data on isolation of *Salmonella* strains in Bari, Italy.
Materials and methods: Strains of *Salmonella* were isolated from various clinical samples according to standard protocols. Data regarding serotyping and antibiotic resistance were analyzed by software Epi-Info 6.4.
Results: During 1996–2001 253 strains of *Salmonella* were isolated. *S. typhimurium* (30.4%), *S. enteritidis* (25.3%), *S. typhi* (11.9%) were the most common serotypes isolated. We have also observed the presence of *S. hidalgo*, *S. kimuenza* and other serotypes that are rare in our area. Resistance to ampicillin and trimethoprim–sulfamethoxazole occurred in the 36.8 and 17.4%, respectively. No strains resistant to quinolones, including ciprofloxacin were detected.
Conclusion: In our area no increase of incidence of human *Salmonella* infection has been demonstrated in the course of the years under study. The prevalence (23.3%) of serotypes of *Salmonella* uncommon in our area, but reported in developing countries, suggest a possible introduction of these strains as the result of presence of immigrants, international travel and increased consumption of imported food.

P628 Effect of different temperatures on the plasmid profile and antibiotic susceptibilities of *Salmonella* strains

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After the incubation at 5, 37 and 45 °C for 18 h of *Salmonella enteritidis* and *Salmonella typhimurium* strains, plasmid profile analysis was carried out by the method described by Kado and Lui. It was found that three *S. typhimurium* DT 104 strain was found to carry serovar specific plasmid (SSP) of 60 MDa; one *S. typhimurium* DT 204b strain contained SSP of the 60 and a 110 MDa plasmid, *S. typhimurium* U^{302} strain contained 100 MDa plasmid. Among the *S. enteritidis* strains, three *S. enteritidis* PT4 and one *S. enteritidis* PT21 strains contained the 38 MDa plasmid which was serovar specific, one *S. enteritidis* PT 6a strain contained the serovar specific plasmid of 38 and a 30 MDa plasmid. According to plasmid profile analysis after 3 days incubation at 5 °C, it was found that the *S. typhimurium* U^{302} strain lost the 100 MDa plasmid, at the same time it lost the chloramphenicol resistance also. After 3 days incubation at 45 °C, except *S. typhimurium* DT 204b strain, all the strains lost their plasmids. At the same time, it was found that the *S. typhimurium* DT 104 strain lost chloramphenicol resistance and the *S. typhimurium* U^{302} strain lost chloramphenicol and gentamicin resistance. After 3 days incubation at 5 and 45 °C, all strains were incubated at 37 °C and plasmid profile analysis was performed and it was found that all strains gained their plasmids and antibiotic resistance again. After incubation at 5 °C of the *S. typhimurium* U^{302} strain integron PCR was performed. It was found that the plasmid which is

100 MDa was not lost completely, in fact it was integrated to bacterial chromosome.

P629 Distribution and antibiotic susceptibility of human isolates from nontyphoidal *Salmonella* serotypes, other than Enteritidis and Typhimurium, in Greece

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Objective: The aim of the present study was to determine the distribution and the levels of antibiotic resistance of various nontyphoidal *Salmonella* serotypes other than Enteritidis and Typhimurium. Concise data describing the incidence and the patterns of antimicrobial resistance for each one of these serotypes have not been published until now.
Materials and methods: A total of 219 human isolates were randomly selected from the collection of Greece's National Reference Centre for *Salmonella* and *Shigella*, originated from various parts of the country for an 8-year period (1990–1997). They were almost evenly distributed every 2 years, with 1997 as the last year of the study. The susceptibilities to 10 antimicrobials of various class lines were determined, using the MICs broth microdilution automated method.
Results: A great variation in the antibiotic resistance rates has been noticed among the serotypes. In particular, *S. virhow* and *S. hadar* have proved the most resistant among the examined serotypes, with *S. virhow* to be additionally the most multiresistant one. The vast majority of the isolates, belonging to the rest of the serotypes, were completely sensitive to the examined antibiotics. All isolates were sensitive to third generation cephalosporins (ceftriaxone) and to quinolones (ciprofloxacin) tested. Some of the results are summarized in the following Table 1.

Table 1 Incidence of antibiotic resistance in the main nontyphoidal *Salmonella* serotypes from humans

Serotype	No. of isolates referred to NRCSS[1]	No. of the isolates tested	% DR	% MR
S. infantis	83	53	1.2	0
	55	51	33.3	11.8
S. virhow				
S. newport	52	27	0	0
S. bredeney	33	15	6.7	0
S. hadar	13	12	33.3	8.3
Other serotypes	293	61	1.6	0
Total	529	219	11.4	2.7

[1]During the studied years: 1990, 1992, 1994, 1996 and 1997; DR, drug-resistant (resistant to 1 or more antimicrobial); MR, multiresistant (resistant to three or more antimicrobials).

P630 DNA fingerprinting for food-related salmonellosis: a European collaboration

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Objectives: Much salmonellosis prevention and control depends on early outbreak recognition through a suitable surveillance system. The value of phenotypic typing methods as surveillance tools is well established and gene-printing is used as an adjunct in outbreak investigations in which enhanced strain discrimination is needed. Our study, involving nine national reference laboratories within Europe, investigates the potential for improvement in the current surveillance system when gene-printing is used to routinely subtype *Salmonella* isolates. The aim was to develop standard laboratory operating procedures for pulsed-field gel electrophoresis (PFGE) and for computer recognition of the results.
Methods: Initially each participating Centre received a set of 16 *S. enterica* strains to be used for quality assurance of the methods. Each laboratory then selected 500 strains of *S. enterica* representing currently defined serotypes of epidemiological importance within their country. An agreed protocol for rapid PFGE was used which involved proteinase K, *lysis* of cells, a series of washes at 50 °C followed by digestion with *Xba*I. Gel images were exchanged in tag image file format (tiff files) for comparison between centers.
Results: We have harmonized a method for PFGE that gives reproducible results and is currently being applied in selected European reference laboratories. By using defined parameters for electrophoresis the gel images produced were comparable between each Centre despite slight variations in DNA preparation. Electronic recording and transmission of data between laboratories has enabled the formation of an international database of gel profiles.
Conclusions: For the control of *Salmonella*, precise identification of the organism is an essential prerequisite. We used a harmonized PFGE protocol that takes into account some of the differences between different European centers. Although standardization of DNA preparation and digestion were not considered to be essential, standardization of the parameters for electrophoresis was considered to be an absolute requirement. We are compiling a searchable database of gene-print information that will allow us to describe the range and incidence of subtypes for the major salmonella serotypes within Europe. The use of internationally agreed methods for DNA fingerprinting allows countries in the EU to rapidly compare subtypes of *Salmonella* organisms responsible for international food-related outbreaks.

P631 High prevalence of antibiotic resistant nontyphoidal *Salmonella* strains isolated from hospitalized Italian children

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Salmonellosis is usually a self-limiting infection and does not require antibiotic treatment. However, systemic infections occur in 5–10% of cases; mainly among children, elders and immunocompromised patients. For these patients an effective antibiotic therapy may be lifesaving. In children, since fluoroquinolones have not been approved for use, few drugs are available for the empirical treatment. Antibiotic resistance among *Salmonella* isolates is increasing world-wide. Epidemiological data about *Salmonella* strain susceptibility should be considered by pediatricians when starting an empirical antibiotic treatment.
Methods: Over the period November 1999–November 2001, 491 children with diarrhea were admitted to our Division. *Salmonella* spp. was isolated from stool cultures in 53/491 (10.8%) patients (median age: 2.2 years; range: 2 months–10 years). All strains were identified and serotyped by standard tests. Susceptibility to ampicillin, ceftriaxon, ciprofloxacin, chloramphenicol, neomicin, tetracycline and trimethoprim was performed using the Kirby–Bauer method.
Results: Among 53 *Salmonella* spp. strains, *S. thyphimurium* was isolated in 27 cases; *S. enteritidis* in 10; *S. coeln* in 2; *S. kimuenza*; *S. bredeney*; *S. mons*; *S. movanjum*; *S. thompson*; *S. bareilly* and *S. israel* in 1 case each; 7 *Salmonella* strains could not be serotyped. 75.5% strains showed resistance to at least one antibiotic. In particular, 85.2% *S. typhimurium* and 50% *S. enteritidis* strains were resistant to at least one antibiotic. Twelve strains were multiresistant (>3 drugs). Twenty-nine (55%) strains were resistant to ampicillin, two (3.8%) to ceftriaxon, four (7.5%) to ciprofloxacin, eight (15.1%) to chloramphenicol, two (3.8%) to neomicin, 34 (64%) to tetracycline, three (5.7%) to trimethoprim.
Conclusion: This study provides evidence for high prevalence of drug-resistant *Salmonella* spp. strains in Italy. Resistance to ampicillin was particularly frequent. We found 50% of *S. enteritidis* isolates to be antimicrobial resistant, which is a high rate with compare to previously reported 2.2% in central–southern Italy. Our data support evidence for increasing resistance among *S. enteritidis* strains which were previously thought to be usually antibiotic sensitive. Resistance to ceftriaxone has been reported to range from 0.5% in the United States to 32% in Turkey. We disclose an intermediate resistance rate of 3.8%.

P632 Epidemiological investigation of human campylobacteriosis by PCR–RFLP

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Objectives: *Campylobacter enteritis* is predominantly caused by the two closely related species *C. jejuni* and *C. coli*, but *C. jejuni* accounts for about 90% of all human infections. We have developed a method for identification and

subtyping *Campylobacter* isolates based on the restriction fragment length polymorphism (RFLP) of polymerase chain reaction (PCR) products of the *flaA* and *flaB* genes. This subtyping technique was optimized for detection of *Campylobacter jejuni* and the potential value of this PCR–RFLP typing was established.

Methods: A total of 32 unrelated isolates including four NCTC reference strains were analyzed. Extraction of DNA was performed using the cetyltrimethylammonium bromide (CTAB) method. Primers for PCR were designed from the nucleotide sequence of the flagellin gene sequences of *C. jejuni*: *flaA*: 5′-ATGGGATTTCGTATTAACAC-3′ and *flaB*: 5′-CTATTGTAATAATCTTAAAA-3′. The PCR product was digested with the restriction endonucleases *Dra*I and *Dde*I in separate reactions. Molecular size of fragments generated by the genotypic methods was estimated by comparison with the molecular size marker, and computed similarities among strains was tested using Dice coefficient. Clustering of strains was based on the nonweighed average pair group method (UPGMA) to facilitate the plotting of a dendrogram. All computations were performed using the NTSYS-PC program.

Results and conclusions: The PCR–RFLP typing technique was highly discriminative between strains. In our work with 32 isolates, a total of seven different *Dde*I profiles were yielded. *Dra*I was less discriminatory grouping only in four profiles. Using both endonucleases together, the number of *Dra*I–*Dde*I profiles was increased to 12, indicating a high discrimination level. The use of RFLP–PCR–*Dra*I–*Dde*I on the *flaA* and *flaB* genes is a suitable technique for identification of campylobacters and a practical typing scheme for clinical and epidemiological investigation.

P633 Electrolyte and mixed acid–base disturbances in cholera

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Objectives: The presence of metabolic acidosis with an increased anion gap during the diarrheal phase of cholera has been documented but there is no notification on mixed acid–base disturbances in cholera.

Methods: Acid–base disturbances in 56 patients with *Vibrio cholerae* serotype Ogawa) who were admitted in emergency ward of Imam–Khomeini hospital, Tehran, in the summer of 1998 were studied. At time of admission and 24 h after rehydration, electrolytes, renal function tests, pO_2, pCO_2, pH and serum bicarbonate were measured.

Results: At time of admission, the mean serum concentrations of sodium was 136.6 meq/L, that of potassium was 3.47 meq/L, and that of BUN was 58.1 meq/dL. All of the patients had metabolic acidosis with a mean serum bicarbonate value of 11.33 ± 3.13 mML and pH of 7.19 ± 0.08 which were changed to 14 ± 2.7 mML and 7.28 ± 0.06, respectively ($P < 0.05$). Thirty-three (58.9%) of patients had mixed respiratory acidosis and metabolic acidosis. In comparison, the severity of acidosis and the mean of $\log pCO_2$ of those with mixed acidosis were more than the patients with pure metabolic acidosis ($P < 0.05$).

Conclusions: Acid–base disturbance in cholera is mixed respiratory and metabolic acidosis and the paradox of the presence of severe acidosis and low bicarbonate loss in diarrhea of patients with cholera is due to respiratory acidosis.

P634 The determinants of cholera epidemic in Tehran in 1998

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Objectives: A case–control study was conducted in Tehran in 1998 for determining factors related to Contracting cholera disease in the capital of Iran. This study was conducted in the peak of the epidemic.

Methods: Thirty cases and 52 controls matched for age and sex were entered in this hospital based study. Data about exposures during 72 h prior to cases onset of disease were obtained by questionnaires that were filled by four trained interviewers. Data were analyzed by STATA software and OR, 95% confidence interval for each independent variable was calculated after adjustment of possible confounders.

Results: History of eating salad food (OR = 0.15, 95% CI = 0.04–0.54) and washing hands before eating (OR = 0.17, 95% CI = 0.04–0.65) were associated with lower chance and history of traveling out of Tehran (OR = 7.3, 95% CI = 1.4–37.6) and consumption of artificial ice (OR = 4.08, 95% CI = 1.02–16.2) were associated with higher chance of contracting cholera.

Conclusion: Although for determining the cause of cholera epidemic such a study should be conducted early in epidemic, we were able to show the probable role of hand washing and consuming salad (probably because of its low pH level) as protective factors and travel (probably because of drinking contaminated water or contact with infected people) and consumption of artificial ice, which purchases from the shops, as risk factors of cholera.

P635 Molecular epidemiology of *Vibrio cholerae* strains in Iran during 1998–2000

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Objectives: Cholera remains an important public health problem in many developing countries. Several investigations indicated that the best way of study about the history of cholera in geographic area, requires the information about epidemiological patterns of infection and the disease with molecular analysis of the different strains of *V. cholerae* prevalent in the area.

Methods: A total of 506 stool samples of patients from different cities of Iran had been analyzed and a total of 318 *Vibrio cholerae* O1 isolated from these specimens.

Results: Resistance degree of strains to the following antibiotics which was tested by the agar dilution method, were: 83.3, 73, 43.7, 41.4, 5.4, 1.2 and 1.2% for Co-trimoxazoleole, oxytetracycline, tetracycline, furazolidone, chloramphenicol, erythromycin and doxycycline, respectively. The plasmid profile detection and polymerase chain reaction (PCR) for the genes encoding the A subunit of choleratoxin (CTX A), zonnula occludens toxin (ZOT), accessory cholera entrotoxin (ACE), toxin coregulated pillus (TCP) and restriction fragment length polymorphism (RFLP) on the PCR product and their plasmids has been done. the existence of CTX, ZOT, ACE and TCP genes in all strains was 84.4, 66.8, 72.9 and 65.4%, respectively. In RFLP of PCR products, we had no polymorphism. 70.7% of these strains had a 100-kb plasmid.

Conclusions: This study provides evidence that *Vibrio cholerae* have significant public health implications and we are objected to changing the new antibiotic resistance pattern.

P636 Characterization of viable but nonculturable halophilic noncholera vibrios

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Objectives: The viable-but-nonculturable (VBNC) physiological state of bacteria was first described by environmental microbiologist. Cells in this state appear dormant and cannot be cultured, but are able of returning to the actively metabolizing state. The VBNC state has been found in numerous human pathogens. The aim of present work is to analyze this state of dormancy in halophilic noncholera vibrios (NCVs), recognized as human pathogens.

Methods: *Vibrio vulnificus*, *V. alginolyticus*, *V. harveyi*, *V. damsela*, *V. mediterranei* obtained from fresh and frozen seafood were used. The microcosm water method described by Rollins and Colwell (Appl. Environ. Microbiol. 1986; 58 : 531–38) was used to obtain nonculturable cells. The effects of factors such as temperature, pH and NaCl concentration on VBNC state were studied by staining preparations with CTC-DAPI in order to detect and enumerate VBNC cells (Cappelier et al. Vet. Res. 1997; 28 : 547–55). The culturability of cells was determined by spread plate counting on Columbia agar containing 5% lysed horse blood (Federighi et al. Food Microbiol. 1998; 15 : 539–50). To examine both entry to and resuscitation from the VBNC state in the natural environment, we employed the membrane diffusion chambers described by McFeters and Stuart (Appl. Microbiol. 1972; 24 : 805–11). The virulence and the cytotoxicity of NCVs were determined as described by Ottaviani et al. (Appl. Microbiol. Lett. 2001; 33 : 61–4). Measurements were obtained for late-log-phase cell suspensions and for VBNC cell suspensions of NCV cells after 10, 20, 30 and 40 days of starvation in microcosm water.

Results: The VBNC state was documented in *V. vulnificus*, *V. alginolyticus* and *V. harveyi*. NCVs showed different morphological and physiological characters during starvation state (environmental conditions, time and bacterial cells

required to entry into VBNC state; seasonal disappearance). NCVs cells undergo a variety of metabolic changes on entering the VBNC state. *V. vulnificus* and *V. alginolyticus* are able to retain virulence during entry into and resuscitation from the VBNC state.
Conclusions: The environmental conditions which induce the VBNC state differ from bacterium to bacterium, and it is likely that the physiology involved varies among different cells.

P637 Evaluation of five commercial systems in the identification of a new emerging pathogen: *Vibrio vulnificus* biotype 3

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Background: In the mid summer of 1996 we reported the first isolation of a new human pathogen, *Vibrio vulnificus* biotype 3, that causes septicemia and severe soft tissue infections following contact with fish from artificial fresh water ponds. Because then, the identification of this new emerging pathogen has become a challenge for clinical laboratories. Our laboratory maintains a unique collection of this new biotype strains, all of them isolated in Israel. In this study, the ability of five commercial systems in identifying this new species has been evaluated.
Methods: Fifty-one documented *V. vulnificus* biotype, three isolated during the first known outbreak during 1996–97, were processed by five commercial identification systems: API 20 NE, Vitek 1, Vitek 2 (BioMerieux, France), Microscan (Dade-Boering, USA) and Phoenix (Beckton Dickinson, USA).
Results: The number of strains correctly identified by Phoenix, Vitek 2 and Vitek 1 were: 50 (98.0%), 46 (90.2%) and 7 (13.7%), respectively. Microscan and API 20 NE were unable to correctly identify any of the strains of this new emerging pathogen and mostly misidentify them as other species of the Vibrionaceae family.
Conclusions: Phoenix and Vitek 2 proved to be the best systems in order to identify the new biotype of *V. vulnificus* correctly. Microscan, API 20 NE and Vitek 1 failed to do so. However, since all of them presented a consistent phenotypic pattern, the ability of these systems to correctly identify the strains could be easily improved by supplementing their software with the data presented in this study.

P638 Isolation of the major clinical *Vibrio* species from water and plankton of the Persian Gulf

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Objectives: Vibrios are one of the dominant bacteria on plankton. Plankton have also been studied extensively as a potential aquatic zoological micro-habitat for *V. cholerae*. An environmental study was done to isolate the major clinical vibrios from the Persian Gulf.
Methods: Sixteen coastal sites from Bushehr Port, in the north-west of the Persian Gulf were sampled at 1 m below the surface. A total of 30 water and 12 plankton samples were collected. Water subsamples were filtered through 0.22 µm membrane filter. Collected plankton samples were added to alkaline peptone water, after homogenization.
Results: A total of 56 strains, 34 vibrios from water samples and 22 vibrios from plankton samples were characterized. Twelve isolates of *Vibrio alginolyticus* and 11 isolates of *V. parahaemolyticus* from plankton and water samples were identified. *V. cholerae* was not isolated from water (salinity: 37–42 ppt) and plankton samples.
Conclusions: The pattern of high salinity observed was that *V. cholerae* was not detected in regions of the study. But, the occurrence of *V. alginolyticus* and *V. parahaemolyticus* in the Persian Gulf suggest that these bacteria are a potential public health hazard in this geographical area.

P639 Aspects pleading for relationships between hemagglutinins and other virulence factors in *V. cholerae* O1 strains

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Objectives: The present work was performed on 166 *V. cholerae* O1 strains isolated from clinical cases (161) and environment (five water sources) during the cholera epidemics of Romania between 1987 and 1995, in the purpose to detect any relationship between HA patterns and other virulence factors.
Methods: All strains were simultaneously investigated for: HA aspects, adhesion ability (by slime test) and the presence of nine enzymatic virulence factors (spot, CAMP and Kanagawa hemolysis, esculin hydrolysis, mucinase, amylase, lecithinase, lipase and DNAse production).
Results: Out of 166 *V. cholerae* strains, 95% exhibited HA activities with chicken erythrocytes, i.e. 81% MSHA, 8% FSHA, 65% MSFRHA; 21% exhibited atypical HA with human (O group) erythrocytes, these atypical HA aspects (accompanied by a great phage type diversity) being concentrated in strains isolated in 1993. Out of 166 *V. cholerae* strains, 87, 86, 85, 83, 75 and 60% were positive for lipase, amylase, lecithinase, DNAse, Kanagawa hemolysin and caseinase, respectively; 41, 12, 7.8% were positive in spot hemolysis, esculin and mucinase, respectively; 48% showed adherence ability to abiotic (borosilicate) surfaces evidenced in slime test, but only 19% of them exhibited a surface pellicle.
Conclusion: Our results demonstrated a high correspondence of 62% between MSHA and caseinase activity, 48% between slime agglutinin and caseinase and 44% between the same MSHA and slime agglutinins. All the five *V. cholerae* strains isolated in water sources showed Kanagawa and lecithinase positivity, but their adhesion aspects were poorly represented suggesting that in the external medium, *V. cholerae* O1 strains can preserve a high potential of toxigenicity (Kanagawa toxin), but scant adhesion ability to abiotic surfaces (this aspect probably explaining the lower resistance in the external medium of *V. cholerae* O1 as compared to non-O1 strains). It is to be mentioned that one strain isolated in a child with acute diarrhea exhibiting MSHA was negative for all the other investigated virulence factors. The low correlation (8.5%) between caseinase and mucinase suggests the existence of two distinct enzymes, our results being different from data cited by Finkelstein et al. 1992. Our results versus other data from the literature (Ingole et al. 1998) proved to be similar for caseinase (69% vs. 60%) and lipase (87% vs. 65.38%), but showed discordances especially for lecithinase (85% vs. 0%) and DNAse (83% vs. 19.23%).

P640 Epidemiological considerations on *Vibrio cholerae* infection in Romania in the last 25 years

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Background: For the past century and a half, cholera has remained endemic in the delta of Ganges, with annual epidemics in major population centers in West Bengal and Bangladesh. The disease has made periodic incursions into other portions of Southern and South-east Asia, and it has given rise to seven major pandemics since 1817. Since then, until early in the 1960s the disease contracted in extent across the globe remaining regularly present only in Southern Asia.
Objective: To study the epidemiological aspects regarding *Vibrio cholerae* infection in Romania in the last 25 years.
Materials and methods: We performed a retrospective study on 2279 patients with *Vibrio cholerae* infection who were diagnosed in the Departments of Infectious Diseases between 1975 and 2000 in Romania. The etiologic agent was isolated from the stools by cultures on TCBS agar. Final identifications required agglutination with group- and type-specific antiserums.
Results: In Romania, after 1975, seven epidemics spread across the country: 115 cases in September 1997, 746 cases in August–September 1981, 745 cases in 1987, 298 cases in 1990, 247 cases in 1991, 20 cases in 1993 and 100 cases in 1994. Only five, respectively, three imported cases were registered in 1984 and 1992 (contact with persons from southern Asia). Most of the cases were noticed in Constanta and Tulcea County and in the Danube Delta Area. In all cases *Vibrio cholerae* 0:1, El Tor biotype, Ogawa serotype was identified.

Water, fresh fish and sea fruit plays a major role in the transmission of *Vibrio cholerae* in endemic areas. During major epidemics, the direct contamination of food with infected excreta was also important. Mild and moderate clinical forms predominated; lethality was ranged between 1.5 and 2%.

Conclusions: In Romania, in the last 25 years, during seven epidemics, a great number of cases were registered, especially in south–east of the country and Danube Delta Area (contaminated water and fish). Despite the treatment, a high lethality was noticed in severe cases.

P641 Evaluation of different methods for detecting the toxin profile and other virulence factors in *Vibrio cholerae* strains

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The purpose of this work was to evaluate by comparison the efficiency of PCR, Reversed Passive Latex Agglutination (RPLA) and NAD degradation (as a marker for ADP-ribosylating toxin) methods for detecting the toxin profile of 13 *V. cholerae* O1 (11 clinical/2 waters) and six non-O1 (4 clinical/2 waters) strains. There were also used additional tests (cytotoxicity on VeroE6 cells, adherence to HEp-2 cells, slime test and 10 enzymatic assays) to investigate the presence of other virulence features. The results showed a concordance between PCR-RPLA (84%), PCR-NAD degradation (73%) and RPLA-NAD degradation (84%). The sensitivity of RPLA and NAD degradation methods was comparable to that of PCR in detecting CT + *V. cholerae* O1 strains. In case of one *V. cholerae* strain isolated in a patient with diarrhea RPLA positive (up to the titer of 10–6) and PCR negative for toxin box (*ctx*, *ace*, *zot*), the NAD degradation method confirmed the RPLA positive result, this aspect pleading for the presence of a new cholera toxin (NCT) in *V. cholerae* O1 strains with different genetic sequences from classical CT, but similar to CT from immunologic and biologic point of view. The negative result in RPLA and positive in PCR concerning one *V. cholerae* strain isolated in a contact, could be explained by the repression of the phenotypic expressing of *ctx* gene, the NAD degradation being probably determined by another toxin with ADP ribosylating activity. The two *V. cholerae* O1 CT +water strains, positive in PCR, RPLA and NAD degradation as well as all 6 *V. cholerae* non-O1 strains negative in PCR and RPLA, but positive in NAD degradation (even in the absence of CT) are pleading for the existence of a reservoir of toxigenic strains in the aquatic medium. The NAD reaction could not be ascribed exclusively to CT, but can be attributed to any other factor with ADP ribosylating activity, being very useful in detecting certain virulent clones of *V. cholerae*. NAD degradation and biofilm-forming (slime test) abilities might offer to the bacterial cell advantages in surviving and dissemination in natural ecosystems. The highest positivity of cytotoxicity on Vero E6 cells and of the enzymatic tests (Kanagawa, sheep erythrocytes hemolysis, lecithinase, caseinase, esculin hydrolysis) in *V. cholerae* non-O1 CT- by comparison to *V. cholerae* O1 CT + strains, suggests that these virulence features could act as substitutes for CT in *V. cholerae* non-O1 CT-strains.

P642 The antimicrobial susceptibility test among clinical isolates of *Shigella sonnei* in Ankara, summer 2001

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During the summer of 2001 an outbreak of *Shigella sonnei* was observed in Ankara. Among the patients admitted with gastroenteritis during March to October 2001, the ones with dysenteric diarrhea increased. Of the 1496 total stools were examined, there were 289 mucus and bloody stools (19.3%). *Shigella sonnei* was isolated in 54 patients (18.7%). The results of these patients stools were examined at Bayindir Hospital, Ankara. Specimens were cultured for *Shigella* and other enteric pathogens using standard microbiological techniques. The incidence of *Shigella sonnei* related diarrhea had increased since July 2001. Antimicrobial susceptibility test were performed using the Kirby–Bauer disk diffusion method in accordance with the standard of NCCLS guidelines. All *Shigella sonnei* isolates were susceptible to ampicillin, chloramphenicol, ciprofloxocin and broad spectrum cephalosporins. Trimethoprim-sulfamethoxazole (TMP–SXT) resistance was observed in all strains of *Shigella sonnei*. As a result, especially in children with mucus and bloody diarrhea, the use of TMP/SXT in prophylaxis of shigellosis, needs to be questioned.

P643 Antimicrobial resistance of Shigella, 1994–2000

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Objectives: It is estimated that shigellosis is responsible for 600 000 deaths each year, mostly in children. *S. sonnei* and *S. flexneri* are reported as the two commonest species. Resistance has been reported to ampicillin (AMP), chloramphenicol (CAF), cotrimoxazole (SXT) and tetracycline (TET). Recently beta lactamase production has been observed leading to cefotaxime resistance. Shigellosis represents a diarrheal disease for which antimicrobial therapy is indicated. We decided to investigate resistance to several antibiotics during the period. Our aim was to assess resistance profile of strains isolated during a 7-year period.

Methods: Here we report antibiotic resistance on 1198 *Shigella* isolates collected during the period 1994–2000 in Bahrein including *Shigella sonnei* (N: 620), *S. flexneri* (N: 403) *S. boydii* (N: 108) *S. dysenteriae* (N: 67). Testing was performed by agar disk diffusion method on strains isolated according classical identification tests using home made reagents.

Results: *S. sonnei*, the most common isolate, was highly resistant to SXT with constant percentage during the observation period (88.4 in 1994 and 85.7 in 2000). Resistance to AMP was maximal in 1997 (28.1%) declining to 5.1% in 2000, the lowest value in the in the 7-year period. One strain was found resistant to cefotaxime and one to cefuroxime. *S. flexneri* was highly resistant to both SXT (max 82% in 1997, min 52.8% in 2000) and AMP (max 95% in 1996, min 52.6% in 1999). CAF resistance also markedly decreased in the last 3 years (down to 38% from 90% in 1996). No resistance was found for all the species tested to ciprofloxacin (CIP) and ceftriaxone.

Conclusions: Contrary to general feeling and commonly reported data we observed a sometimes very significant decrease in resistance to 'old' antibiotics likely linked to their restricted use and consequent limited selective pressure. In some cases (such as SXT) this apparent advantage is impaired by the sickle cell trait/disease present in our patients. The persistent exceptional activity of CIP is hampered by the side-effects in children, the most exposed population. A careful rotational use of antibiotics might be the best policy to make old drugs again active limiting the vicious spiral of continuos research for use (and abuse) of new molecules.

Acknowledgement: This study was supported by a research grant from AGU to GAB.

P644 *Yersinia enterocolitica* infection in children in Crete, Greece

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Background: *Yersinia enterocolitica* can cause illness ranging from self-limited enteritis to life-threatening systemic infection. The aim of the present study was to review the epidemiology, clinical course and outcome of *Y. enterocolitica* enteritis in children seen at the University General Hospital of Crete, Greece.

Methods: Medical and microbiologic records of all children with stool cultures positive for *Y. enterocolitica* were retrospectively reviewed during the 7-year period, 1995 through 2001.

Results: The review included 41 patients aged less than 14 years with *Y. enterocolitica* enteritis. Most patients (71%) presented during the cold months November to April. A clear history of exposure to any potential source of contamination was not elicited. *Y. enterocolitica* accounted for 6% of bacterial intestinal pathogens isolated during the study period. Of 43 isolates tested almost all were resistant to ampicillin, amoxycillin-clavulanic, cephalothin, cefuroxime, ceftazidime, aztreonam, and imipenem. The majority were susceptible to trimethoprim-sulfamethoxazole, gentamicin, amikacin, tetracycline, chloramphenicol, and the quinolones. An intermediate resistance was commonly shown for rifampin. A total of 13 children were hospitalized and 28 were treated as out-patients. Among the 13 hospitalized children 7 were aged less than 2 years. Blood cultures were obtained for the hospitalized children and all were negative. Children commonly presented with fever and

enteritis. Bloody stools were observed in 23% and abdominal distension and pain mimicking acute abdomen in 38% of the hospitalized patients. No child had sickle-cell disease or thalassaemia and no one presented with extra-intestinal manifestations. Only seven children received antibiotics and all had an excellent outcome.
Conclusions: *Y. enterocolitica* was a considerable cause of enteritis in the children in Crete, especially during the winter months. A certain risk factor was not demonstrated. Young children were more prone to severe manifestations. Acute abdomen was often the presenting manifestation. The role of antibiotics needs further evaluation.

P645 Clonal dissemination of a *Yersinia enterocolitica* strain, with different susceptibility to nalidixic acid

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Objective: To study the epidemiological relationship among 10 clinical isolates of *Yersinia enterocolitica* either susceptible or resistant to nalidixic acid as well as the mechanisms of nalidixic acid resistance.
Materials and methods: Ten strains of *Y. enterocolitica* collected from diarrhoeagenic patients in different towns in the area of Madrid (Spain), were studied from July 2000 to March 2001. In order to perform the epidemiological analysis, two methods were used: (1) Digestion of chromosomal DNA with low frequency restriction enzymes and pulsed-field gel electrophoresis (PFGE) and (2) REP-PCR. The susceptibility to nalidixic acid and ciprofloxacin was evaluated by a microdilution method. Moreover, the presence of mutations in the *gyrA* and *parC* genes were analyzed by PCR amplification of the quinolone resistance-determining region (QRDR) of these genes and their subsequent DNA sequencing.
Results: These strains showed the same PFGE and REP-PCR patterns, despite their different geographical and temporary origin. Six strains were nalidixic acid-resistant (NalR), exhibiting a MIC of ciprofloxacin ranging between 0.25 and 1 mg/L. These strains showed mutations in the QRDR region of the *gyrA* gene; four strains with one mutation in the amino acid codon Ser-83 that changed to Arg; one strain with a change from Ser-83 to Ile; and one strain with a change from Asp-87 to Tyr. Mutations in the *parC* genes were not found in any of the studied strains.
Conclusions: The nalidixic acid-resistance shown by these strains is due to the presence of mutations in the *gyrA* gene. These results showed a clonal dissemination of a nalidixic acid-resistant *Y. enterocolitica* strain derived from a susceptible strain in the area of Madrid (Spain), probably because of selective pressure with fluoroquinolones.

P646 The microflora of the small bowel in health and disease

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Objectives: To compare the microflora in the proximal jejunum in healthy volunteers with the microflora in patients with gastrointestinal symptoms suggestive of spontaneous bacterial overgrowth (diarrhea, abdominal pain, bloating) in the small bowel.
Methods: Twenty healthy subjects with no gastrointestinal complaints were included in the study. Eighteen patients with gastrointestinal complaints suggestive of spontaneous bacterial overgrowth in the small bowel were recruited from the out-patient clinic at Center of Gastroenterology, Huddinge University Hospital. The participants had not taken any antimicrobial agents within 1 month preceding the study. Biopsies from the proximal jejunum were taken with a Watson capsule. In order to prevent bacterial contamination from the gastrointestinal tract proximal to the small bowel, the capsule was flushed with 5 mL sterile 0.9% saline solution, 5 min before releasing the spring-loaded cutting device. During retraction, the biopsies were protected from bacterial contamination within the capsule. The biopsies were transported to the microbiological department in Brain Heart Infusion (BHI) medium. The samples were weighed, homogenized and diluted in pre-reduced media and inoculated on nonselective and selective agar. The plates were incubated aerobically and anaerobically. All isolates were identified to genus level.
Results: Seven of the patients suffered from irritable bowel syndrome, six from chronic intestinal pseudo-obstruction, two from mitochondrial myopathia with diarrhea and the remaining three patients from malabsorption, diabetes mellitus with diarrhea and functional dyspepsia, respectively. The small bowel microflora in both healthy subjects and in patients was similar to the oropharyngeal microflora. *Streptococcus intermedius* and *Haemophilus parahaemolyticus* were only found in the microflora of healthy subjects. *Lactobacillus* spp. was detected in samples of 2 of 20 healthy subjects and in samples of 9 of 18 patients ($P < 0.05$). The numbers of *Veillonella* were slightly higher in patients than in healthy subjects. No other statistically significant differences between numbers of species or of total numbers of aerobic and anaerobic microorganisms were found.
Conclusions: The jejunum microflora in health and disease resembles the oropharyngeal microflora. There were no major differences in numbers of most species but some species were only isolated in healthy persons whereas others were mainly found in patients.

P647 Changes in the infant intestinal micro flora associated with an anthroposophic lifestyle

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Objectives: Children with an anthroposophic life style have been shown to develop atopic allergy more seldom than control children with a Western lifestyle. The intestinal flora is supposed to have an impact on the development of the immune system. In the anthroposophic life style a vegetarian diet comprising vegetables spontaneously fermented by lactobacilli, and a restrictive use of antibiotics, antipyretics and vaccinations is typical. The aim of this study was to assess the gut flora in infants in relation to certain life style characteristics associated with anthroposophy.
Methods: Fecal samples from 69 children below 2 years of age with an anthroposophic life style and 59 similarly aged infants with a traditional life style were analyzed by bacterial enumeration (cfu) and bacterial typing through biochemical fingerprinting (The PhenePlate (TM) method) of coliform bacteria, enterococci and lactic acid bacteria; eight isolates per group and sample, i.e. >3000 isolates were typed.
Results: The numbers of enterococci and lactic acid bacteria were significantly higher in children who had never been exposed to antibiotics. The number of enterococci was significantly higher in breastfed and vegetarian infants ($P < 0.01$). The diversity (Simpson's diversity index) of lactobacilli was higher in infants born at home, than in infants born in hospital and in vegetarians ($P < 0.01$). The diversity of coliform bacteria and of lactobacilli did not change with age in the anthroposophic group. The data were interpreted as to indicate an earlier establishment of a 'mature' flora in anthroposophic children than in control children. This finding could not have been obtained without fingerprinting methods.
Conclusion: In conclusion, life style factors related to the anthroposophic way of life influenced the composition of the gut flora in the infants, which in turn indicates that the modern Western lifestyle has influenced the colonization of the intestinal tract of the newborn. These differences may contribute to the lower prevalence of atopic disease previously observed in children in anthroposophic families.

P648 Gastroenteritis outbreaks due to infections with 'Norwalk-like viruses' in Switzerland 2001

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'Norwalk-like viruses' (NLVs) are a genus belonging to the family of Caliciviridae. These viruses are mainly transmitted by the fecal-oral route and by aerosols. They are one of the most common causes of outbreaks of nonbacterial gastroenteritis. In the US, NLVs are thought to be responsible for an estimated 67% of foodborne infections and for 96% of nonbacterial cases of gastroenteritis. By outbreak investigation in the German part of Switzerland, an attempt has been made for the first time to assess and describe the epidemic potential of NLVs in our country. In cooperation with different Cantonal Laboratories and public health specialists, outbreaks with NLVs as possible causative agent, were recorded, analyzed and classified during the year 2001. In total, seven NLV outbreaks could be documented. In five cases, NLVs were

detected by RT-PCR in stool samples, in one case the PCR product could not be confirmed by sequencing and in another case, no samples were provided. The clinical symptoms were typical and in accordance with the definition of the NLV-infection. The outbreaks occurred in four different settings: four outbreaks concerned different school and boy scout's camps (158 patients), one was a municipal outbreak (a few hundred patients estimated), one occurred in a health resort (approx. 40 patients) and one was a family and-friends outbreak (25 patients). The described outbreaks – except the municipal outbreak, which was thought to be waterborne – pointed out clearly the relevance of person-to-person transmission in the epidemiology of the NLVs. Furthermore, one outbreak in a school camp was prolonged and showed the importance of disinfection of contaminated environments (e.g. beds, toilets) after a NLV-outbreak to prevent further infections.

Tissue helminths and lice

P649 Electroencephalographic findings in cerebral cysticercosis patients

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During the last 5 years (from 1997 to 2001), 13 patients with the diagnosis of brain cysticercosis have been treated in the Clinic for Infectious Diseases. Headache existed in eight patients (61.54%), while faintness occurred in five patients (38.46%). In most of the patients, the disease was clinically manifested by the presence of focal centers. Eight patients (61.54%) had epileptic seizures, seven patients (53.85%) felt malaise and numbness in one-half of the body, while talk disorders occurred in two patients (15.38%). In eight patients (61.54%) neurological findings were pathological. Besides computerized tomography and nuclear magnetic resonance, 11 patients were subjected to electroencephalography (EEG). EEG findings were changed in 10 patients (90.91%), while only 1 patient (9.09%) exhibited normal bioelectrical brain activity. In five patients (45.45%), the signs of mild unspecific cerebral dysfunction existed (diffuse in one patient, over the centroparietal, frontal or temporal regions in four of them). One patient expressed cerebral dysfunction more heavily, over the frontal and temporal regions. In four patients (36.36%), the EEG was specifically changed, with actually present epileptic activity in the form of isolated spikes. All four patients with specifically changed EEG clinically manifested epileptic seizures. The anti-epileptic therapy was administered to 10 patients (76.92%) and yielded favorable results.

P650 Inflammatory reaction in neurocysticercosis patients

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During the period from 1997 to 2001, 13 patients with the diagnosis of neurocysticircosis have been treated in our clinic. Besides the routine laboratory and other additional findings (computerized tomography (CT) and magnetic resonance), 11 patients were subjected to lumbar puncture and cytological and biochemical analyzes of cerebrospinal fluid (CSF) were carried out. In five patients (45.45%) cytobiochemical findings of CSF were normal, while in six patients (54.55%), it was pathologically changed, showing the signs of inflammatory reaction. Pleocytosis was present in six patients (54.55%), the number of elements reaching 3×10^6 to 215×10^6/L. Lymphocytes dominated in the CSF concentrate, while very small numbers of granulocytes, eosinophils and monocytes existed. CSF protein values were increased in five patients (45.45%), being about 0.64–1.52 g/L. Values of glycorachia were normal in four patients (36.36%), while in two patients (18.18%) they were decreased. The presence of inflammation signs in CSF correlated with the findings of active cysticercosis forms, as found by CT in four patients (66.67%) (in addition to calcific forms). The presence of lymphomonocytic pleocytosis and mild proteinorachia was established in four patients (66.67%), who exhibited hydrocephalus as well. According to our results, the occurrence of inflammatory reaction was ascertained in the patients who had active brain cysticercosis, as well as hydrocephalus as a neurocysticercosis complication.

P651 Incidence of parasitic infections in an onchocerciasis-endemic village in northern Nigeria

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Objectives: To determine the prevalence of intestinal parasitic infection in an onchocerciasis endemic area and to investigate the possible association between the intensity of parasitic infections.
Methods: Cross-sectional study of 235 adults in Gongon Maliki village in Northern part of Nigeria was undertaken for this study. A total of 116 people were found positive for onchocerciasis by the skin snip method. All the 116 subjects was screened for the presence of intestinal parasites using the formol ether faecal concentration technique. The chi-square test was used to compare the proportion of positive individuals between groups. Disease intensity among different age groups and sex was compared to determine if the differences were statistically significant.
Result: *Entamoeba coli*, Hookworm, *Schistosoma mansoni*, *Strongyloidies stercoralis*, *Enterobius vermicularis* and *Taenia saginata* eggs and ova were seen in the stool sample of the 116 (49%) individuals positive for onchocerciasis. While the prevalence of *E. coli* (6.03%) *E. vermicularis* (1.72%) and *T. saginata* (0.86%) were negligible those of Hookworm (31.8%) and *S. mansoni* (37.93%) were of significant importance. Hookworm and *S. mansoni* occur together more frequently as a multiple infection than as a single specie infection and also as triple species infection with *E. coli* but very rare. There is no direct correlation between the intensity of the infections.
Conclusion: The result reflect the poor hygiene level of the community. It also imply that joint treatment of Hookworm and schistosomiasis infection would be beneficial to the community along with the ongoing onchocerciasis control program in the area.

P652 A particular case of eosinophilic pneumonia *Toxocara canis* IgG/M positive

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Tradate, I

We describe a case of a young woman 26-year-old with bilateral interstitial recidivanting pneumonia. The patient had a history of intrinsic asthma from 2 years in treatment with inhalatory drugs (low dose of inhalatory steroid and β_2 agonist), she lived in a small town and she had got a dog. She presented fever, cough, dyspnea, chest pain, artromialgies, polipnea, O_2 saturation 93%, tachycardia 100/min, bilateral crepitant rales, distal cutaneous maculo-papulosus rash at the fore limbs. Laboratory data on admission revealed leukocytosis 37×10^9/L with peripheral blood eosinophilia 19.3×10^9/L, VES 62, IgE 6911 IU/mL (normal values 0–150). All other laboratory findings, including auto-antibodies, rheumatoid factor level, serology for CMV, Chlamydia, Laegionella, Mycoplasma, HIV, Rubella, Hepatitis ABC, tuberculin skin test results were negative. Blood cultures and also sputum, urine, bronchoaspirate cultures were negative. Stools examination was negative, the stools were checked for evidence of ova or parasites and appropriate examinations and cultures excluded deep fungal infections. The patient was treated empirically, full-dose, consecutively with many types of antibiotics (ev ceftriaxone, ev netilmicine, ev teicoplanine, oral claritromicine) without any

resolution. She had a complete resolution in a few days with oral 50 mg/die metilprednisolone. Biopsy of a skin lesion was made with evidence of vasculitis. The histological examination showed: necrotizing vasculitis and necrotizing granuloma with a prominent eosinophil infiltrate compatible with Churg–Strauss Syndrome. Sierology for *Toxocara canis* was positive: IgG/M ELISA 1.042 (positive >0.300). Churg–Strauss Syndrome is a rare systemic vasculitis occurring in patients with asthma and blood eosinophilia; lungs, skin, are the most common sites of involvement, but many other organs are affected frequently. The diagnosis often in established from clinical findings or biopsy of extrapulmonary sites. The prognosis is good, with remission occurring in the majority of treated patients. *Toxocara canis* pneumonia may resolve spontaneously. Today, after 2 years, she is in health. The question is unresolved: our patient was really affected by Churg–Strauss Syndrome?

Keywords: *Toxocara canis*, Churg–Strauss Syndrome, eosinophilic pneumonia

P653 *Tunga penetrans*

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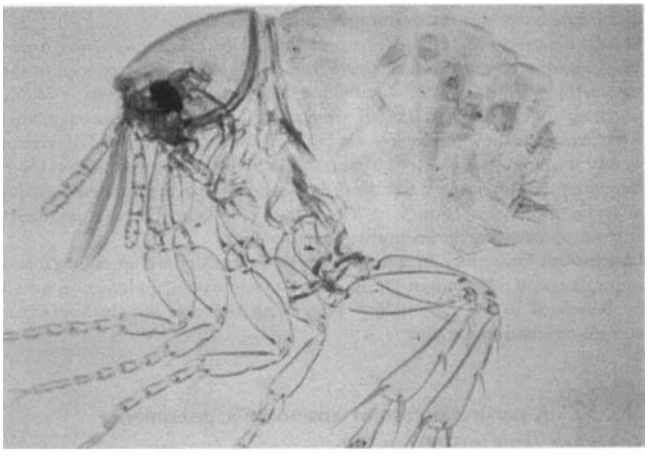
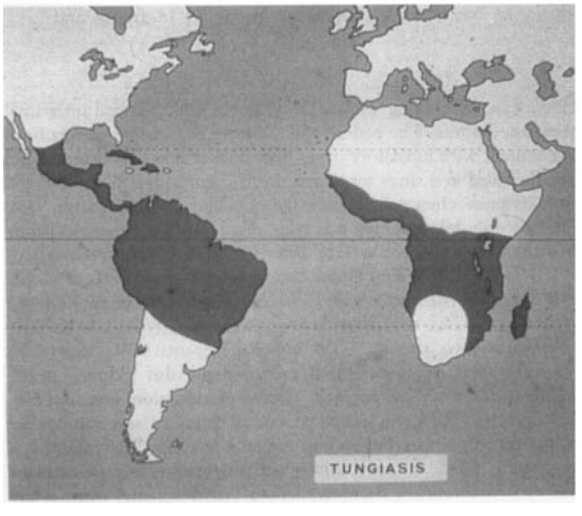
Rome, I

Tunga penetrans, also known as "sand flea, jigger flea, chigoe, chique" causes a particular infection of the cutaneous tissue, called Tungiasis. Adult fleas can be found in sand and soil and are free-living. After copulation females look for a host (humans and other animals such as pigs and poultry) and find their way in cracks of the skin, typically on the feet between the toes and under the toenails. They burrow in the hosts skin between the stratum corneum and the stratum granulosum leaving only their breathing organs (spiracles) uncovered. The fleas then grow in size remaining attached to the host with their mouth parts until they resemble a small white pea with a black spot in the middle. The growth of the jiggers causes skin irritation and itching in the host that through scratching determines the dispersion of the eggs. *Tunga penetrans* (Fig. 1) was originally discovered in Central and South America, however, now it has spread to the African continent and to India (Fig. 2)





From 1990 to 2001, we observed 18 cases of *Tunga penetrans* infection in patients coming from Tanzania, Zanzibar, Gabon, Guinea Bissau and the Ivory Coast. All the patients showed characteristic lesions located around the toenails and under the feet, particularly in the crevices of the skin between the toes, which contained the fecund female jiggers. The sizes of the injuries were different, all were painful and some showed signs of bacterial super-infection, especially the ones located around the toenails. Diagnosis was suspected while examining the lesions, and was confirmed by identifying a small black point on the summit of the "small white pea" lesion, which corresponds to the posterior respiratory spiracles of the flea. The mature female jiggers were carefully removed and the sores were disinfected.

P654 Subcutaneous myasis

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From January 1990 to September 2001, in our department we have diagnosed and treated 27 cases of Subcutaneous myasis. The patients we observed came from Africa, Central and South America and for the most part were tourists. At presentation, the patients showed a bullous lesion, that was described as itching and painful. Some also developed low grade fever and general malaise. Most patient could not remember being in close contact with flies or maggots, but all had slept and lived in poor hygienic conditions. The sores started as a small vesicle, painless and un-itching, that grew in size becoming painful in 1–2 weeks. During physical examination, we found a characteristic 1–3 mm dark-line on top of the lesion. This corresponds to the respiratory spiracles of the maggot. Once the skin surface was covered with vaseline or oil, oxygen bubbles appeared confirming our diagnosis. Patients who presented with early manifestations were advised to wait for the larva to develop, abstaining from any kind of treatment, and to return after 1–2 weeks. Treatment of the full-grown lesion consisted in covering the spiracles with a thin film of paraffin oil to stop the maggot from breathing and exerting firm digital pressure on each side of the bulla to remove it. We observed and identified each maggot analyzing its peculiar morphological features under an optic microscope. The larvae belonged to two species, *Dermatobia hominis* and *Cordylobia anthropophaga*, both known agents of Subcutaneous myasis. Once the maggots were removed the lesions healed spontaneously in a few days. Local application of antibiotics may be useful to avoid bacterial super-infection.

P655 Confirmation of *Tatera indica* (Rodentia: Gerbillideae) as the main reservoir host of zoonotic cutaneous leishmaniasis in west Iran

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Introduction: During the Iran–Iraq war, ZCL was one of the most important infectious diseases in the area of our study.
Methods: During our study (summer of 1994) 22 *Tatera indica* were collected and two out of them (9.09%) were naturally infected with amastigote. Parasites were cultured in NNN + LIT medium and isolated promastigotes were sent to the Medical University of Montpellier in France for identification.
Results: The result of isoenzyme test showed, the parasite is *Leishmania major* zymodem MON 26-(LON 1).
Conclusion: Therefore, *Tatera indica* was known as the main reservoir host of zoonotic Cutaneous Leishmaniasis in the west of Iran. This is the first report on the isolation and identification of *L. major* zymodem MON 26-(LON 1) from this species of rodent mean time. *Rhombomys opimus* was absent in these areas.

P656 A study of seven echinococciasis cases in adult patients during 2000–2001

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Objective: To study seven echinococciasis cases in adult patients during 2000–2001.
Methods: Samples of serum from patients were examined for echinococcus antibodies by indirect hemagglutination test (IHA). The diagnosis was based also on imaging procedures.
Cases report: During the study period, echinoccociasis was diagnosed in seven cases. The common causes of admission were persistent epigastric pain (5/7), nausea/vomiting (2/7), cholestasis/cholangitis (1/7), weight loss (1/7), suppurative liver echinococcus cyst (1/7) and fever (3/7). All patients had hydatid cysts in the liver and all had positive echinococcus antibodies by IHA test. In 5/7 patients, the title of antibodies was 1:3200. Primary disease was found in 6/7 patients and recurrence in 1/7. All patients were treated by surgery.
Conclusions: Echinococciasis is still being a health problem in Greece. IHA test is a sensitive and specific method for diagnosis of echinococciasis.

P657 Evaluation of IgG-ELISA and IHA and their correlation with ultrasound appearance in the long-term follow-up of cystic echinococcosis of the liver

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Objectives: Cystic echinococcosis (CE) poses important diagnostic and follow-up problems. Diagnosis of relapse may be difficult, especially after medical treatment. The lack of serological patterns predictive of the patient's post-treatment response is a contributing factor. This is due to the short of follow-up in most studies published so far.
Methods: Since usefulness of serology in follow-up of CE is questioned by some for the reasons aforementioned, we evaluated retrospectively 42 patients with abdominal CE (23 male and 19 female), with a follow-up ranging from 12 to 48 months. IgG-ELISA and IHA are routinely used in our parasitology laboratory both for diagnosis and follow-up; their values were plotted in graphs together with ultrasound (US) appearance at the time of blood drawings. Fluid in the cysts at US (Gharbi types I, II, III) was a marker of viability. Solid cysts (Gharbi types IV, V) were rated non-viable.
Results: The graphs obtained showed four different patterns.
(1) ELISA and IHA run parallel, increasing or decreasing in accordance with US appearance.
(2) ELISA values decrease months before IHA while cysts become nonviable.
(3) ELISA values are steady while IHA increases indicating relapse (reappearance of fluid).
(4) Both IgG-ELISA and IHA fluctuate against a stable US appearance; these fluctuations seem to have no clinical importance.

Conclusions: IgG-ELISA and IHA are useful in follow-up after medical treatment if combined with US examination. Further study of the patterns described and comparison with other serological markers of relapse (IgG subclasses, IgE, Cytochines) are needed to define reliable indexes of relapses and to help in clinical decision-making with these patient.

P658 Hydatid disease: ruptured cyst

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Hydatid disease is a parasitic infection, most frequently caused by *Echinococcus granulosus*. The geographic distribution of the disease is cosmopolitan, in temperate climate countries. A 38-year-old man appeared in the emergency department with dyspnea, fever, cough, hemoptysis and left-sided pleuritic pain. The symptoms began 5 days ago with no response to the treatment of second generation cephalosporins. A chest X-ray revealed cavitary lesion in the left lower lobe and bilateral basilar acinar infiltrates in lower lobes. Full Blood Count showed Hct 45.5%, WBC 15.000/mm^3 (PMN 87%, LYM 5%, M 4%, EOS 4%), Plt 195.000/mm^3 and ESR was 56 mm. A gastropscopy was performed, but no potential site of bleeding was found in the upper digestive tract. Bronchoscopy revealed the characteristic endobronchial whitish-yellow membranes, considered specific for ruptured hydatid disease, with an abnormal orifice in a subsegmental division of the posterior of the left lower bronchus, that correspond to the fistula between the cavity and the bronchian tree. On microscopical examination (wet and stained preparations) of bronchial washing the pathogenomonic hooklets of *Echinococcus granulosus* were found. The diagnosis was also confirmed by serology. Investigation of the upper abdomen (CT scan) revealed a liver hydatid cyst. Albendazole was introduced and patient was referred for surgical treatment of both the lung and the hepatic cysts.
Conclusions:
1. In lung infections, protoscolices or hooklets may sometimes be demonstrated in bronchial lavage fluid or sputum although the absence does not rule out the possibility of hydatid disease.
2. Eosinophilia is present in fewer than 25% of cases.
3. Surgical excision of the cyst is the treatment of choice for hydatid disease. Long-term treatment with Mebendazole is mainly recommended.

P659 A new experimental approach to form the unilocular liver cystic echinococcosis in rabbits

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Objectives: Cystic echinococcosis is still endemic in developing countries and remains to be an important cause of morbidity especially in Turkey. Beside surgical and percutaneous radiological methods new experimental therapeutic modalities with the use of antiparasitic drugs are undergoing. Therefore, to constitute an effective model of cystic echinococcosis is gaining importance. The aim of this study is to present a new practical and time saving experimental model for treatment of cystic echinococcosis.
Methods: Seven New Zealand rabbits were used in this study. Naturally infected cystic cattle liver obtained from slaughterhouse, explored under sterile conditions. Appropriate cysts opened and protoscoleces examined under light microscope with 0.1% eosin. Approximately 0.5 cm in diameter daughter cysts were taken from big cysts, which has high viability rates of protoscoleces. Suitable cavities were performed in anterior surfaces of right lobes of rabbits' livers under general anesthesia. Daughter cysts put into these cavities and after the liver capsule sutured separately.
Results: Livers were evaluated with computerized tomography and ultrasound monthly for 3 months postoperatively. Settled cysts were observed in all livers at the end of the first month. Mean diameter was 8.8 ± 0.8 mm. Five cysts diameters were increased, mean diameter was 9.8 ± 1.0 mm while only two cysts diameter was decreased at the end of the third month.
Conclusions: The main advantage of this method is to form a successful unilocular liver cyst surgically, then classical intraperitoneally protoscoleces injection methods. As a conclusion, this method is found an applicable approach with the decrease of the cyst development time and high inoculation rate of cysts into the liver.

P660 An unusual case of hydatid disease in the thigh

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Objective: Hydatid cyst disease (Echinoccosis) is a zoonotic disease caused by the tapeworm *Echinococcus* which have the ability of implanting in any organ of the body but mostly in the liver producing a cystic mass. It rarely involves soft tissues with a frequency ranging between 2.4 and 5.3% of all hydatid disease cases. This is a very rare case initially presenting with a soft tissue mass in her thigh with unusual localization of echinococcosis. In the literature not more than 10 cases with unusual localization of echinococcosis is reported.
Case: A 57-year-old female admitted with the complaints of pain and increasing size of her left leg. A plain X-ray showed water density mass in the left thigh. It was learned from the history that 30 years ago she was operated from the same region for the same complaints. We performed an MRI test to left thigh region. A cystic multiloculated mass was identified on

MR images. Albendazole was started to the patient in order to prevent anaphylactic reaction due to rupture of the cyst during the operation. She was submitted to surgery again and a hydatid cyst was extirpated from the thigh. Histopathological examination revealed typical hydatid sand which is compatible with the diagnosis hydatosis. The patient continued the treatment for 45 days after surgical procedure. Blood eosinophils elevated initially returned to normal and no more complaint was present after treatment.
Conclusion: Patients with musculoskeletal lesions of cystic structure as soft tissue masses should be evaluated by physicians especially in areas where echinococcosis is endemic. These cystic structures resemble abscesses morphologically. Essentially ultrasonography, was suggestive of the diagnosis. Magnetic Resonance Imaging (MRI) provides more precise information about the localization, number and morphologic structure of the lesion also helps to differentiate the mass from neoplastic diseases. Surgical excision of the cysts and fine-needle aspiration biopsy usually lead to the definitive diagnosis. Since puncture of these cysts may cause an anaphylactic reaction due to spillage of hydatid fluid and/or dissemination of infection, the use of fine-needle aspiration biopsy is not preferred but this procedure may represent a real aid in the definitive diagnose of clinically unsuspected hydatid cysts.

P661 Pulmonary hydatid disease: clinical presentation and outcome

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Introduction: *Echinococcus granulosis* causes hydatid disease and is an increasing public health problem in parts of Asia. The lung is the most common site of cyst formation after the liver.
Objectives: Ascertain demographics, clinical features, treatment and outcomes of patients with pulmonary hydatid disease.
Methods: Retrospective descriptive case series using data-extraction sheets for patients presenting in a Karachi Tertiary Care Hospital, between January 1995 and August 2001.
Results: A total of 19 patients had pulmonary hydatid cysts out of a total of 100 patients with hydatid disease. Demographics of the 19 patients: male (68%), female (32%), mean age; 21 years, Pakistanis (63%), Afghanis (37%).
Clinical features: Productive cough (84%), fever (73%), haemoptysis (52%), dyspnoea (42%), chest pain (42%), coexisting liver cysts (11%).
Treatment: Albendazole (12%), Albendazole and surgical removal (88%).
Outcomes: Mean length of admission; 1 week. No intraoperative cyst rupture, mortality or recurrence of cysts were documented.
Discussion: Presentation is more common in young males with symptoms of cough, fever and haemoptysis. Surgical removal with Albendazole cover is the treatment of choice, and this carries low mortality and recurrence rates. Early clinical detection, sterilization of the source and interrupting transmission of the parasite should be combined with increased awareness as necessary prevention strategies to combat hydatid disease in Pakistan and other parts of Asia.

P662 Do healthcare professionals contribute to ineffective control of head lice?

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Objectives: Myths and stigma surrounding head lice have generated controversy over treatment and prevention methods. In 1998, the Public Health Medicine Environment Group, UK produced guidance on the treatment and prevention of head lice. This was adapted for local use in South Staffordshire, an English health district, and disseminated among local healthcare professionals (HCP). The aim of this study was to examine healthcare professionals use and awareness of the local policy and their knowledge of the treatment and prevention of head lice 18 months after dissemination.
Methods: Printed copies of the policy were posted to primary healthcare professionals (general practitioners and practice nurses), community healthcare professionals (community pediatricians, health visitors and school nurses) and pharmacists in February 1999. A self-administered questionnaire was then sent to all HCP in September 2000 to gather data on their use of the guidelines and their knowledge of the treatment and prevention of head lice. Responses were classified as 'full', 'some' or 'no' knowledge of treatment and prevention methods.
Results: The policy was well disseminated with 76% (173/228) of respondents indicating that they referred to it. A significantly greater proportion of CHCP would refer to the guidelines when asked about head lice by a patient or parent ($P < 0.001$) compared to primary healthcare professionals (PHCP) or pharmacists. When describing preventive measures for head lice, all HCP groups appeared to have similar 'full knowledge'. However, significant differences between the groups in the category 'no knowledge' ($P < 0.00001$) were seen with PHCP accounting for most respondents. Overall, treatment for head lice was poorly described with significant differences seen between the groups ($P = 0.0096$). Most of those with 'no' knowledge of how to treat head lice were PHCP. Altogether, respondents were significantly more likely to have 'full knowledge' of prevention methods (53.8%, 95% CI 47.2–60.2%) ($P < 0.0001$) than they were of appropriate treatment methods (4.6, 2.3–8.1%).
Conclusions: Our results suggest that HCPs lack of knowledge of appropriate treatment and prevention methods contributes to ineffective control of head lice infections. This lack of knowledge was most marked in PHCP and to a lesser extent in pharmacists. These results deserve further investigation in other populations.

HIV I

P663 Trends in the costs of HIV management: 1996–2001

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Cadiz, E

Background: Increased use of protease inhibitors (PIs) has been associated with declines in AIDS mortality and opportunistic infections (OIs). These benefits have been obtained at the expense of an enhanced cost for the oral antiretrovirals, specially PIs.
Objective: We have retrospectively evaluated a cohort of 100 HIV patients before and after the start of HAART including PIs (HAART-PI) to assess the effects on healthcare costs of HAART-PI in a 450-bed teaching hospital.
Methods: Costs were obtained from the medical records, the Office for Control and Management, Central Laboratories Service and Pharmacy of the Hospital. Other costs were estimated from the standards of the Andalusian Health Service and the Spanish Ministry of Health. Mean costs per patient per month were compared by semester since July 1996 to July 2001. Mortality and the incidence of opportunistic infections were correlated with the use of PIs.
Results: Hospital admissions and length of stay decreased by 47 and 21%, respectively. In-hospital cost per day decreased by 9%, partially due to a 33% decline of admissions with ≥ 1 major HIV-associated diseases. Admission costs per patient per year declined by US$ 2823. Outpatient visits per year increased by 67%, with a cost increase of US$ 339 per patient per year. The cost of laboratory tests increased by US$ 2341 per outpatient per year. Cost of pharmacy per patient per year increased by US$ 3578. Estimated saving per patient per year was US$ 1597 during the year 1997, US$ 779 in 1998, US$ 419 in 1999, US$ 234 in 2000, and US$ 121 in 2001.
Conclusions: HIV patients on HAART with PIs not only had considerably less admissions to hospitals and milder HIV-associated complications but, an important global general management cost reduction in the short term (1–3 years) after initiation of HAART-PI. Although, clinical benefits persist after 3 years of therapy, global healthcare savings tend to decrease afterwards due to enhanced costs of pharmacy and laboratory tests.

P664 Quality of life and use of complementary medicine among HIV positives

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ELECTHIV 2 Study Group

Introduction: In the pre-HAART period, 43% of individuals with HIV in Europe had had the opportunity or expressed their intention of using

complementary therapies (CT). Since 1996, HAART changed the use of CT among HIV-positive people.
Objectives: ELECTHIV2 is a case–control study, sponsored by European Commission and Italian Institute of Heath, comparing the characteristics and the quality of life of CT users and non-CT users, HIV-infected subjects.
Methods: In seven European countries (Italy, Belgium, France, Germany, Greece, Spain, UK), anonymous, self-administered questionnaires were distributed which included sociodemographic information, clinical, immunological, therapeutical and side-effect data. The Hat-Qol Questionnaire was chosen to compare the quality of life of CT users and non-CT users.
Results: Total 1066 self-administered, anonymous questionnaires were collected. Total 632 were CT users and 434 non-CT users. Slightly better scores on the quality of life were obtained by those who use CT compared to non-CT users. CT users speak more easily about themselves and their illness to others, hide less and are less afraid that colleagues or family, may learn of their seropositive condition, compared to non-CT users (t-test $P < 0.05$, covariance analysis (CA) $P < 0.05$). Those using CT accept their illness more easily than those who do not use CT (t-test $P < 0.05$; CA $P < 0.05$). The quality of life is more satisfactory for CT users than for nonusers. The significance appears statistically correlated to the asymptomatic clinical status (t-test $P < 0.05$; CA $P < 0.05$). The analysis shows that those using CT perceive the use of antiretroviral therapies as more difficult than those who do not use nonconventional therapies. The antiretroviral therapy seems to be accepted less by CT users. The statistical significance appears correlated with the clinical status ($P = 0.03$) and, in particular, with the definition of the AIDS case ($P < 0.05$). It is probable that the diagnosis of AIDS corresponds to a longer illness and therefore more difficult therapeutic regimes.
Conclusions: The level of quality of life seems to increase with the level of education of NATC users, whilst amongst nonusers it appears to remain constant. From the scores of the Hat QoL, it is also possible to notice a more problematic relationship between NATC users and their doctor, especially if the former has long-term side-effects (in particular peripheral neuropathy). This is one more reason to seek refuge in nonconventional therapies.

P665 Seroprevalence study of HIV HTLV-I/II HBV and HCV infections among immigrant prostitutes in Madrid, Spain

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Madrid, E

Objectives: To evaluate the seroprevalence of the HIV-1/2 HTLV-I/II HBV and HCV infections among immigrant prostitutes in Madrid.
Methods: We analyzed 391 samples from women who had recently arrived in Spain to work as prostitutes (aged 18–41 years). Total 360 came from sub-Saharan Africa: Sierra Leona (185), Nigeria (123), Liberia (30), Sudan (19), and 31 from Latin American countries. Most of them from Ecuador 28/31. Their sera, collected from January 1998 to November 2001 during their first 2 months in Spain were brought to our laboratory by 'Medicus Mundi' screening tests were determined by detection of antibodies using EIAs (Axsym Abbott, Ortho) and confirmatory tests by lineal immunoassays (RIBA Inno-LIA Pepti-LAV) and Western blot.
Results: Nineteen (4.9%) were infected with HIV-1 (8/185 from Sierra Leona, 6/123 from Nigeria, 4/30 from Liberia, and 1/28 from Ecuador), 14 (3.6%) with HBV (HBsAg-positive). One in 154 was infected with HTLV-I/II. No cases of HCV infection were identified. Total 150 (38%) had serologic markers of past infection with HBV.
Conclusions: In this study, it is worthy to note the high prevalence of HIV-1 among the women from Liberia (13%). None of the women tested were infected with HCV despite the high prevalence of the HCV in these African countries. In addition, one woman from Sierra Leona was infected with HTLV-I/II (relatively rare).

P666 Comparison of two RNA extraction methods prior to HIV genotypic resistance testing

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Valladolid, E

Objectives: A critical point in the development and performance of molecular diagnosis techniques is the extraction of genetic material in the purist conditions. We evaluated the use of two RNA extraction methods prior to the genotypic detection of HIV-1 resistance by line probe assay (LiPA).
Methods: Fifty plasma samples were tested for the determination of viral load (VL) by means of Cobas Amplicor HIV-1 Monitor™ (Roche Diagnostics, Branchburg, NJ, USA), preserving the obtained RNA extracts. RNA extraction by means of SV Total RNA Isolation System (Promega Corporation, Madison, WI, USA) was also carried out. Both RNA extracts were processed in parallel for the detection of HIV resistance by LiPA, and bands were recorded comparatively.
Results: Findings obtained by Roche extraction method were superior in all the studied parameters: proportion of amplified samples (66.0% by Promega vs. 98.0% by Roche for LiPA RT and 76.0% vs. 100.0% for LiPA P), percentage of mutated samples (32.0% vs. 58.0% for LiPA RT and 30.0% vs. 42.0% for LiPA P) and band intensity, this being superior in 66.7% of the samples for LiPA RT and in 18.4% for LiPA P.
Conclusions: The outcome obtained by LiPA after RNA extraction by Roche methodology was remarkably superior to that of Promega. More studies focused on the optimization of extraction methods prior to genotypic detection of HIV resistance are needed.

P667 Testing of HIV-1 viral load with the bDNA assay using low volumes of plasma

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Madrid, E

Introduction and objectives: The branched DNA assay has proved to be one of the most used systems for quantification of HIV plasmatic viral loads and it is probably the most reproducible one. The main disadvantage of the test may be the high plasma volumes needed (1 mL), which makes it unsuitable for pediatric tests. We assayed two smaller volumes and have compared their results with those of the standard procedure.
Methods: Eighty samples from HIV-positive patients ranging from 300 to 300 000 copies/mL were included. All the samples were run with the standard recommended volume (1 mL) as well as with 0.5 and 0.2 mL. All remaining procedures were those recommended by the manufacturer. In both cases of reduced volumes, results were converted to a volume of 1 mL. All results were compared and analyzed.
Results: Average viral loads for all three volumes were 4.21 log (52 430 C/mL) for 1 mL, 4.32 log (58 308 C/mL) for 0.5 mL, and 4.39 log (72 316 C/mL) for 0.2 mL. Analysis of paired volumes showed the following correlation coefficients (R^2): 0.95, 0.95 and 0.99 for the pairs 1/0.5, 1/0.2 and 5/0.2 mL, respectively.
Conclusions: Average quantification was quite similar among all three tested volumes, since differences were under accepted reproducibility values of the technique (0.2 log). An almost perfect correlation was achieved with lower volumes, although the remaining pairs also produced very high correlation coefficients. Although standard protocol must be used whenever possible, we think lower volumes may be used without considerably affecting the results of the assays.

P668 Interpretation of genotype methods for testing antiretroviral resistance

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Granada, E

Objective: To compare different algorithms for interpretation of resistance mutations in the sequence of protease and reverse transcriptase genes.
Methods: A total of 43 patients suffering HIV-1 infection have been genotyped for HIV protease and reverse transcriptase (Trugene HIV-1 genotyping kit, Visible Genetics). Total 23% of them were studied for first failure, 32% second failure, and 37% third failure. The remaining 8% were pregnant women. All sequences were subjected to 11 interpretation systems using as reference the software from Visible Genetics.
Results: The 11 algorithms tested for the 15 different drugs gave the same results in more than 80% of the cases for AZT, 3TC, NVP, DLV, EFV, SQV, RTV, NFV and LPV; 54% for ddI; 55% for ddC; 59% for d4T; 53% for ABC; 79% for IDV and 71% for APV. The most similar algorithm to Visible Genetic's was Retrogram with a 82.02% concordance, and the lesser was ADRA with 53%.

Conclusions: There are important differences in interpretation to resistance to ddI, ddC, d4T, ABC and APV, likely because the mutations that give resistance to these drugs may not be yet well established. In general, there is not a good matching between algorithms and Visible Genetic's. Interpretation is still the main problem for the genotypic tests of resistance.

P669 Prevalence of genotypic resistance in HIV-naïve patients

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Objectives: The aim of this retrospective study is to asses the prevalence of primary resistance to antiretroviral drugs, both nucleoside analogs and protease inhibitors in untreated patients from Spain, and to determine their possible relation with several epidemiological variables.
Patients and methods: One hundred and twelve samples belonging to the same number of patients were processed by means of the genotypic technique line probe assay (LiPATM) in order to study the presence of mutations at codons 41, 69, 70, 74, 184 and 215 of reverse transcriptase gene (LiPA RTTM) and at codons 30, 46, 48, 50, 54, 82, 84 and 90 of protease gene (LiPA PTM). Patient's epidemiological variables, which are relevant in HIV infection, were also analyzed.
Results: The successful amplification rate was 79.5% for LiPA RTTM and 89.3% for LiPA PTM. In case of LiPA RTTM, statistical significance ($P < 0.05$) was observed when successful amplification was related to sex ($P = 0.04$) and to viral load level ($P = 0.005$). Global prevalence of primary resistance was 12.5%. Primary mutations in reverse transcriptase gene were found in two samples (2.2%), being M41L, K70R and M184V in one of them and K70R in the other. With LiPA PTM, primary mutations were detected in 12.0% of cases, V82A being the most frequently detected mutation (11/12, 91.6%). In one case (8.3%), mutation M46I was detected. Primary mutation V82A was found alone in eight samples (66.6%) and together with the secondary mutation I84V in three samples (25.0%). No statistical significance was found for any of the epidemiological variables.
Conclusions: As for amplification, the profitability of this technique should be improved, because problems were found in a high percentage of samples. The prevalence of resistance detected is around the mean of that found by other authors.

P670 Selective genotype mutations elicited by nelfinavir (NFV)-based antiretroviral therapy (ART): frequency, relationship with other codon mutations, and consequences on subsequent treatment options

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Objective: To assess the frequency, ART background, and outcome of the occurrence of the specific protease gene mutations at codons 30 ± 88 among HIV-infected patients (p) receiving the protease inhibitor (PI) NFV.
Methods: Among 1017 p treated with ART since January 2000, 233 p who received NFV for >6 months ensuring a >90% adherence were considered. Virologic or immunological failure prompted viral genotypization in 68 cases (29.2%): the protease gene mutations were assessed according to eventual previous ART, subsequent ART modification, and laboratory follow-up.
Results: Of 233 p treated with NFV, 36 started NFV when naïve to ART, 41 were naïve to PI and non-nucleoside reverse transcriptase inhibitors (NNRTI), 84 at their first switch of PI- or NNRTI-based ART, and 72 p after two or more ART changes. On the whole, only 3 p of 233 (1.3%) developed the isolated D30N mutation (with or without the N88D), concurrently with virologic failure (as expressed by a rise of $>1 \log_{10}$ of HIV-RNA levels). All cases occurred among the 77 ART- and PI- or NNRTI-naïve p, and prompted an ART change, including a different PI in 2 p, a NNRTI in 1 p, and concurrent switch of ≥ 1 nucleoside analog: the 6–15-month follow-up showed a favorable virologic response (undetectable viremia in 2 p, and a $1.8 \log_{10}$ drop of HIV-RNA in the remaining p). On the other hand, all the 156 p with one or more failures of triple ART had a broad and varied resistance spectrum, including multiple cross-mutations impairing the activity of most PI, but failed to present the D30N mutation ($P < 0.04$ compared with the 77 p who never received PI and/or NNRTI).
Discussion: In our series, the specific primary genotypic mutations conferring resistance to NFV had a very low frequency, and were limited to p who were never exposed to PI- or NNRTI-based ART before starting NFV, while failure of second or rescue NFV-based ART was due to a growing spectrum of PI cross-resistance, excluding those at codons 30/88. The specific primary NFV-resistance profile ($D30N \pm N88D$) distinguishes NFV from other PI, and allows its use in either first-line or subsequent ART, although NFV efficacy is often reduced when salvage therapy of p treated for a long time with PI is of concern (and a broad spectrum of protease mutation are expected, such as those involving codons 90, 36, 46, 48, 71, 77, 82, 84). Since most of the shared PI-evoked mutations are absent when NFV is used as first-line PI, the limited cross-resistance frequently allows successful second-line or salvage PI-based ART.

P671 Comparison of Organon Tekknica nucleic acid sequence-based amplification and Roche Monitor assay for HIV-1 viral load quantification

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Murcia, E

Objective: To compare the performance of Organon Teknika nucleic acid sequence-based amplification (NASBA-QT) and the Roche Amplicor Monitor assay (AMPLICOR HIV-1) for monitoring HIV-1 RNA levels.
Methods: Plasma samples were obtained from adult patients. Plasma was separated from anticoagulated blood within 2 h of collection in EDTA and stored at −70 °C until analyzed. All samples were tested in the first thaw. Both the NASBA-QT and AMPLICOR HIV-1 assays were performed following the manufacturers' instructions. For the correlation analysis, the Spearman's correlation coefficient was calculated.
Results: Quantitative HIV-1 RNA analysis was performed on plasma samples from 70 HIV-seropositive patients. Log-transformed HIV-1 RNA values that were above the levels of detection limit of both assay ($N = 65$) were compared. There were 52 and 54 positive samples in AMPLICOR HIV-1 and NASBA-QT, respectively. There were 41 samples positive by both methods, 11 samples were positive only by AMPLICOR HIV-1 and 13 were positive only by NASBA-QT. The quantification values obtained in the 41 positive samples by both assays were significantly correlated with one another (Spearman's correlation coefficient of 0.741).
Conclusion: This study showed a good correlation between the results obtained with the two assays.

P672 The trend of plasmatic HIV-1 viral loads: a way to assess evolution of efficacy of antiretroviral therapy in a large population

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Introduction and objectives: HAART was introduced and generalized in Spain in early 1997. Since then, our patients have benefited clinically from these regimens. New and simple methods to estimate the evolution of large populations with HIV infection in the era of HAART are now necessary. Our aim was the evaluation of HIV-1 viral load trends since 1996, just before the introduction of HAART, to the present.
Methods: We included data from 22 970 viral load tests. Plasmatic viral loads were determined by the QUANTIPLEX HIV bDNA system (Bayer, Germany), according to the manufacturer's instructions. Six different viral load segments were established in order to analyze chronological trends (<500, 500–10 000, 10 000–30 000, 30 000–100 000, 100 000–500 000, and >500 000 copies/mL). Additionally, frequency of poor evolutions (rise in more than 0.5 log or maintenance over 20 000 copies/mL) was also considered for followed-up patients.
Results: During 1996, frequencies from all viral load levels, with the exception of the highest (>500 000 copies/mL, 2.7%), were homogeneously distributed from 13 to 25%. From 1997 to the present, the most represented segment was the lowest (<500 copies/mL) with frequencies ranging from 45% to almost 60%, followed by the second level (500–10 000 copies/mL), with around 20%. The remaining segments always accounted for less than 10% each. The only segment whose frequency increased over time was the first one (<500 copies/mL), while frequencies for all remaining levels were clearly decreasing. When considering follow up of treated patients,

poor evolution rate was kept almost constant throughout the period (average 23%).

Conclusion: Viremia has been much reduced in our HIV-positive patients since the introduction of HAART. This response has been globally maintained over the last 5 years and values are slightly tending to the lowest viral load level. The mentioned approach with easy-to-measure parameters has permitted a rapid estimate of the efficacy of HAART in a large HIV population.

P673 HIV-1 plasma virus load is increased in HIV-infected women with vulvovaginal candidiasis and *C. trachomatis* cervical infection

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Background: Sexually transmitted infections (STIs) increase transmissibility to HIV-1 and are associated to increased HIV-1 viral loads in genital (semen and cervicovaginal fluid) specimens. There is still unproven association between STI and increase in plasma viral loads of HIV-1. Plasma virus load is a major risk factor for progression to AIDS and death.

Objective: To define biological variables associated to increased prevalence and incidence of vulvovaginal candidiasis (VVC) and *Chlamydia trachomatis* (CT) infection among women with HIV infection.

Methods: Prospective study of HIV-infected women at the Infectious and Tropical Diseases Clinic in Brescia, baseline prevalence and cohort incidence of VVC and CT have been assessed. CVV was diagnosed by direct observation of Gram-stain preparations and culture from vaginal samples. CT was searched in cervical swabs by the LCR technique. Incidence rates were calculated using the person/month method. Association between CVV and CT with demographic and biological variables was performed using multivariate logistic regression, and a Cox regression model.

Results: From January 1998 to July 2001, 192 women were enrolled. The median age was 35.6 years (SD + 6.1 years), 14.6% were immigrants from Africa. The route of acquisition of HIV was drug addition (IVDU) and heterosexual intercourse in 44.8 and 42.2%, respectively. Total 28.6% had CD4 cell count below 200, and in 20.4% HIV-RNA levels were over 10 000 copies. A diagnosis of AIDS had been done in 25%. The baseline prevalence of CVV and CT was 14.6 and 9.6%, respectively. Among 106 women who were followed for a mean period of 19.3 months, the incidence of CVV was 12/100 women/year (29 new episodes). Among 86 women who were followed for a mean period of 18.5 months, the incidence of CT was 5/100 women/year (seven new cases). In the multivariate logistic regression model, incidence of CVV and CT was not related to age, clinical stage or CD4 cell counts. However, the probability of HIV-RNA to be above 10 000 copies was significantly higher among women with CVV at baseline (OR = 2.63; 95% CI, 1.03–6.68; $P = 0.04$) and with new CVV episodes (RR = 3.16; 95% CI, 1.31–7.61; $P = 0.01$) and new CT infections (RR = 11.8; 95% CI, 1.33–104; $P = 0.02$).

Comments: We report a significant association between incidence of CVV and CT and plasma HIV-viral loads. Whether CVV and CT are the cause or a consequence of increased viral loads need to be further investigated.

P674 HIV viral load in blood and cervicovaginal secretions in pregnant women

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Objective: To relate viral load in blood with viral load in cervicovaginal secretions in pregnant women.

Methods: The viral load in blood and cervicovaginal secretions was studied in 33 HIV-infected pregnant women at their first visit (initial viral load) and at 38th week of pregnancy (final viral load) with Monitor HIV System (Roche®). If an inhibition of reaction occurred the viral load was detected by bDNA (Quantiplex System 340 Bayer®). We considered as undetectable an HIV viral load <100 copies/mL.

Results: Pregnant women that showed a final viral load of <100 copies/mL, the vaginal and cervical viral load was undetectable too. Only one patient had a final vaginal load of 220 copies/mL. Pregnant women with final viral load of >100 copies/mL showed highest rates of undetectable viral load in vagina (initial undetectable viral load rate of 82.4% vs. final undetectable viral load rate of 92.9%) and endocervix (initial undetectable viral load rate of 58.8% vs. final undetectable viral load rate of 78.6%). Cesarean was the main delivery way and only one patient had a vaginal delivery with an undetectable viral load in vagina and endocervix. There were no perinatal transmission.

Conclusions: The viral load in blood does not predict the viral load in cervicovaginal secretions. This situation has an important significance when the viral load in cervicovaginal secretions is detected in pregnant women with undetectable viral load in blood.

P675 Treatment of pregnant women infected with HIV-1: study on treatment interruption during the first trimester of pregnancy

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One of the treatment recommendations for pregnant HIV-1-infected women is the maintenance of effective antiretroviral drugs when pregnancy occurs. The maternal benefit is indisputable, the risk of perinatal HIV-1 transmission is minimum but what about the toxicity for the newborn? The purpose of our study is to analyze another option chosen by Toulouse University Hospital in France. If a clinical, immunologic and virologic status of woman is good when pregnancy occurs, any antiretroviral therapy is stopped during the first trimester (we avoid the period of embryo-fetal toxicity). The ZDV and 3TC is introduced at 24th, 28th or 32nd week of gestation. The method used consisted of reporting the different therapeutic regimen before and during the pregnancy and to note the rate of CD^4 and the viral load in the beginning of pregnancy and at the delivery. Data have been analyzed on Excel tab computer to extract the results. A total of 40 women had an antiretroviral therapy in early pregnancy. A total of 38 patients stopped their treatment during the first trimester then followed this therapeutic regimen (23 women had ZDV + 3TC, 4 ZDV, and 11 ZDV + 3TC + NVP). At the delivery, the rate of CD^4 of 27 patients had increased and the viral load of 31 women had decreased, including 28 to a significant degree (i.e. a variation with >0.5 log). A total of 22 patients had their CD^4 count increased and their viral load decreased at the same time. This study shows that stopping of the therapy during the first trimester of pregnancy maintained the viral load of <10 000 copies/mL (i.e. risk of perinatal HIV-1 transmission reduced) in 32 cases over 38. Further, this interruption treatment had no impact on the health of the mother during the pregnancy but its necessary to supervize the immunovirological marker for a long duration. The lack of data on antiretroviral fetal toxicity should incite us to minimize the exposure of the embryo and fetus to these molecules. The treatment interruption programmed in the first trimester could be one of the strategies. But, if its impossible to stop the therapy because of the bad health of the mother, that would be interesting to proportion in the placenta and the cord the antiretroviral drug to have data on the human passage placenta.

P676 Kaletra treatment in patients from a Neapolitan area who failed previous HAARTs

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Background: HAART was introduced in Italy 5 years ago; until today many patients (pts.) failed previous PI- or NNRTI-based treatments. Lopinavir/ritonavir (Kaletra) is a new PI combination that has shown to be effective in experienced and naive HIV-infected patients.

Aim: To evaluate the safety, tolerability and the efficacy of combinations containing Kaletra in multiexperienced anti-HIV-positive patients.

Methods: We enrolled in an early access program 33 consecutive anti-HIV-positive patients, who failed at least two previous HAARTs observed, as outpatients from December 2000. The median observation period was 32 weeks (range 4–38). Of the 33 enrolled patients, 26 were male; the median age was 36 years (range 31–63). All CDC clinical groups were represented (A1: 4 pts.; B2: 12 pts.; B3: 5 pts.; C2: 1 pt.; C3: 11 pts.). Twenty-eight patients received Kaletra + 2 NRTI and five patients received Kaletra + 1 NRTI + 1 NNRTI. In all the patients, the following were performed every 4 weeks: clinical examination, viral load, CD^4 cell count, liver, pancreas and kidney function tests, hematology, cholesterolemia and triglyceridemia. The

efficacy of Kaletra was evaluated using 'on-treatment' analysis. Adherence to treatment was evaluated at the fourth week of treatment using a standardized questionnaire (QL0721 modified).
Results: No new AIDS defining event has been observed during the treatment. Thirty-one patients but two well tolerated the Kaletra-based regimens. One anti-HCV-positive patient who received an NNRTI-containing regimen discontinued treatment for hypertransaminasemia. A hypersensitivity syndrome probably due to abacavir occurred in the second patient. Abnormalities of kidney and pancreatic function tests were not observed. The adherence to treatments was high (88%). No significant modification in cholesterol, triglycerides and glucose levels were observed during treatment. The mean $\pm$ SD CD^4 cells number raised from 233 ± 201 at baseline to 584 ± 380 at 32nd week. A 3 log decrease of HIV viral load was observed from baseline to 28th week (5.17 ± 2 and 2.01 ± 1.05, respectively). The prevalence of patients with undetectable viral load was 55% at the 4th week, 78% at the 16th week and 89% at the 28th week.
Conclusions: Our ongoing data show that the Kaletra-containing regimens are effective, tolerated and safe in multiexperienced patients. Adherence to treatments has been high. Thus, we suggest to extend the use of Kaletra in PI naive patients.

P677 Correlations between HIV subtypes and antiretroviral treatment failure

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Objective: To correlate genotype with response to antiretroviral treatment.
Methods: Study was performed on outpatients in Chelsea & Westminster Hospital, London, UK. Genotype with quantitative phenotypic analysis and subtyping were performed in patients who had experienced ART failure. Treatment history (antiretroviral combinations, length and switches) and demographic parameters were recorded for each patient.
Results: We analyzed 45 patients (15 November 2000–15 March 2001), who presented antiretroviral resistance for the clinical, immunologic and virologic point of view. The average age of patients studied was 34.5 (range from 28 to 58 years). Forty-one of 45 patients were men, out of which 38 were men having sex with men. For the studied patients, the average length of antiretroviral treatment was 7.13 (range from 1 to 11 years). The duration and composition of antiretroviral treatment that had been used were similar among the subtypes. We found next subtypes B ($n = 35$), A ($n = 5$), C ($n = 4$), and D ($n = 1$). After analysis of genotyping and phenotyping assays, we observed that most of the patients (26) had NRTIs and NNRTIs resistance and only 10 patients presented resistance to all three classes of antiretrovirals. We found only one patient with virus resistance to all drugs from all three classes. We noticed that there are some mutations that are significantly more prevalent in non-B subtype, comparative with subtype B (in RT: V106A, $P = 0.016$, G190A/I, $P = 0057$). Conversely, some other mutations are more frequent in B subtype comparative with non-B subtype (in PR: L90M, $P = 0.004$, M36I, $P = 0.006$, V77I, $P = 0.0001$; in RT: Y181C, $P = 0.0007$, M41L, $P = 0.0001$, T215Y/F, $P = 0.01$). Some specific cluster was more prevalent in patients with B subtype comparative with non-B subtype (in PR: M36I + V77I + L90M, $P = 0.004$).
Conclusions: To interpret the different mutations correctly, it is necessary to consult an expert in viral resistance and/or continuously actualized databases, e.g. Stanford University, AlamosResistanceDB and VircoDB in daily routine. The presence of distinct genetic patterns among HIV subtypes in patients who experienced antiretroviral failure emphasizes the clinical need to advance our understanding of drug resistance in different subtypes and further clinical investigation is warranted.

P678 HIV-subtypes analysis in the *pol* gene sequence

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Objective: To know the HIV-circulating subtype using the *pol* gene sequence and to compare the results with three different alignment systems.
Methods: We have studied 43 patients with HIV-1 infection from the Infectious Diseases Unit in our hospital. A fragment of 1.3 kb from the *pol* gene was transcribed and amplified by PCR. In the amplification product, the protease gene (codons 10–99) was sequenced and a part of the reverse transcriptase (codons 41–239) by bidirectional sequencing with primers labeled with Cy5.5 and Cy5.0 in a semiautomatic sequencer (Visible Genetics Inc, Opengene System). The protease and reverse transcriptase sequences were analyzed in a new subtyping method that compares the obtained sequence with the reference sequences for the subtypes, using BLAST algorithm (Basic Local Alignment Search Tool) and phylogenetic analysis (Los Alamos Database). Two other methods for knowing the subtype from the *pol* gene sequence were used (from ABL Networks and Stanford database algorithms).
Results: Subtype B was found in 68% of patients with the Los Alamos Database and in 95% of them using the databases from ABL and Stanford. Los Alamos Database showed mixed subtypes in 21% of the samples. One patient identified as a CRF 02 A–G (circulating recombinant form) by two systems was identified as a B/F/K mixture by Los Alamos Database.
Conclusion: The B subtype is the most prevalent as shown by *pol* gene sequencing. This method may be useful to search for CRF, but this must be confirmed for sequencing longer genomic regions.

CMV, EBV and herpes viruses

P679 Seroprevalence of cytomegalovirus antibodies among various age groups in Konya, Turkey

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Objective: Although cytomegalovirus rarely causes disease in immunocompetent individuals, it can cause severe morbidity and mortality in congenitally infected newborns and immunocompromised patients. Cytomegalovirus infections which are endemic throughout the world are especially more common in the developing countries and in the areas of low socioeconomic conditions. This study was designed to show the prevalence of CMV antibodies (CMV IgM and IgG) among children aged 1 day to 15 years and in adults upper than 15 years of age.
Methods: The antibodies to CMV (IgG and IgM) were analyzed in serum samples of 713 children (357 female, 346 male) and 585 adult (349 females and 236 males) by means of Enzyme-Linked Fluorescent Assay (ELFA) technique (bioMerieux sa, France).
Results: For the overall study population, we found that 2.38% were seropositive for CMV IgM, and 95.9% for CMV IgG antibodies. The seropositivity of CMV IgM antibodies was 3.13% among children aged 0–15 years and 1.78% among individuals over 15 years of age (no statistically significant difference $P > 0.05$). The seropositivity of CMV IgG antibodies was 86.2% among children aged 0–15 years and 97.6% among the individuals over 15 years of age and there was statistically significant difference between the age groups ($P < 0.05$) but no difference between males and females ($P > 0.05$).
Conclusion: Although symptomatic infection is rare most of the children are infected before puberty because the seropositivity rates of CMV IgG antibodies rise to 97.6% after the age of 15. Our findings confirm that CMV is highly endemic in our population.

P680 Prevalence of cytomegalovirus, herpes simplex 1, and herpes simplex 2 in cancer patients with infection

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Objectives: The purpose of this study was determinate in serum of the cancer patients for antibodies IgM, IgG, gBIgG against CMV as well as for IgG, IgM against HSV1 and HSV2.

Materials and methods: Sera from 65 cancer in-patients of the Saints Anargyri Oncological Hospital who showed fever and leukopenia (Group A) as well sera from 15 cancer out-patients of 'Hippokrateion' General Hospital without symptoms of infection (Group B) were tested for the detection of antibodies: gBIgG against CMV and IgM, IgG against HSV1 and HSV2 by the micro-ELISA in plates method (Biotest)—ALPHADIA SA/NN. IgG and IgM of CMV were tested by the ELISA method (AXSYM-ABBOTT).
Results: Of the 65 cancer patients with symptoms of infection, 62 (95.3%) were positive for IgG CMV antibodies; Ten of these patients (15.3%) were positive for IgM CMV. In Group B, all were found to be positive for IgG CVM, while they all were negative for IgM CMV. The gBIgG CMV were positive in all the patients with IgG positive antibodies in both Groups A and B. Of the 65 patients of Group A, 13 (20%) were positive for the IgG HSV1 antibodies and one patient (1.5%) were positive for the IgM HSV1. Of the 15 patients in Group B, 2 (13.3%) were positive for IgG HSV1 and 1 patient (6.6%) were positive for IgM HSV1. As regards HSV2 in Group A, one patient (1.5%) tested positive for IgG HSV2; in Group B, all were negative; and the IgM HSV2 were negative for both Groups A and B.
Conclusion: This study showed that there is a high frequency of CMV in cancer patients (84%) with a great degree of relapse (15.3%) in patients with symptoms of infection. The frequency of HSV1 IgG in cancer patients was found to be high (19%) whereas the frequency of HSV2 IgG was very low (1.5%).

P681 Mononucleosis-syndrome: cytomegalovirus (CMV) infection in immunocompetent adults

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Objective: Cytomegalovirus (CMV) seroprevalence rates in adults range from 50 to 90%. While CMV may cause severe disease in immunosuppressed patients (e.g. AIDS, organ transplant recipients), most CMV infections in immunocompetent individuals remain subclinical. However, about 0.1% of CMV infections in immunocompetent adults cause a prolonged mononucleosis-like illness which is often difficult to diagnose and which can easily be missed. This problem has rarely been addressed in a few case series in the literature.
Methods: We describe the clinical course and the diagnostic features of a nonimmunocompromised patient with acute CMV infection who presented to our tropical medicine outpatient clinic.
Results: A 63-year-old lady presented with a 3-weeks history of recurrent fever with night sweats and general fatigue. On presentation she already started to feel better but was still weak and had night sweats. The clinical examination showed no abnormalities. The patient did not have weight loss. The symptoms had started 1 week after she had returned from a holiday in Tunisia.

CMV serology was positive for IgM and IgG antibodies. CMV was isolated from the urine while plasma PCR and antigenaemia assay were negative. The patient had an abnormal liver function with increased liver enzymes, as well as increased atypical lymphocytes in the blood. Other causes for the elevation of liver enzymes were excluded. The patient made an uneventful recovery within 7 weeks.

We diagnosed five other patients with 'mononucleosis-syndrome' suffering from an acute CMV infection this year. They all presented with a prolonged period of fever (2–6 weeks), elevated liver enzymes and atypical lymphocytes. Besides the clinical findings, diagnosis was based on the presence of CMV IgM and IgG antibodies; virus isolation or PCR have not been performed. They all recovered after a mean period of 50 days.
Conclusion: CMV infection may cause relevant clinical disease ('mononucleosis-syndrome') in immunocompetent adults. It should be considered in patients presenting with a prolonged febrile illness, atypical lymphocytes and increased liver enzymes. The diagnosis is straightforward (serology), so that laborious, unpleasant and expensive workup can be avoided. Further investigations are rarely needed. Careful follow-up is important to detect complications (pneumonitis, thrombocytopenia, meningoencephalitis). In immunocompetent patients treatment is usually symptomatic.

P682 Primary CMV infection in an outpatient setting

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Objective: Despite being common throughout the world, CMV infection is rarely a cause of clinical illness. CMV-related morbidity mostly affects immunocompromised patients (e.g. transplant recipients and HIV-infected individuals) and is probably related to insufficient immunological control of persistent infection. However, primary CMV infection may give rise to severe clinical illness in immunocompetent adults. We retrospectively analyzed cases of acute CMV infection in medical outpatients.
Methods: During a 6-year period, we identified a total of 17 patients who were diagnosed as suffering from primary CMV infection in our medical outpatient clinic. This was diagnosed on the basis of a strongly positive CMV IgM antibody test result, with or without detectable IgG antibodies, together with a febrile illness accompanied by hepatitis. From the medical records we evaluated the presenting signs and symptoms as well as some relevant laboratory results.
Results: At first consultation, all patients (6 female, 11 male; aged between 26 and 58 years) had malaise and fever higher than 38 °C. Cephalgia was present in 10 out of 13 cases (four not known). In seven out of nine patients who underwent abdominal ultrasound investigation, there was a mild enlargement of the spleen. None of the 17 patients had a palpable splenomegaly. Five patients had a mild leukocytosis but all 17 had a relative or absolute lymphocytosis. C-reactive protein was within normal limits in 4 patients and only slightly elevated in the remaining 13. All patients showed elevated levels of AST and γ-GT to varying degrees. In all 15 patients whose LDH levels were determined these were markedly elevated. Seven patients reported travel to areas outside Europe, mostly to tropical areas, within the preceding 2 weeks.
Conclusions: CMV is a relevant differential diagnosis in feverish illnesses accompanied by hepatitis in adults. A substantial proportion of our patients seem to have acquired their CMV infection abroad, so that a diagnosis of CMV infection needs to be taken into account in travelers, in addition to infectious illnesses more commonly considered in this context such as dengue or hepatitis.

P683 Hemorrhagic pericarditis due to cytomegalovirus (CMV) presenting as cardiac tamponade in a noncompromised patient

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Background: Isolated CMV infection of the pericardium is extremely rare in patients with an intact immune system.
Results: A 45-year-old black woman presented with progressive dyspnea, cough, and fatigue for weeks. Adult-onset diabetes mellitus and hypertension were well controlled. An extensive pericardial effusion was noted on spiral chest computed tomography scan. Echocardiography revealed normal left ventricular systolic function and diastolic collapse of the right ventricular free wall, cardiac tamponade. She was afebrile, and had distant heart sounds on examination. Laboratory studies showed the following: WBC 8.7 K/μL, CD3+4+ (42.6%) 760 cells/μL, platelets 574 K/μL, AST 44 U/L, CMV IgG 102 IU, and ESR 61 mm/h. An emergent pericardiocentesis revealed 1300 cc of blood colored pericardial fluid with RBC 3400 K/mm^2, WBC 999/mm^2, neutrophils 64%, lymphocytes 13%, monocytes 21%, and glucose 111 mg/dL. She underwent localized pericardectomy 4 days later due to reaccumulation. A negative workup included HIV I and II antibodies (Ab), HTLV I and II Ab, antinuclear Ab, native DNA Ab, anti-SM Ab, rheumatoid factor, CMV IgM, and urine H. capsulatum antigen. Her complement levels were normal (C3, C4, CH50). Pericardial fluid and blood cultures remained sterile, and negative for CMV and *M. tuberculosis* by DNA amplification analysis. Histologically, organizing hemorrhage, prominent lymphocytic stromal infiltration, and characteristic CMV intranuclear and cytoplasmic inclusions were abundant within the reactive fibroblasts throughout the pericardial thickness. Her infection resolved after ganciclovir 5 mg/kg every 12 h for 2 weeks, than every 24 h for another 2 weeks. On 12-month follow-up no recurrence was noticed.
Conclusions: Cytomegalovirus reactivation and end-organ infection is rare in individuals with intact cellular immunity. In contrast, to the common viral (enteroviruses) pericardial infection, CMV pericarditis is often subacute and

may be considered as a potentially treatable cause of life-threatening complication in this setting.

P684 Mononucleosis infectious-bacterial super infections

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Introduction: Infectious mononucleosis is a common expression Epstein-Barr virus infection. Clinical manifestation includes the classic tread of fever, pharyngitis and cervical lymphadenopathy as well as lymphocitosis with a predominance of a typical lymphocytes. The diagnosis is based on the clinical, hematological and serological data. The EBV infection with a clinical expression is usually associated with the group of adolescent population, but with a significant increase of the frequency in young children.
Material and methods: The 4 year long period reporting (1997–2000) includes 38 patients with infectious mononucleosis. The bacterial super infections were the subject of our interest. Membranous tonsillitis occurred in 34 patients (91.9%), peritonsillar abscess in 6 (16.2%), otitis media 5 (13.5%), pharyngotracheitis 11 (29.7%), bronchitis 9 (24.3%) and pneumonia 7 (18.9%). Bacterial super infection was evident and in 31 of the cases confirmed with the most frequent isolation of *Streptococcus beta haemoliticus*—23 isolates (71.3%), *Pneumococcus*—5 (16.3%), *Staphylococcus aureus*—3 (9.4%). From the thought swab/sputum sample.
Conclusion: Infectious mononucleosis is a frequent expression of the primary EBV infection in the childhood and adolescence, especially among patients with a chronical/recidivant tonsillitis. All patients were commonly treated for the basic illness with a complementation of antibiotics if necessary based on the antibiogram.

P685 Evaluation of the Platelia Epstein-Barr virus viral capsid antigen (VCA) IgM, VCA IgG, nuclear antigen-1 (EBNA-1) IgG, and early antigen diffuse (EA-D) IgG ELISA assays

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Commercial enzyme-linked immunosorbent assays (ELISA) for the specific serological diagnosis of Epstein-Barr virus (EBV) were developed based on affinity-purifed viral capsid antigen (VCA; gp125), or recombinant polypeptides consisting of the carboxyterminal 200 amino acids of EBV nuclear antigen-1 (EBNA-1) or early antigen (diffuse-type) (EA-D). We evaluated the performance of the Platelia EBV VCA IgM, VCA IgG, EBNA-1 IgG, and EA-D IgG ELISA assays on a panel of 160 clinical samples (33 EBV nonimmune samples, 43 acute or late infection EBV samples, 40 EBV-reactivations or chronic, persistent EBV infections, and 44 EBV-immune samples). For the Platelia EBV VCA IgM assay, a sensitivity of 97.0% was obtained and a specificity of 100%. For the Platelia EBV VCA IgG assay, sensitivity was 100% and specificity was 97.4%. The Platelia EBNA-1 assay showed a sensitivity of 93.8% and a specificity of 98.8%. The Platelia EA-D assay had a sensitivity of 81.7%, and a specificity of 90.7%.
Conclusion: The Platelia EBV assays provide the means to classify EBV-samples into clinically relevant categories, allowing for standardization and automation of EBV-specific serology.

P686 Studies on association of Epstein-Barr virus (EBV) and human papilloma virus (HPV) with tongue carcinoma

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Objectives: At present, not only HPV but also EBV are thought to oncogenesis in epithelioid malignancies. The aim of this study was to evaluate whether EBV or HPV might be involved in cases of cancers of the tongue.
Methods: The studies were performed on 14 patients, aged 43–66 years, with histopathological diagnosis of squamous epithelial cancer of the tongue. Sera of the patients were tested for IgG-anti-EA antibodies (ETI-EA-G; DiaSorin), IgG-anti-VCA antibodies (ETI-VCA-G; DiaSorin) and IgG-anti-EBNA (ETI-EBNA-G; DiaSorin). In parallel, DNA extracted from cancer material was tested for the presence of EBV DNA and HPV DNA (Sharp Signal System; Digene). In addition another technique for HPV 16 and HPV 18 detection by PCR amplification was employed.
Results: Basing on serological markers, in 13 patients an earlier experienced EBV infection was documented (serum IgG-anti-VCA, IgG-anti-EBNA antibodies). In 12 of the patients, presence of EBV DNA was identified and, among the latter patients, two exhibited a mixed EBV/HPV infection. In no case could HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 or 58 be identified.
Conclusion: The results indicate that EBV may be involved in tongue cancer aetiopathogenesis.

P687 EBV hepatitis: clinical aspects

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Objectives: To investigate the clinical presentation, laboratory data and course of hepatitis with EBV etiology.
Methods: A total of 30 patients (26 males, 4 females) hospitalized in the Infectious Diseases Hospital during the period 1998–2001 were included in the study. The clinical signs and symptoms, liver disorders biochemical markers and serological data were analyzed. The diagnosis was confirmed on the presence of anti-EBV VCA IgM.
Results: The age of the patients ranged from 5 to 40 years, mean 19.60. Greater than 21 years were 26.6%. A great variety of symptoms was find: fever, headache, pharyngitis, mialgia, anorexia, nausea, vomitings, malaise, lymphadenopathy, periorbital edema, papular red and petechial rash, jaundice, hepatosplenomegaly. The course with moderate severity was with 86.66%,with levels of bilirubinemia from 17 to 272 M/L, increased serum aminotransferases activities (ASAT 60–1159 μ/L, ALAT 94–1140 μ/L) and high levels of GGT (147–1419 μ/L and AP/207–2007 μ/L). The complete blood count and differential showed atypical lymfocytes with all persons. Thrombocytopenia was with 10%. Antibody test indicated with all persons recent infection with EBV. An anicteric course was registered with 12 persons. Continuous subfebrility, extreme splenomegaly and lymphadenopathy was with 1 p and diagnosis was performed by lymph node biopsy. Protracted course was registered with 3 p. The patients were treated from 7 to 42 days. Hepatoprotective drugs, antibiotics, corticosteroides were administered. Clinical and laboratory checks 1 and 3 months after the acute phase revealed normal aminotransferases activities.
Conclusions: EBV hepatitis is diseases with moderate severity, with no transition to chronic hepatitis, but the versatility and variability of the EBV infection is a challenge for the differential diagnosis.

P688 Anti-herpetic action of the interferon inducing yeast RNA-tilorone molecular complex

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Antiviral effect of a yeast RNA—tilorone molecular complex (MC) has been studied in mice infected by herpes simplex virus type I (HSV-1). A specific antiviral MC effect has been estimated from the animal survival rate, the HSV-1 level being determined by a CPR approach. The MC has been found to prolong the animal life duration, the levels of HSV-1 reproduction in serum and brain tissue having become lover on the second day p.i. by 1.75 $\lg \pm 0.07$ and 1.08 $\lg \pm 0.02$, respectively, and by 2.08 $\lg \pm 0.05$ and 1.47 $\lg \pm 0.02$, respectively, on the 5th day p.i. The others conclude the MC to be a promising antiviral compound possessing antiherpetic action.

P689 Detection of Epstein-Barr virus (EBV) by PCR assay in biopsy samples from pediatric patients with Hodgkin's disease in Spain

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Background: Epstein-Barr virus (EBV) is associated with some cases of Hodgkin's disease (HD). EBV has a pathogenic role in HD by inhibiting apoptosis or allowing cells to escape from cytotoxic T lymphocytes response. Detection and typing of EBV in patients diagnosed with HD has epidemiologic relevance.
Objectives: To analyze biopsy samples from HD pediatric patients for EBV DNA by PCR assay and immunohistochemistry (IH) techniques. To carry out EBV typing in EBV-positive samples. To investigate the presence of several proteins related to the cellular cycle and apoptosis. To correlate results with epidemiological features.
Patients and methods: Seventeen pediatric patients (aged up to 21 years) diagnosed with HD between 1989 and 2000 were included in the study. Detection of EBV was performed in DNA extracted from paraffin-embedded biopsies by PCR assay (*Bam*HI L region). EBV typing was carried out by nested-PCR (*Bam*HI WYH region) and subsequent 2% agarose gel electrophoresis. Detection of LMP-1, EBNA-1, p53, p21CIP/WAF1, p27KIP1, bcl-2 and Ki-67 (MIB-1) was performed in neoplastic cells (Hodgkin and Reed-Sternberg) by IH using specific monoclonal antibodies.
Results: The mean age of patients was 14.5 years (range 5–21 years), nine were male and eight female. The HD histiotypes found were: nodular sclerosis (12), mixed cellularity (2), interfolicular (1) and unknown (2). EBV was detected in 8/17 patients (47.05%). Six patients carried EBV-A and in two patients EBV was untypable. Distribution of EBV positivity according to clinical HD stage at the time of diagnosis was: I (100%), II (0%), III (66.6%) and IV (75%). Distribution of EBV positivity according to age was: 0–7 years (100%), 7–14 years (50%) and 14–21 years (28.5%). Mortality occurred in 1/9 EBV-negative and in 0/8 EBV-positive cases, respectively. p21CIP/WAF1 and p53 proteins were more often detected in EBV-negative than in EBV-positive HD cases.
Conclusions: Overall, EBV was detected in 47.05% of pediatric HD patients. EBV-A was the only subtype found. The highest rate of EBV positivity was found in patients under 7 years of age. The different patterns of protein expression and EBV status found are discussed. The differences in EBV positivity found in the different age groups should support the hypothesis of the multifactorial etiology of HD.

P690 Herpes simplex virus as agent of central nervous system infections

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Herpes simplex virus (HSV) is one of the most common agents of encephalitis in otherwise healthy older children and adults. HSV encephalitis (HSVE) is a severe disease associated with significant morbidity and mortality. The aim of this study was the evaluation of PCR for detection of HSV DNA in cerebrospinal fluid (CSF) samples, in parallel with serological analyzes of simultaneous drawn serum and CSF samples in the diagnosis of suspected encephalitis or meningoencephalitis cases.
Methods: Fifty-two paired samples (serum and CSF) from 48 patients (29 males, 19 females) with suspected encephalitis or meningoencephalitis aged 2 months to 65 years old (24 children, 24 adults) were studied. All patients were admitted to the hospital with neurological symptoms between June 1998 and November 2001 (approximately 3.5 years). CSF analysis from all patients revealed leukocyte count and protein levels mild elevated. PCR assay was used to detect HSV DNA in CSF samples. Primers from the thymidine kinase gene were used yielding a 320-bp amplicon. Serum and CSF samples obtained from the patients were examined for IgM and IgG antibodies against HSV by enzyme-linked immunosorbent assay.
Results: Nine cases of HSVE (seven adults, two children) were diagnosed by positive CSF-PCR (rate 18.8%) and eight of them by serum and CSF serology; one had HSV IgM and IgG in serum and IgG in CSF, one had IgM and IgG only in serum, three had only IgG in serum and CSF and three had IgG only in serum. In one patient (infant 4.5 month) CSF was positive by PCR, but both serum and CSF were HSV antibody-negative. In three patients, PCR failed to detect HSV DNA in the CSF samples, but IgM and IgG antibodies were detected only in the serum. Thus, in nine patients, the diagnosis of HSV encephalitis was confirmed, whereas in the other three patients, the HSV encephalitis was suspected but it was not confirmed.
Conclusions: Specific diagnosis of HSVE was possible by a combination of PCR and detection of HSV antibodies in serum and CSF samples, since most forms of viral encephalitis show similar clinical findings. However, serial CSF samples should be examined from patients with clinically suspected HSVE for both PCR and intrathecal HSV antibody analysis.

P691 Diagnosis of herpes virus and enterovirus central nervous system infections in cerebrospinal fluid samples using genetic amplification techniques

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Background: Herpesviruses are associated with central nervous system infections, being herpes simplex virus the most common cause of sporadic acute encephalitis. Early diagnosis of herpesvirus infections allows accurate therapy and reduces mortality. Enteroviruses are also involved in sporadic viral meningitis and meningoencephalitis.
Objectives: To investigate the role of herpesviruses and enteroviruses in central nervous system infections in patients from our geographical area.
Patients and methods: One hundred and six cerebrospinal fluid samples (CSF) from 101 patients with suspected herpesvirus encephalitis (HE) were collected between February 1998 and April 2001. Sixty-seven were male and 34 female; their mean age was 45.6 years (range 6–87 years). Seventy-one were immunocompetent patients and 30 were known to be infected with HIV. HE was suspected on clinical findings, imaging techniques (EEG, CT scan, MRI) and CSF analysis. CSF samples were tested for herpesvirus DNA (herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus 6 (HHV6) and Epstein-Barr virus (EBV) by multiplex nested-PCR (Radar-REAL, Spain). Enterovirus investigation was carried out using the NucliSens Basic Kit system (Organon Teknika, Boxtel, NL).
Results: Herpesviruses DNA were detected in 25/101 patients (24.75%). Fourteen of them were immunocompetent patients: HSV ($n=9$), HSV + EBV ($n=3$), CMV ($n=1$), VZV ($n=1$); and 11 were infected with HIV: HSV ($n=5$), EBV ($n=4$), CMV ($n=1$), VZV ($n=1$). Herpesvirus positivity rates were: 14/71 (19.7%) for immunocompetent patients and 11/30 (36.66%) for HIV-positive patients. On admission, fever and/or decreased level of consciousness were present in15/25 patients. Imaging techniques supported EH diagnosis in 5/25 patients. Abnormalities in CSF proteins, glucose or leukocyte count were found in 15/25 patients. Mortality occurred in 6/25 patients (20%). Thirty-four out of 69 CSF samples negative for herpesviruses were further screened for enteroviruses. Enteroviruses were detected in 2/34 samples.
Conclusions: Although HSV and EBV were the herpesviruses most often found, other herpesviruses (CMV and VZV) have a role in herpesvirus encephalitis. Enteroviruses testing in CSF samples negative for herpesviruses may allow assessing the involvement of these viruses in CNS infections in our area.

P692 A PCR-assay for detection and species identification of five human herpes viruses

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Objectives: To develop a method for simultaneous detection and species identification of five human herpesviruses (*Herpes simplex* virus (HSV) types 1 and 2, Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus type 8 (HHV8)).
Methods: PCR-based amplification was used for detection of the viral DNAs. DNAs were isolated from virus-infected cell cultures. Species identification was accomplished by restriction endonuclease digestion of amplicons obtained after a second round of PCR with enzymes *Taq*I or *Rsa*I. Restriction fragment patterns were analyzed by agarose gel electrophoresis.
Results: Three highly conserved regions within the DNA polymerase gene were identified according to a multiple alignment of DNA sequences of human herpesviruses generated by the MACAW program. Three consensus primers targeted to each of the three conserved regions were designed. This primer set enables specific amplification of HSV1, HSV2, CMV, EBV, and HHV8. Amplification with P1 and P3 primers yielded an approximately

870 bp long DNA fragment that was used as a template for a second round of PCR with primers P1 and P2. The generation of the final PCR products of expected size (531 bp for HSV1 and 2, 537 bp for EBV, 603 bp for CMV, 517 bp for HHV8) attested to the specificity of the PCR assay. No amplification was found with HHV6 under the given conditions. VZV and HSV7 templates were not tested in the PCR assay, since analysis of the corresponding nucleotide sequences allowed us to make a conclusion that they should not be amplified with P1/P2/P3 primer set. The sensitivity of the penta-specific PCR assay was assessed using serial dilutions of plasmid DNA containing the cloned P1/P3 amplified products of each herpesvirus. For all herpesviruses, the sensitivity of the assay was 1–10 genome copies. Clinical specimens of saliva, serum, blood, endocardial biopsies, urogenial scrabs obtained from public health institutions of different profile will be used for validation of the assay in clinical application.
Conclusions: A herpesvirus penta-specific consensus PCR assay coupled with restriction enzyme digestion, which allows one to identify five human herpesviruses, was developed. This PCR assay is a specific, sensitive, and cost-effective method for the evaluation of patients with different herpesvirus induced disorders.

P693 Detection of human herpes virus 6 late mRNA using NucliSens (TM) Basic Kit NASBA

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Objectives: The aim of this study was to develop an assay based on NucliSens (TM) Basic Kit NASBA for identification of clinically relevant human herpesvirus 6 (HHV6) infection in immunocompromised individuals. Such an assay would be useful in the management of post-transplant infections and enhance our understanding of HHV6 pathogenesis.
Methods: Kit-based reagents were utilized for extraction, amplification and detection of amplified products (NucliSens (TM) Basic Kit, bioMérieux (Ltd.). The primers and probe were designed to amplify and detect the HHV6-specific late gp105 mRNA. Detection of amplified products was by electrochemiluminescence (ECL). Titrated HHV6 variant A (U1102) and B (Z29) reference viruses, synthetic RNA (prepared by cloning of the target and in vitro transcription) and a range of blood and respiratory specimens ($n = 100$ to date) were utilized to evaluate the assay.
Results: The assay proved to be very sensitive and specific. Less than or equal to 100 HHV6 synthetic RNA copies were detectable in the assay with no amplification from purified HHV6 DNA. Variant A and B viruses were picked up with equal efficiency and there was no cross-reaction with closely related herpesviruses. Extracts prepared for the cytomegalovirus pp67 assay were suitable for HHV6 NASBA allowing significant infection with either or both viruses to be assessed in a single sample.
Conclusion: The HHV6 NASBA will prove useful in identification of patients with active infection and will help in studies of the association of this common betaherpesvirus with significant disease.

P694 Incidence of *Herpes zoster* ophthalmicus 1996–2000

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Aim: To present the clinical expression of this disease in its different forms, the possible complications on the eye which may led to serious and lasting damaging of the function of seeing as well as the therapy of this diseases.
Material and methods: A total of 55 patients have been examined and treated within a period of 5 years in 1995–2000.
Methods of examination: Anamnesias, clinical expression, blood test, dermatology examination, examination of the anterior segment of the eye with and diffuse light, biomicroscopic examination, ophthalmological examination of the fundus of the eye.
Results: In 23 cases, the disease went on with symptoms as blepharoconjunctivitis, photophobia, epiphora, a more expressed, mixed injection of the eye, but without recidives or serious complications. In 18 cases, keratitis developed which was cured with the therapy that was applied. There were nine cases of recidivent keratoconjunctivitis, and in five cases a inflammation of the optic nerve developed (neuritis nervi optici).
Conclusion: This is a serious disease with possible very difficult complication on the eyesight. The patients are usually at the age of 50–70 years with equal distribution of the sexes.

Hemorrhagic fevers

P695 Immunological characteristics of eye lesion in hemorrhagic fever with renal syndrome

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The pathogenesis of hemorrhagic fever with renal syndrome (HFRS) suggests the possibility of involvement of the optical analyzer into the pathological process, about which testifies often revealed symptoms such as: optic weakness, eye pains sclera vessels and conjunctive injections. In 25 patients with HFRS, serious and grave condition was observed. In all cases, HFRS diagnosis was confirmed in MFA reactions by increasing titers of specific antibodies to four and more times. All observed patients were men aged 18–45 years. In this condition, we carried out the analysis of subpopulation of peripheral blood lymphocytes and the presence of tissue-specific humoral and cellular response to basic antigens of cornea (bovine corneal protein-54), lens (L-crystalline) and retina (S-antigen). Immunotypation was performed by indirect immunofluorescent method with monoclonal antibodies usage. Antibodies to antigens were revealed in steady phase UFA, using highly purified antigens as immunosorbents. Cellular response was evaluated in 5 days 'RBTL' adding the antigens in (10 mko/mL culture) dose. During acute period (5–14 days of disease), we revealed the reduction of the general number of T cells (CD^{3+}), the increase in CD^{8+} lymphocyte number (suppressors (STA)) and the shift of immunoregulator factor to reduction. The number of mature B-cells (CD^{72+}) and NK-cells (CD^{16+}) was also increased. None of patients had specific tissue antigens. In 50% of the patients were the signs of cellular sensibilization to BCR-54, in 17% patients to L-crystalline lens. Cellular response S-antigens were not discovered. Within the period of reconvalescence (15–26 days of disease), all revealed disturbances were normalized. Described changes in lymphocyte population were typical for 'acute phase' of virus infections. As for cellular sensibilization to BCR-54 and L-crystalline lens, it can certify the possibility of similar antigen determinants of cornea and other tissues (kidneys, myocardium) which are involved in the pathological process or modifications of antigen of cornea by HFRS virus.

P696 Prostaglandins metabolism in acute renal insufficiency in patients with hemorrhagic fever with renal syndrome

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Renal pathology occupies a specific place in the clinical course and outcome of hemorrhagic fever with renal syndrome (HFRS), its clinical manifestation being acute renal insufficiency (ARI). However, the causes of ARI remain unclear. The purpose of our study was to investigate the character of prostaglandin metabolism in ARI in patients suffering from HFRS. The results of our study revealed a significant reduction in the activity of prostaglandins depressive series in the initial period of HFRS: prostaglandins E_2 value (PGE_2) was 23.67 ± 0.98 pg/mL ($P < 0.001$); 6-keto-prostaglandin F1α value (6-keto-PG F1α) was 26.83 ± 3.74 pg/mL. At the same time, increase of thromboxane B2 (TXB2 345 ± 9.8 pg/mL, $P < 0.001$) which possesses vasoconstrictive and aggregation action was recorded. In oligoanuric period, the observed disturbances went on aggravating: levels of PGE_2 and 6-keto-PG F1α were 7.8 and 7.1 times, respectively, lower compared to control values of these prostaglandins were increased, but still their levels remained 2.3 and 2.0 times lower in comparison with control ones even in the period of clinical convalescence. While studying TXB2 content, it was determined that its value was six times higher in oligoanuric period, the trend towards its reduction was marked in the period of restored diuresis. The correlation index of TXB2/6-keto-PG F1α testified to the prevalence of vasoconstrictive

prostaglandins in blood serum (31.79 ± 0.54 compared to control value of 0.78 ± 0.07). Thus, the obtained data point out to the participation of vasoactive prostaglandins in the pathogenesis of ARI in patients with HFRS. It is disbalance in their correlation that plays a significant role. The results of our study can be regarded as pathogenetic evidence for the use of medicines possessing corrective effect on prostaglandins metabolism in the treatment of ARI in patients suffering from HFRS.

P697 Crime-Congo hemorrhagic fever (CCHF) in Kosova during the period 1995–2001

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Objectives: Presentation of CCHF cases, Kosova (period 1995–2001), analysis done for above-mentioned time frame, with special emphasis during the years 1995, 1996 and 2001 when this disease appeared as outbreak in Kosova.
Methods: Descriptive–retrospective analysis of the data taken from official surveillance system of the Institute of Public Health, Department of Epidemiology, Pristina, from the Clinic of Communicable Diseases, Medical Fakulty of Pristina; and serology data taken from the Mikrobiology Institute, Medical Fakulty, Ljubljana, Slovenia.
Results: The first case of CCHF in Kosova was registered in the village of Nishor of Suhareka municipality in 1954. During 1995–2001 period, 129 confirmed cases were registered, with 21 death cases resulting with fatality rate of 16.2%. CCHF occurred as outbreak during the year 1995 with 65 cases or incidence 3.07/100.000 and seven death cases with fatality rate of 10.7%; during the year 1996 with 23 cases, or incidence 1.06/100.000 and five death cases with 21.7% fatality rate. After a quiet period for 4 years with few sporadic cases in the year 2001, this disease appeared again in epidemic form with 31 cases or incidence 1.44/100.000 and seven death cases with fatality rate of 22.5%. Out of 129 cases of disease in this period, in 74 cases or 57.3% the mode of transmission was tick bite, in 14 cases or 10.8% contact between people was the mode of transmission, and the way of contracting the virus is unknown in 14 cases or 31.7% – scientific explanation is available. The largest number of the confirmed 129 cases in this period is in the age group of 15–45 years with 79 cases or 61.2%. During the time period 1995–2001, 61.2% of the cases were male and 38.8% were female. The largest number of cases as anticipated in all time periods was in the group of farmers 52 cases or 40.5%, housewives 39 or 30.2%.
Conclusions: The territory of Kosova, dating from year 1954, when first cases were registered is endemic zone for this disease. Taking into consideration that Kosova is an endemic–epidemic and epizootic area with CCHF, also with enormous population of HP ticks, permanent surveillance of disease and appropriate prophylactic and epidemic prevention measures were taken. Despite that we can conclude that epidemiological situation in Kosova is uncertain and in the future, we can expect new cases of disease or outbreaks.

P698 Serosurvey of arbovirus among Bedouins in the Sinai in Egypt

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Introduction: Recent outbreaks of West Nile (WN) in humans in Israel stress the need to determine the current impact of this virus and other arboviruses in the Sinai that shares a border with Israel.
Objective: The objective of this study was to determine the prevalence of WN and other arboviruses among Bedouins (nomadic with limited mobility population) of north (El-Arish) and southeast (Nuweiba) Sinai, where there is a lack of information about arboviral activity among this unique population.
Materials and methods: This study was established in collaboration with the Egyptian Ministry of Health and Population. Eight-hundred and sixty-six (866) serum samples collected in 2001 from Bedouins living in El-Arish ($n = 197$) and Nuweiba ($n = 669$) were tested for IgG by ELISA using antigens for WN, Sindbis (SIN), sandfly fever Scilian (SFS) and sandfly fever Naples (SFN) viruses using standard methods.
Results: The overall seroprevalence rate (SPR) of WN was 10% ($n = 19$) and 4% ($n = 24$) in El-Arish and Nuweiba, respectively. Interestingly, SPR of WN in both sites was not age dependent as previously reported in other governorates of Egypt. The SPR of WN was 5% (2/43) and 4% (5/136) in children (4–10 years) in El-Arish and Nuweiba, respectively, and peaked to 15% (8/53) and 6% (8/143) among ages 21–30 years in these same areas, then declined. The overall SPR of SIN was 7% ($n = 14$) and 3% ($n = 21$) in El-Arish and Nuweiba, respectively. No SIN antibodies were detected in ages of >60 years ($n = 34$) for either site. Antibodies to SFS virus were detected in EL-Arish and Nuweiba with relatively low SPR [3% ($n = 5$) and 2% ($n = 12$), respectively]. Significant SFN activity was noted through all age groups in both El-Arish and Nuweiba with an overall SPR of 9% ($n = 17$) and 8% ($n = 54$), respectively, and a peak of 55% (6/11) in ages 61 to >70 in El-Arish and 14% (20/143) in ages 21–30 in Nuweiba.
Conclusion: To our knowledge, this study is the first to report the activities of the studied viruses, particularly WN and SFN among Bedouins in the Sinai. Further investigations are required to study the epidemiology and ecology of the above viruses in the Sinai.

P699 Epidemiological characteristics of dengue in Trujillo, Peru

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Objective: From January to August 2001, an epidemic of dengue fever was presented for the first time in Trujillo city in the North of Peru. In this epidemic, we determined the principal epidemiological characteristics of dengue.
Methods: To determine the epidemiological characteristics of dengue, we studied 360 cases of the 6152 presented with an epidemiological and standardized questionnaire to ask the principal risk factors to the disease. This were confirmed with serological studies by the National Institute of Health.
Results: Sera that had been collected previously by the Peruvian Ministry of Health were tested by enzyme-linked immunoabsorbent assay (ELISA) by DEN virus type I and II. The major morbidity was in Florencia de Mora (FM) district with 22.41×1000 ha. The positive indices in FM was 25.4%. There was no statistical difference by sex. The age with more cases was from 40 to 60 years old. The fever symptom was 94.4%, muscle and join pain 60.8%, headache 51.9%, bone aches 39.4%, skin hemorrhages 27.2%, rashes 0.8%, echimosis 0.2%, nasal bleeding 0.8%, hematuria 0.3%, etc.
Conclusion: We concluded that the characteristics of dengue in Trujillo city, Peru were the same as in other districts of the country.

P700 Development of DNA vaccines against dengue infection and evaluation of their protective mechanisms by transgenic mice

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To develop a safe and effective DEN vaccine becomes a high priority of the World Health Organization, health ministries in some affected countries. Since the phenomenon of antibody-dependent enhancement of infection by antivirion antibodies has been implicated in the development of severe dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), NS1 has been considered as a potential candidate for DEN vaccine. The laboratory of Prof Sytwu previously demonstrated DNA immunization with DEN2 virus NS1 elicited protective immunity in C3H mice against subsequent viral challenge. Intravenously challenged with lethal DEN2, mice vaccinated with NS1 DNA exhibited a delay onset of paralysis, a marked decrease of morbidity, and a significant enhancement of survival. Despite having a weak NS1-specific antibody titer measured by ELISA, no anti-NS1 antibody response was detected in NS1-vaccinated mice by radioimmunoprecipitation or immunoblot analysis. In contrast to humoral immune response, DNA immunization elicits strong cellular immune response, an NS1-specific T-cell proliferation. To further dissect the mechanisms and components involved in this protection, we will perform the NS1-specific cytotoxic T-lymphocyte (CTL) assay and adoptive transfer experiments. Sera, CD^4 T cells, CD^8 T cells, or B cells from DNA-immunized mice will be individually or combinatorially transferred into recipients and the protection in subsequent viral challenge will then be evaluated. We will investigate the host defense mechanisms against dengue virus by using transgenic and knockout mice. Dengue-infected Th1/2 double transgenic C3H mice or IL-12 knockout C3H mice will provide the best models to directly dissect the role of Th1 or Th2 differentiation and the regulation of IL-12 during the host–virus

interactions. It has been reported that the efficacy of a DNA vaccine can be greatly improved by simultaneous expression of interleukin-2 (IL-2) or other cytokines. Therefore, in the future, we will construct new DEN 2 DNA vaccines by a bicistronic vector separately encoding the NS1 and IL-2 (or other cytokine genes, e.g. GM-CSF, IL-4, IL-12), B7, CD^{40}, CD^{40} ligand or heat shock protein (HSP), etc. The immune responses and protective efficacy elicited by these immunomodulatory DNA vaccines will be further evaluated.

Automated methods

P701 Evaluation of the VITEK 2 system for routine identification of clinical isolates in a university hospital laboratory

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Objective: The objective of the study was to assess the performance of the fully automated VITEK 2 system, dedicated to identification and susceptibility testing in the context of the hospital microbiology laboratory.
Methods: Over a period of 3 months, 255 Gram-negative rods (Gnr) and 185 Gram-positive cocci (Gpc) were tested using the VITEK 2 ID-GNB and ID-GPC cards. The sealed cards, which ensure user safety, contain phenotypic fluorescence tests for the identification of clinical strains in 3 h maximum. The strains tested belonged to Enterobacteriaceae ($n=207$), non-Enterobacteriaceae ($n=48$), Micrococcaceae ($n=124$), and Streptococcaceae ($n=61$). The Gnr were mainly cultured overnight on BCP agar (58%) and CPS ID 2 agar (35%), and the Gpc on TSA blood agar (94%). The strains were collected by primo isolation (85% for Gnr and 48% for Gpc) or after subculture (15% for Gnr and 52% for Gpc). The comparison was performed using ID 32 E and ID 32 GN strips for Gnr, and rapid ID 32 STREP and ID 32 STAPH strips for Gpc.
Results: Of the 255 strains of Gnr, 239 (93.7%), including low discriminations, were correctly identified. Ten strains (3.9%) were not identified and six (2.4%) were misidentified. The strains not identified belonged to *Klebsiella pneumoniae* (2), *Enterobacter* (3), *Salmonella* (1), *Proteus mirabilis* (1) *Shigella* (1). The system generated misidentifications for: *Enterobacter cloacae* (1), *Morganella morganii* (1), *Escherichia coli* (1), *Klebsiella pneumoniae* (2), *Proteus vulgaris* (1). For non-Enterobacteriaceae, only one strain of *Pseudomonas aeruginosa* was not identified. Of the 185 Gpc strains, 171 (92.4%), including low discriminations, were correctly identified. Two strains (1.1%) were not identified and 12 (6.5%) were misidentified. The two strains which were not identified belonged to the *Streptococcus* genus (one *S. anginosus*, one *S. pneumoniae*). The misidentified strains belonged to the *Enterococcus* and *Staphylococcus* genera (one *E. avium*, one *E. faecium*, one *E. gallinarum*, five *S. epidermidis*, one *S. haemolyticus*, one *S. hominis*, one *S. warneri*, one *S. xylosus*).
Conclusion: This study shows that VITEK 2 is an accurate system for the routine identification of clinical bacteria. Identification of *S. epidermidis* should be improved with the next database version. Identification in 2–3 h and the level of automation of the VITEK 2 system improve the laboratory work flow, and consequently meet the physician's requirements.

P702 Preincubation at 35 °C gives false negative results in BACTEC 9240 blood culture system

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Objectives: Concern about the performance of BACTEC 9240 blood culture system arose when about 10% of 69 bottles indicated as negative in the BACTEC instrument were positive on terminal subculturing when used for educational purposes. Official recommendations from the manufacturer are not clear regarding the advisability of preincubation at 35 °C, but at our hospitals the bottles have traditionally been preincubated at 35 °C, and are not seldom more than 20 h delayed. Therefore, we wanted to determine the influence of preincubation time and temperature on the performance of BACTEC 9240.
Methods: Blood culture sets (two BACTEC 9240 Plus Aerobic/F and one Anaerobic/F bottles) from two hospitals were preincubated at 35 °C and from one hospital kept at room temperature before entering the BACTEC instrument. Preincubation time in hours was registered. On arrival the bottles were visually inspected for signs of growth. Bottles seen to be positive were subcultured. One aerobic bottle from visually negative sets was also subcultured to detect growth not yet visible. All bottles whether visually positive or negative were incubated in BACTEC for 5 days or until detected positive. Bottles not detected positive after 5 days were removed from the instrument and subcultured. Positive bottles were defined as bottles giving growth detected by any of the above means.
Results: During the first 4 months (July–October 2001) 535 bottles were culture positive. Of these, 261 (49%) had been kept at room temperature and 274 (51%) had been preincubated at 35 °C before entry into the BACTEC instrument. Of the 38 (7%) culture positive bottles not detected by BACTEC, three, visually negative, had been kept at room temperature before entry, while the remaining 35 had been preincubated at 35 °C. Of these 35, 20 were visually negative prior to entry into BACTEC, and 15 were visually positive; 12 of the latter had been preincubated for more than 20 h.
Conclusions: Preincubation of blood cultures at 35 °C reduced the positive yield by approximately 10% when only instrument detection was utilized. Careful visual inspection of bottles is mandatory when prolonged preincubation at 35 °C is employed.

P703 Comparison of Phoenix to Vitek2 with a diverse group of bacteria which are found in clinical microbiology labs

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Objectives: Two rapid automated identification (ID) and susceptibility test systems, Phoenix and Vitek2, were evaluated for the identification of Gram-negative bacilli, *Staphylococcus*, and *Enterococcus*.
Methods: A total of 176 Gram-negative bacilli, 121 *Enterococcus* and 400 Staphylococcal strains were tested, composing a sample of species that are commonly seen in clinical microbiology. This included: *S. aureus* (172), *Staphylococcus* coagulase-negative species (228), *E. faecalis* (51), *E. faecium* (56), other *Enterococcus* spp. (13), *E. coli* (20), *Enterobacter/Citrobacter* (54), *Klebsiella* (35), *Proteus/Morganella* (23), *Serratia* (9), *P. aeruginosa* (22), *Hafnia* (5), and *Acinetobacter* (8). All isolates were tested in both systems following normal procedures recommended for each product. This included recommendations for supplemental tests. When needed the results of the supplemental tests were used to select a final result. Phoenix and Vitek2 ID results were compared and when discordant, the strains were retested and the ID result arbitrated by a third method. Arbitration used API 20E for Gram-negatives, API 32S for *Staphylococcus*, and API StrepRapid for *Enterococcus*.
Results: Phoenix ID results were equal to Vitek2 ID results in 96% of the tests. The comparability by organism group were 95% for Gram-negative *Bacillus*, 95% for *Staphylococcus*, and 97% for *Enterococcus*. Arbitration tests of the few discordant results were in favor of Phoenix = 8×, Vitek2 = 8× times, and neither system 5×. Overall, supplemental manual ID tests were required for 6.7% of the Vitek2 tests and 1.4% of the Phoenix tests. With Gram-negative bacteria the requirement for supplemental tests was 20.6% for Vitek2 and 1.1% for Phoenix.
Conclusions: The Phoenix system is highly equivalent to Vitek2 in the identification of common Gram-positive and -negative strains. Vitek2 frequently requires supplemental testing with Gram-negative bacteria whereas with Phoenix supplemental testing is only occasionally required.

P704 Comparison of Phoenix to Vitek2 antimicrobial susceptibility test performance with a diverse group of bacteria which are found in clinical microbiology labs

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Objectives: Two rapid automated identification and antimicrobial susceptibility test (AST) systems, Phoenix (BD Diagnostics), and Vitek2

(BioMérieux), were evaluated for the AST performance using a challenge set of Gram-negative bacilli and Gram-positive Cocci.
Methods: Standard commercially available Phoenix panels and Vitek2 cards were used with appropriate panel types for Gram-negative and Gram-positive organisms. A total of 165 Gram-negative bacilli, 102 *Enterococcus* (34 VRE) and 340 Staphylococcal strains (158 *S. aureus* and 49 MRSA), were tested composing a sample of species that are commonly seen in clinical microbiology. All antibiotics evaluated were clinically relevant for organism being tested and included 10 antibiotics for Gram negatives, 11 for Staphylococci, and eight for *Enterococcus*. Isolates were tested in both systems following normal procedures recommended for each product. Individual MICs were interpreted following DIN standards except for agents where there were no interpretive standard, in which case SFM or NCCLS standards were used. Phoenix and Vitek2 AST results were compared and major discrepancies were retested and the AST result arbitrated by microdilution MIC testing (Biotest AG, Dreieich, Germany). Overall performance by organism group and by significant resistance mechanisms were compared.
Results: Overall, Phoenix AST results were comparable to Vitek2 in 94% of the tests. The comparability by organism group were 92% for Gram-negative *Bacillus*, 96% for *Staphylococcus*, and 88% for *Enterococcus*. MRSA was detected in 49 strains in both systems with two strains being resistant in Phoenix and susceptible in Vitek2, and two strains susceptible in Phoenix and resistant in Vitek2. VRE was detected in both systems 32 times, and in Vitek but not Phoenix two times, and in Phoenix but not Vitek one time. Arbitration testing was in favor of Phoenix in the two cases where Phoenix was susceptible and Vitek2 was resistant.
Conclusions: The Phoenix system is generally equivalent to Vitek2 in the AST testing of common Gram-positive and -negative strains.

P705 The BD Phoenix automated identification and susceptibility testing system in a clinical microbiology laboratory

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Objectives: Phoenix™ Automated Microbiology System (BD Biosciences) is a new fully automated system for rapid identification and antimicrobial susceptibility testing of Gram-positive and Gram-negative bacteria. The objective of this study was to evaluate the quality of performance of Phoenix™ panels used for identification and antimicrobial susceptibility testing.
Methods: Two hundred and sixty-one Gram-negative ($n = 175$) and Gram-positive ($n = 86$) isolates collected from Polish hospitals in recent years were used in the study. Two Phoenix™ ID/AST panel types, NMIC/ID-5 for Gram-negative rods and PMIC/ID-4 for Gram-positive cocci, were used in the analysis according to manufacturer's recommendations. Results of this study were compared with data obtained by standard or conventional microbiological methods.
Results: A high rate of agreement between Phoenix™ identification and the conventional methods was observed. It ranged from 97.3% for Gram-positive cocci to 96.0% for Gram-negative nonfermenters, and 91.0% for Enterobacteriaceae isolates. A high level of agreement was demonstrated by the susceptibility data obtained with Phoenix™ and the standard agar dilution method. For staphylococci, enterococci and Enterobacteriaceae isolates the 100% concordance of the isolate susceptibility categorization was observed with the majority of antimicrobials tested. The category agreement value of below 90% was found only in the case of ciprofloxacin susceptibility in enterococci (84.6%) and Gram-negative nonfermenting rods (88.5%). There were no major' (false resistance) or very major' (false susceptibility) errors in the detection of methicillin-resistance in staphylococci and resistance to glycopeptides and high concentrations of aminoglycosides in positive for ESBL by 'double-disc' method.
Conclusion: The Phoenix™ system is a reliable system for clinical microbiology diagnostic.

P706 Detection of glycopeptide resistance in *Enterococcus faecium* using Phoenix Automated Microbiology System

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Objective: Glycopeptide (vancomycin and teicoplanin; VA and TEC) resistance in *Enterococcus faecium* (GR-EF) has become a worldwide concern, especially in nosocomial infections. The purpose of this study was to evaluate the performance of Phoenix™ Automated Microbiology System in the identification and detection of GR in EF.
Methods: The Phoenix System was compared to multiplex PCR, standard broth microdilution (SBM) and disk diffusion methods (DD) per NCCLS recommended procedures. A total of 86 isolates were identified using conventional biochemical tests as a reference method. A multiplex PCR test was then used to determine the glycopeptides resistance genotypes (*vanA* or *vanB*). Sixty-eight strains of GR-EF (46 *vanA* and 22 *vanB*) and 18 strains of glycopeptide susceptible EF (GS-EF) were tested in Phoenix, SBM and disk diffusion methods for their susceptibility to VA and TEC.
Results: The Phoenix identification results of the test strains were 96.5% correlated with the reference identification results. All 18 strains of GS-EF were correctly detected in Phoenix, SBM and DD. Phoenix detected all 68 strains of GR-EF with VA resistance. However, of the 46 *vanA* strains tested, Phoenix gave (1/46–2%) susceptible TEC results. Also, 2% of *vanA* strains showed susceptible result to TEC in all three AST methods tested. Of the 22 *vanB* strains tested, five strains showed resistant results to TEC with both Phoenix and SBM while the other 17 strains were detected as susceptible in all three AST methods to TEC. The average time to results for identification in Phoenix was 2 h. The average time to detection for VA and TEC were 7 and 9 h by Phoenix, respectively.
Conclusion: These results indicate that identification and glycopeptide resistance in EF may be reliably and rapidly detected in the Phoenix system.

P707 Controlled clinical comparison of New BacT/ALERT FN versus standard anaerobic blood culture bottles

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Objectives: To determine the optimal anaerobic companion bottle to pair with the BacT/ALERT (bioMérieux, Durham, NC, USA) nonvented aerobic FA (FA) medium for recovery of pathogenic microorganisms from adult patients with bacteremia and fungemia, we compared BacT/ALERT FN (FN) bottles with standard BacT/ALERT SN (SN) anaerobic bottles.
Methods: Each bottle, FA, FN, and SN, was filled with 8–12 mL of blood.
Results: Of 11 498 blood culture sets received in the clinical microbiology laboratory, 7945 sets had all three bottles filled adequately and 8569 had both anaerobic bottles filled adequately. Of 683 clinically important (based on previously published criteria) isolates detected in one or both adequately filled anaerobic bottles, significantly more staphylococci ($P < 0.001$), including *Staphylococcus aureus* ($P < 0.001$); Enterobacteriaceae ($P < 0.001$); and all microorganisms combined ($P < 0.001$) were detected in FN bottles. In contrast, significantly more *Pseudomonas aeruginosa* ($P < 0.01$) and yeasts ($P < 0.001$) were detected in SN bottles. More *Bacteroides fragilis* group bacteremias were detected only in the FN (6) versus the SN (1) anaerobic bottle (P = NS). Overall, the mean time to detection was shorter with FN (16.8 h) versus SN (18.2 h). This difference in time to detection was greatest for the *B. fragilis* group: FN, 28.1 h versus SN, 60.0 h. Many of the facultative microorganisms recovered in either FN or SN were also found in the companion FA. When microorganisms found in the companion FA bottle were omitted from the analysis, significantly more staphylococci ($P < 0.001$), including *S. aureus* ($P < 0.001$), and Enterobacteriaceae ($P < 0.01$) still were detected in FN bottles, whereas there were no significant differences for *P. aeruginosa* and yeasts, which were found as expected in FA bottles.
Conclusions: We conclude that the companion anaerobic FN bottle detects more microorganisms than the SN bottle when used in conjunction with the nonvented aerobic FA bottle in the BacT/ALERT blood culture system.

P708 Recovery and time detection of *Mycobacterium tuberculosis* in the BACTEC MGIT 960 system from clinical specimens – comparison with the conventional Löwenstein–Jensen medium

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Objective: To evaluate the automated systems BACTEC MGIT(c) 960 (BM system – Becton Dickinson) in comparison to solid media Löwenstein Jensen (LJ medium); from the recovery and time to detection of *Mycobacterium* used routinely in our laboratory during 12 months.
Methods: Two thousand and fifteen consecutive clinical specimens have been processed in the laboratory. The samples were digested and decontaminated; they were inoculated in flasks of BM system and in LJ medium and introduced in their respective incubation systems. The Statgraphics Plus 4.1 software has been used for the statistical adjustments.
Results: Of the 2015 processed specimens, 136 were positive for some of the two methods. The BM system registered 134 positive specimens (98.53% of the positive specimens) and LJ medium recovered 106 (77.94%). Thirty samples grew alone in the BM system and two (1.47%) alone in LJ medium. There is a statistically significant difference between the means of growth of the two methods at the 95.0% confidence level. The half time of growth is of 13.47 days in BM system while in the LJ medium was of 27.6 days. The time of growth was studied for regression analysis; the correlation coefficient equals 0.66 indicating a moderately strong relationship between the variables. In the patients with *Mycobacterium* infection and LJ-medium negative has been observed that a high percentage of patient were in treatment for the tuberculosis. In the patients with LJ medium positive were not observed differences in relation to the treatment. Sensitivity in BM system was 98.53% compared to 77.94 in LJ medium, this difference was statistically significant ($P < 0.005$).
Conclusion: The BM system showed more sensitivity than LJ medium in the *Mycobacterium* detection, since in view of the results, if we use the analyzing BM system we recover 22.05% of the positive specimens more than that with other methods. LJ medium presents a time of growth higher than automated systems. The less time of detection and the higher sensitivity make BM system a good method for *Mycobacterium* isolations.

P709 Controlled clinical comparison of plastic versus glass bottles of BacT/ALERT PF medium for culturing blood from pediatric patients

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Objectives: The pediatric plastic BacT/ALERT (bioMérieux, Durham, NC, USA) PF (PPF) is a new nonvented aerobic culture medium in a clear plastic bottle designed to prevent breakage. To assess the new PPF bottle versus the current glass BacT/ALERT PF bottle, we compared the performance of these two bottles for the recovery of microorganisms as well as the time to detection of growth in samples of blood obtained for culture from children.
Methods: All bottles were weighed before inoculation and upon receipt; only bottles filled with similar volumes of blood were compared.
Results: Of 3445 bottle pairs received, 2010 (58.3%) contained comparable volumes of blood. Of 97 clinically significant (based on previously published criteria) isolates, 74 were detected in both bottles, 13 only in PPF, and 10 only in PF (P = ns). No organism group was detected significantly more frequently from one medium versus the other. Of 74 sets that were positive in both bottles within 3 days, the mean time to detection of pathogens from pediatric patients was 18.0 h in PPF and 17.8 h in PF bottles. False-positive instrument signals were detected in 10 of 2010 (0.5%) PPF bottles and five of 2010 (0.2%) PF.
Conclusions: We conclude that BacT/ALERT PPF and PF bottles are comparable for recovery of microorganisms and that the safety advantage of plastic bottles can be achieved without compromising performance.

P710 Evaluation of the performance of the VITEK 2 automated identification and antimicrobial susceptibility testing system on Gram-positive cocci

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Objective: An evaluation of the performance of the VITEK 2 (bioMérieux, France) for the identification and susceptibility testing of Gram-positive cocci compared to the currently used routine method was performed.
Methods: A total of 168 fresh isolates of Gram-positive cocci were tested using VITEK 2 including 17 *Streptococcus pneumoniae* (S.p.), 51 enterococci and *Streptococcus* spp., 50 (S.a.), and 50 coagulase-negative staphylococci (CNS).The results were compared to those obtained with the routine system currently used in our laboratory (AutoSceptor, BD, Maryland). Strains discrepant results were compared with API (bioMérieux, France) system for identification, and with E-test (AB BIODISK, Sweden), for susceptibility testing, according to the NCCLS.
Results: The comparison of identification results showed an agreement of 100% for S.p. and S.a., 96.1% for enterococci and *Streptococcus* spp., and 72% for CNS. The results obtained by API, showed the VITEK 2 to be correct for 100% of the enterococci and *Streptococcus* spp., and 90% of the CNS (100% for *S. epidermidis*).For the evaluation of the susceptibility test (1680 antibiotic/organism combinations) category agreement between the two automatic systems was 88% for CNS (11 antibiotics tested),6.5% for S.p. (nine antibiotics tested),70% for S.a. (11 antibiotics tested) and 53% for Enterococci and *Streptococcus* spp. (nine antibiotics tested). No Very Major Errors were found with VITEK 2 for any of the organism/antibiotic combinations tested. In comparison with E-test, testing of the discrepancies between the two automated systems showed the VITEK 2 to give correct results in 100% of the cases with CNS, in 95% of the cases with Enterococci and *Streptococcus* spp. (for Enterococci, no discrepancy for vancomycin was seen), 92% correct results for S.a.(no discrepancies for oxacillin). The agreement between the two systems remained unchanged (76.5%) for S.p. This was due to only one antibiotic, enicillin, and four strains. VITEK 2 gave results of resistant for two strains and intermediate for two strains. Instead, E-test gave intermediate for two strains and sensitive for two strains. Considering the time to complete of susceptibility results, times varied from 7.5 to 10.5 h, with an average time of 8.5 h.
Conclusion: The VITEK 2 provides rapid results and above all is reliable for both identification and susceptibility testing regarding the major part of the analysis of Gram-positive cocci.

P711 Comparison of the Phoenix™ Automated Microbiology System to the Dade-MicroScan WalkAway-96 for identification of clinical bacterial isolates

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Objectives: The Phoenix™ System (BD Diagnostic Systems, Sparks, Maryland, USA), a rapid automated ID/AST system, was compared to the Dade-MicroScan WalkAway-96 System (Dade-MicroScan, W. Sacramento, California, USA) for accuracy of identification (ID) of fresh clinical laboratory isolates as well as a Challenge Set of selected stock strains.
Methods: A total of 328 bacterial isolates, comprised of 192 Gram-negative bacilli (GNB) and 136 Gram-positive cocci (GPC) (27 *Staphylococcus aureus*, 53 coagulase-negative staphylococci, 45 enterococci and 11 streptococci), was evaluated in both the Phoenix (PHX) and MicroScan (MS) systems. Inoculum suspensions were prepared for each strain on the same day from the same subculture plate for both test systems. Standard commercially available PHX and MS ID/AST panels were inoculated and incubated following each manufacturer's procedure. If required, supplemental tests were used to determine a final ID. Results from both systems were compared for genus/species agreement, and discrepancies were arbitrated using a third system, API (bioMérieux, France).
Results: The overall rate of agreement between the PHX and MS systems for species level ID was 95.3 and 95.6% for GN and GPC, respectively. *Enterococcus* and *Streptococcus* species both achieved a 100% rate of species level agreement. The genus level agreement was >99% overall. Arbitration of the nine GNB disagreements resolved with seven in agreement with the Phoenix ID. For the six GPC disagreements, four resolved in agreement with Phoenix. Supplemental manual ID tests were required for four GN strains (2.1%) with MS, while PHX required additional tests for two GN strains

(1.0%). In addition, PHX correctly identified 60/63 (95.3%) of *Staphylococcus* species as beta-lactamase producing strains.
Conclusions: The Phoenix Automated Microbiology System provides a high level of agreement to the MicroScan WalkAway System for identification of Gram-negative and Gram-positive clinical isolates.

P712 Comparison of the Phoenix™ Automated Microbiology System to the Dade-MicroScan WalkAway-96 for antimicrobial susceptibility testing with Gram-negative and Gram-positive bacterial isolates

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Objectives: The Phoenix™ System (BD Diagnostic Systems, Sparks, Maryland, USA), a rapid automated ID/AST system, was comparatively evaluated with the Dade-MicroScan WalkAway-96 System (Dade-MicroScan, W. Sacramento, California, USA) for accuracy of antimicrobial susceptibility test (AST) results with a varied group of Gram-negative bacilli (GNB) and Gram-positive cocci (GPC).
Methods: A total of 306 clinical bacterial isolates, comprised of 183 GNB (family Enterobacteriaceae), and 123 GPC (79 staphylococci including 39 methicillin-resistant (MR) strains, and 44 enterococci) isolates were tested in standard commercially available Phoenix (PHX) and MicroScan (MS) AST panels. Each inoculum suspension was prepared from the same subculture plate for both test systems. PHX and MS panels were each inoculated, then incubated and automatically read in the system's instrument following each manufacturer's recommended procedure. Thirteen antimicrobics were evaluated for GNB, 13 for *Staphylococcus* species (ST), and nine for *Enterococcus* species (ENT). The minimal inhibitory concentration (MIC) of each antimicrobic/organism combination was interpreted based on the 2001 NCCLS Standards. Discrepant AST results were repeated in both systems, and arbitrated using the NCCLS-recommended broth microdilution method. Essential agreement (EA), MIC results within ±1 doubling dilution, and categorical agreement (CA) were determined for the Phoenix System.
Results: Overall, 3610 AST combinations were evaluated in both systems. For GN isolates, the rate of EA was 99.7%, while the CA rate was 95.6%. False-susceptible (VM) and false-resistant (M) rates for PHX were 1.0 and 0.6%, respectively. Arbitration of 25 GNB disagreements resolved with 22 in agreement with the Phoenix MIC. For ST isolates, the EA and CA rates were 97.4 and 95.7%, respectively. All 39 MR strains were correctly identified as such by PHX. With ENT species, the EA was 99.3%, and the CA 95.8%. High-level aminoglycoside detection was 100% in agreement for the two systems. PHX also correctly identified three enterococcal strains as vancomycin-resistant.
Conclusions: The rapid AST performance of the Phoenix System is nearly equivalent to that of the MicroScan WalkAway overnight system, and provides an alternative in automated AST testing of GNB and GPC for the clinical laboratory.

P713 Controlled clinical comparison of plastic versus glass bottles of BacT/ALERT FA medium for culturing blood from adult patients

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Objectives: A new clear plastic nonvented FA bottle, designed to prevent breakage, has been developed for the BacT/ALERT (bioMérieux, Durham, NC, USA). To assess the new plastic FA (PFA) bottle versus the current glass BacT/ALERT FA bottle, we compared the performance of these two bottles for the recovery of microorganisms as well as the time to detection of growth in samples of blood obtained for culture from adult patients with suspected bloodstream infection.
Methods: PFA and FA bottles were each filled with 8–12 mL of blood.
Results: Of 3191 blood culture sets containing both bottles, 2368 (74.2%) met the criteria for adequacy of filling. A total of 176 clinically important (based on previously published criteria) isolates were recovered from 93 patients; 112 isolates were recovered from both bottles, 28 were recovered from the PFA bottle only, and 36 were recovered from the FA bottle only (P = ns). Of cultures found to be positive within the first 72 h of incubation, the mean time to detection was similar for PFA (20.5 h) and FA (20.1 h) bottles. The number of false-positive results was comparable: nine (0.4%) in PFA bottles and 12 (0.5%) in FA bottles.
Conclusions: We conclude that BacT/ALERT PFA and FA bottles are comparable for recovery of microorganisms and that the safety advantage of plastic bottles can be achieved without compromising performance.

P714 Comparison of BacT/ALERT 3D and Bactec 460 for drug susceptibility testing of *Mycobacterium tuberculosis*

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Objective: The aim of the study was to evaluate the nonradiometric BacT/ALERT system (Organon Teknika) against the radiometric Bactec 460 method (Becton Dickinson) for susceptibility testing of *Mycobacterium tuberculosis* to Rifampicin (RIF), Isoniazid (INH), Streptomycin (SM) and Ethambutol (EMB).
Methods: The drug susceptibilities of the fully susceptible reference strain *M. tuberculosis* H37Rv and an earlier established test-panel of 47 clinical isolates of susceptible and resistant *M. tuberculosis* strains were assessed by the BacT/ALERT method. The panel included 23 strains resistant to RIF (2 mg/L); 25 to INH (0.2 mg/L); 16 to SM (4 mg/L); and seven to EMB (5 mg/L), when determined by our conventional standard method Bactec 460. In the BacT/ALERT system, the following drug concentrations were used: RIF 1 mg/L, INH 0.1 and 0.5 mg/L, SM 1 mg/L, and EMB 4 mg/L.
Results: Altogether 240 susceptibility tests were carried out with the BacT/ALERT system. Of these 239 tests could be evaluated. Full agreement between the two methods was seen in 233/239 tests (97.5%). For each drug, the concordance between the two methods was 95.8% for RIF, 97.9% for INH (high and low concentration), 93.8% for SM and 97.9% for EMB. The mean time to achieve a susceptibility pattern from a strain grown on Löwenstein–Jensen media was 11 days using BacT/ALERT compared to approximately 7 days for the Bactec 460 method. For one (resistant) strain, the susceptibility could not be evaluated for INH (low) by the BacT/ALERT method due to the rapid growth in the drug containing culture vials.
Conclusions: A good correlation was seen between the two methods. The result suggest that the BacT/ALERT system is a valid alternative to the Bactec 460 system for drug susceptibility testing of *M. tuberculosis*, especially in laboratories where disposal of radioactive waste is restricted.

P715 Rapid identification of clinical bloodstream isolates of staphylococci with the VITEK 2 system

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Objectives: Staphylococci are an increasingly common cause of bloodstream infections. Rapid and reliable species identification of these organisms is essential in ensuring accurate diagnosis and prompt effective treatment of these severe infections. Our aim was to evaluate the ability of VITEK 2 system (bioMeriéux, Inc., Hazelwood, MO) to identify these organisms rapidly and accurately.
Methods: A total of 350 staphylococcal isolates (*Staphylococcus aureus*, $n = 117$ and coagulase-negative staphylococci $n = 233$), collected from blood cultures between 1999 and 2001 at the 'A. Gemelli' teaching hospital in Rome, were identified with the VITEK 2 system and a reference method consisting in the ATB ID 32 Staph system (bioMérieux, Marcy l'Etoile, France) together with the standard identification scheme of Kloos and Bannermann (W.E. Kloos and T.L. Bannermann, in P.R. Murray et al. ed., Manual Clinic Microbiol, 6th ed., 1995, p. 282–98). VITEK 2 results were considered correct when they were identical to those of the reference method. When discrepancies occurred, isolates were retested with both systems and reference-system results were confirmed using a nucleic acid-based procedure that combined PCR amplification of the gap gene of *S. aureus*, which encodes glyceraldehyde-3-phosphate dehydrogenase, and restriction-length polymorphism (RFLP), using Alu I restriction endonuclease. Primers designed on the gap gene were used to amplify a 933-bp DNA fragment by PCR. Alu I digestion of PCR products gave different RFLP patterns that allowed species identification.
Results: The VITEK 2 system correctly identified 337 of 350 isolates at the species level (96.3%) with the VITEK 2 system alone and 12 other strains (one

S. aureus, one *S. haemolyticus*, six *S. hominis*, three *S. epidermidis* and one *S. warneri*) were identified using simple, rapid manual tests suggested by the manufacturer (clumping factor, hemolysis, anaerobic growth, colony pigment and polimixin susceptibility). Only one strain (0.3%), an *S. hominis*, was misidentified (as *S. epidermidis*). Results obtained with phenotypic methods were confirmed by PCR-RFLP of the *S. aureus* gap gene. Results were obtained within 2–4 h.
Conclusions: The VITEK 2 system can provide rapid, accurate and reliable species-level identification of staphylococci responsible for bloodstream infections.

P716 Evaluation of an automated extraction method (MagnaPure) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Cobas Amplicor PCR from urogenital specimens

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Introduction: Infections with *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are frequently encountered sexually transmitted diseases. Specific diagnosis is important as specific treatment can avoid transmission and therefore, reduce the incidence. Conventional techniques such as immuno-fluorescence or cell culture in case of CT, or specific bacterial culture in case of NG, are cumbersome and lack sensitivity. Newer amplification techniques such as commercially available PCR tests (Amplicor/Roche) have proven useful through better sensitivity and better handling. Better performance along with automation have promoted this approach for STD screening in ambulatory medicine. However, while amplification and detection are today handled by an automated device (Cobas Amplicor/Roche), the preparation of the clinical specimens is still done manually and may, therefore, limit the throughput.
Methods: We evaluated a newly available automated robotic platform (MagnaPure/Roche) for the extraction of DNA from clinical urogenital specimens for the detection of CT/NG, using the Cobas Amplicor PCR system. The automated extraction on 645 clinical samples was done in parallel to the standard manual protocol, as recommended by the manufacturer. Successful amplification and absence of inhibitory factors were monitored through amplification of an internal control (IC) and OD values were recorded for all samples. Positive results for NG were confirmed using the Roche 16S rDNA NG confirmation test. Inhibited samples (OD < 0.2) were re-extracted using silica matrices (Qiagen Mini-Amp DNA) cartridges and amplification was repeated (Cobas Amplicor). Amplification was performed using the same Cobas Amplicor PCR system and the same batch reagents.
Samples: We tested 566 vaginal/cervical Amplicor swabs, 46 urethral Amplicor swabs and 33 native urine samples for presence of CT/NG; the specimens were split and subjected to the automated MagnaPure total DNA procedure and to the manual standard protocol, without delay.
Results: The MagnaPure extraction procedure showed a slightly better sensitivity, in comparison to the standard technique. More unspecific *Neisseria* spp. were detected with the MagnaPure, however, at OD values lower than 2.0. Finally, samples primarily inhibited by the manual method yielded significantly better results via the MagnaPure.
Conclusion: MagnaPure extraction of urogenital specimens for subsequent amplification using the Cobas Amplicor system is a valuable alternative to the standard manual extraction technique. It shows significant better performance with regard to sensitivity and primary inhibition and could be integrated in an overall automated analysis procedure for CT/NG testing.

Molecular diagnostic methods I

P717 Clinical impact of identification and detection of methicillin resistance of staphylococci in blood cultures by multiplex PCR

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Objective: To assess the impact on antibiotic therapy of performing a triplex PCR that discriminates *Staphylococcus aureus* from the coagulase-negative *Staphylococcus* (CNS) and detects their methicillin resistance on positive blood cultures in comparison with routine testing methods.
Patients and methods: Consecutive patients with presumptive staphyloccocal bacteremia (blood culture positive for clusters of Gram-positive cocci) were included. PCR was performed on DNA extracted from blood culture broth using three sets of primers for genes encoding staphylococcal 16S rRNA, thermostable nuclease (*nuc*) and PBP2A (*mecA*), respectively. PCR results were compared to those of conventional techniques of identification (coagulase and latex tests) and oxacillin susceptibility (disc-diffusion or Rapid ATB-staph microdilution test).Endpoints were defined as: (1) turnaround time of the result, and (2) the adaptation of empirical antibiotherapy.
Results: Atotal of 21 patients were enrolled in the study. In these patients, bacteremia was caused by CNS in 14 (methicillin resistant in 13) and *S. aureus* in seven (methicillin resistant in two). Phenotypic and genotypic methods showed 100% agreement. Mean times to communication was: 6.1 h (range: 5 1–7.5 h) for PCR vs. 43.3 h (range: 24.58–50.58 h) for conventional methods ($P < 0.01$). Only three patients (14%) benefited from a modification of antibiotic therapy based on the PCR results: two patients with methicillin-sensible *S. aureus* were shifted from vancomycin to oxacillin, and one patient with methicillin-resistant CNS bacteremia was started with treatment with vancomycin.
Conclusion: This triplex PCR assay was as accurate for diagnosis of staphylococcal bacteremia as the conventional methods. It provided results on average 37 h earlier. However, the therapeutic benefit of this rapid diagnosis was limited given that empirical therapy was appropriate in the majority of cases.

P718 Less than one-hour detection of vancomycin-resistant enterococci directly from fecal samples by real-time PCR using the Smart Cycler®

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Objectives: Nosocomial infections with vancomycin-resistant enterococci (VRE) have become a significant problem in hospitals and institutions worldwide. The emergence and dissemination of VRE has prompted recommendations for surveillance and rapid detection of these organisms. However, the usefulness of surveillance cultures for VRE is limited by delayed definitive detection time (2–4 days), thereby, slowing the identification of patients requiring isolation to limit nosocomial spread. The objective of this study was to develop a real-time PCR screening test for detection of VRE directly from fecal samples in less than 1 h.
Methods: A multiplex PCR assay for real-time detection of VRE using the Smart Cycler® was developed by Infectio Diagnostic. This assay combines *vanA*- and *vanB*-specific PCR primer pairs and two molecular beacon probes targeting the respective amplified regions of *vanA* and *vanB*. Fecal material from rectal or perirectal swabs collected during a VRE surveillance program at the Massachusetts General Hospital were analyzed by (1) selective broth culture method and (2) our real-time PCR assay. The PCR assay was performed directly from the fecal material prepared with a simple 10-min specimen preparation protocol.
Results: A total of 62 rectal specimens were tested by both culture and PCR. VRE was detected in 12 specimens by culture. The 12 samples positive for VRE based on culture were also all detected by our PCR assay. As compared to culture, the PCR assay detected one additional VRE positive specimen for a sensitivity of 100% and a specificity of 98%. Among the PCR positive specimens, nine were positive for *vanA*, three were positive for *vanB* and one was positive for both *vanA* and *vanB*.
Conclusions: This real-time PCR assay for rapid VRE detection represents a powerful screening test for early identification of the colonized patients that should be helpful to control VRE transmission.

P719 Differentiation of *Leptospira interrogans* by IS1533-and IS1500-based PCR assay

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Leptospirosis, caused by pathogenic members of the genus *Leptospira*, is one of the most widespread zoonotic diseases in the world. Potential sources of infection can be known most easily by determining the serovar associated with an outbreak of the disease, because certain serovars are often associated with specific mammalian hosts, and with the severity and complication of the disease. Traditional serological typing of leptospiral isolates is a difficult and labor-intensive process involving the use of cross-absorption agglutination reactions. Growth of leptospires, particularly from clinical isolates, is a slow and labor-intensive process and is often unreliable. A rapid and accurate method for typing leptospires from clinical samples is essential for medical procedure and the control of leptospirosis. Recently, PCR methodology has been used as an alternative approach to typing leptospiral isolates.

Objectives: In this study, we evaluated the PCR-based assay that targets IS1533 and IS1500 sequences for identification of *Leptospira interrogans* sensu stricto serogroups.

Methods: The PCR assay was performed with the following serovars selected from the reference culture collection: *Icterohaemorrhagiae, Copenhageni, Grippotyphosa, Canicola, Pomona, Bataviae, Australis, Autumnalis, Hebdomadis, Castellonis* and *Cynopteri*. These serovars represent the serogroups known to be prevalent by serology in São Paulo, Brazil, although by isolation from clinical material the serovars *Copenhageni* and *Icterohaemorrhagiae* are the prevalent serovars. In addition, an array of microorganisms that frequently are associate with contaminated clinical material were used to contaminate other set of leptospiral cultures in order to verify the specificity of the primers.

Results: The PCR-based assay was unable to differentiate the serovars *Canicola, Bataviae, Australis, Autumnalis* and *Hebdomadis*. The serovars *Grippotyphosa* and *Castellonis* were differentiated by both set of primers. The primers that targets IS1500 sequences differentiated the serovars *Copenhageni* and *Icterohaemorrhagiae*.

Conclusions: The ability to discriminate some serovars will provide a useful tool in epidemiology. These preliminary results showed that the PCR will allow us to focus future pathogenesis studies on different strains of serogroup Icterohaemorrhagiae. The use of this assay eliminates the need for difficult and time-consuming conventional techniques.

P720 Rapid and sensitive detection of *Toxoplasma gondii* from clinical samples by a single-step PCR

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Objectives: *Toxoplasma gondii* can cause severe complications in immunocompromised hosts. Diagnosis of pathogen directly from the clinical samples is desirable. A sensitive two-step nested polymerase chain reaction (PCR) followed by post-PCR manipulation of the product, for example, liquid hybridization or colorimetric detection using internal probes is being used to detect *T. gondii*. Our aim was to develop a rapid, sensitive and simple PCR method to detect *T. gondii* directly from clinical samples.

Methods: Twenty clinical samples of amniotic fluid, cerebrospinal fluid (CSF), vitreous fluid and tissue were tested. DNA was prepared from the samples using Qiagen DNA mini kit with some modification. A portion of DNA sample was amplified by single PCR using specific primers for 35-fold repetitive B1 gene of *T. gondii*. To avoid the false-negative resulting from inhibition, a positive internal control PCR was run in parallel to amplify human β-globin gene, which is present in all biological samples. To detect the sensitivity of the method, we estimated the number of tachyzoid cell by microscopy and performed a series of PCRs on diluted materials. To ensure reproducibility, PCR was performed in duplicate with two different sample volumes. PCR products were analyzed on agarose gel electrophoresis. The procedure is performed in less than 5 h.

Results: Results of blind samples analysis using our method were in agreement with the results where samples were analyzed using two-step nested PCR followed by either liquid hybridization or colorimetric detection using internal probes. The detection limit of the method was as low as one parasite.

Conclusion: This PCR technique is a rapid, sensitive and simple method, which can be applied for amnion fluid, vitreous, CSF, tissue or blood samples without prior culture. A good primer designing and successful optimization of the PCR allowed simplifying the method to a single step PCR.

P721 PCR-restriction fragment length polymorphism for rapid identification of *Nocardia* spp. isolates

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Objectives: Nocardia is a well-recognized human pathogen. Traditional differentiation of *Nocardia* species has involved the evaluation of various biochemical reaction. These methods have not always been reproducible and have been inadequate for the identification of recently characterized *Nocardia* species (*N. farcinica* and *N. nova*), for which additional antibiogram and specialized biochemical data are required for differentiation. All of these methods are time consuming and laborious. The objective of this study is to evaluate the PCR-restriction fragment length polymorphism (PCR–RFLP) as a rapid method for rapid identification of *Nocardia* isolates.

Methods: DNA was obtained by suspending one loop full of bacteria in 300 mL of distilled water, subjected to sonication for 15 min, boiled in water bath for 15 min Primers Tb11 and Tb12 were used for the amplification of 439 bp fragment of the gene hsp 65. For restriction fragment analysis 20 µL of PCR products was digested by 5 U of either *MspI* or *HinfI*. The type strains *Nocardia asteroides* CECT 3051 and *N. farcinica* CECT 3053 were used as control strains.

Results: We identified four *Nocardia* isolates from clinical samples by this method: two *N. nova* isolates (one from a respiratory infection and the other one from skin infection), one *N. farcinica* and one *N. asteroides* (both were isolated from respiratory samples from the same patient, with 10 years interval between the first and the second isolate. The first isolate was identified 10 years ago by traditional methods as *N. asteroides*). The PCR–RFLP analysis of the isolates: (1) By digestion with *HinfI*, all isolates produced only one band of 439 bp (2) By digestion with *MspI*: *N. nova* produced three bands of 130, 110, 75 bp; *N. asteroides* produced three bands of 180, 145, 120 bp; and *N. farcinica* produced one band of 439 bp.

Conclusion: PCR–RFLP represents a rapid, sensitive, reliable and reproducible method for the identification of *Nocardia* isolates in clinical practice. By this method, *Nocardia* isolates can be identified within 8 h.

P722 Efficiency of PCR and RT-PCR for detection of *Treponema pallidum* DNA in plasma

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Objective: For improvement of detection of *T. pallidum*, it is necessary to use highly effective methods of molecular diagnostic. The PCR approach is able to detect single copy of DNA target. The application of RT-PCR with 16S RNA as a target may greatly increase the sensitivity of assay. The aim of our study was to evaluate these two approaches for detection of *T. pallidum* in plasma of infected patients.

Methods: Blood plasma samples from 157 patient with different stage of syphilis (14 cases of primary syphilis, 74 cases of secondary syphilis, 14 cases of secondary syphilis relapse, 51 cases of early latent syphilis and 4 cases of late latent syphilis) were tested for the presence of treponemal DNA and RNA. Nucleic acids were purified with silica-GuTC method. To increase the sensitivity of the assay bacteria from 1 mL of plasma were concentrated by centrifugation so that each PCR mixture contained nucleic acids from 250 µL of plasma. Isolation of nucleic acids, reverse transcription and amplification were carried out in presence of recombinant internal RNA and DNA controls. We designed oligonucleotide primers which amplified 273-bp fragment of the gene encoding 47-kDa lipoprotein and 382-bp fragment encoding 16S RNA gene of *T. pallidum*. The detection limit of PCR test was measured using the PCR-based limiting dilution assay with purified DNA *T. pallidum* Nichols strain. Working concentration of internal control was twofold higher of detection-limit value.

Results: Detection limit of the PCR assay with chosen primers was equal to four copies of treponemal DNA in PCR reaction mixture. In primary syphilis, the treponemal DNA and RNA were detected in 100% of samples. In secondary syphilis, DNA was detected in 73% and RNA in 78% of samples. In secondary syphilis, relapsers DNA and RNA were detected in 79% of samples. In early latent syphilis, the DNA and RNA were detected in 17 and 20% samples, respectively. In all, late latent syphilis samples neither DNA nor RNA were detected.

Conclusion: The efficiency of RT-PCR assay for detection *T. pallidum* 16S RNA in plasma in comparison with PCR assay is higher in some stages of syphilis. The active forms of syphilis (primary and relapse to secondary) are accompanied with increasing frequency of positive results in molecular biology tests.

P723 PFGE typing of *Salmonella* strains

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Objectives: Salmonellosis is the most common food- and water-borne disease in Spain. Serotyping is one of the methods frequently used for epidemiological studies. In our work, we have evaluated the usefulness of PFGE method for detecting DNA polymorphisms in *Salmonella* strains.
Methods: A total of 44 isolates were used in this study. Serotypes were analyzed at Instituto de Salud Carlos III, Madrid, Spain. Thirteen different serotypes were detected serotype anatum being the most common. The macrorestriction DNA of the isolates was performed by *Xba*I endonuclease and the digestion products were analyzed by PFGE using Contour-clamped Homogenous Electric Field System (CHEF III). Images were processed with TDI software and clusters analyzes were carried out using UPGMA algorithms. All computations were performed using the NTSYS program, version 2.0 (Exeter Software, NY, USA).
Results: Four isolates were untypeable by using the *Xba*I enzyme (one *S. goldcoast*, two *S. ohio* strains and one *S. brandenburg*). A total of 20 different *Xba*I pulsed-field profiles were observed for the other 40 typeable strains.
Conclusion: PFGE patterns allowed the discrimination of serotypes. Moreover, strains belonging to the same serotype showed different PFGE patterns disclosing a close-related homology, as they shared a high number of DNA fragments. These results indicate that PFGE technique may be used as a epidemiological tool for the subtyping of different *Salmonella* strains.

P724 Identification of *Salmonella enteritidis* strains of human and food origin by phage-typing and by analysis of plasmid profile

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Objectives: To identify the epidemiologically unrelated *S. enteritidis* strains isolated from clinical samples (stools, blood) ($n = 35$) and from food ($n = 38$) using the phage-typing and analysis of plasmid profile.
Methods: All isolates were phage-typed according to the international scheme of Ward et al. (1987) using of 10 standard bacteriophages. The plasmid DNA was isolated by the method of alkaline lysis (Maniatis et al. 1982). The samples were analyzed in 0.7% horizontal agarose gels electrophoretically at 60 V for 5 h and visualized by staining with ethidiumbromide. The plasmids harboring *E. coli* strain V517 was used as a control.
Results: The human strains belonged to four phage types – PT1, PT2, PT4, PT8 and the food isolates to five phage types – PT1, PT2, PT8, PT13a, PT26, respectively. The highest number of strains from both groups represented the strains of phage-type PT8 (88.6 and 76.3%, respectively). However, the strains were epidemiologically unrelated. Thirty-five human and 38 food strains could be divided into six groups by the analysis of plasmid profile. The 74.3% of human and 65.8% of food isolates harbored a 59-kb plasmid.
Conclusions: Although the *S. enteritidis* strains were epidemiologically unrelated, they belonged mostly to phage-type PT8, which has been the most frequent in the Slovak Republic since 1995. The plasmid analysis revealed six different plasmid profiles. The 59-kb plasmid found in the most of isolates corresponds, probably, to the serotype-specific virulence plasmid.

P725 Sensitivity and specificity of the polymerase chain reaction (PCR) method for the rapid diagnosis of meningococcal disease

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Objectives: The aim was to evaluate the sensitivity and specificity of the polymerase chain reaction (PCR) as a diagnostic tool for meningococcal meningitis compared to conventional methods.
Methods: A total of 574 cerebrospinal fluid (CSF) or blood samples collected between 1998 and 2000 from patients with symptoms of bacterial meningitis were examined. Each sample was analyzed by PCR and standard culture methods. Patients were classified into seven categories on the basis of their clinical findings: (1) culture positive ($n = 45$); (2) culture negative but positive for direct smear, antigen detection or stain of skin lesion ($n = 27$); (3) culture negative and antigen detection negative or not done ($n = 274$); (4) culture positive for other bacterial species ($n = 29$); (5) clinically diagnosed viral meningitis with ($n = 38$)and 6: without antibiotic treatment ($n = 105$); and (7) patients with no symptoms of meningitis (controls) ($n = 56$). Extraction of DNA from whole blood was carried out by IsoQuick extraction kit (ORGA, USA). CSF samples were boiled and a modification of the procedure of Zambardi et al. was used. PCR was carried out with the following primers: IS0116 specific for *N. meningitidis*. The gene amplified was *siaD* for serogroups B, C, Y, W-135. For serogroup A the amplified gene *orf2* was used. The amplification products were 650 bp (NM), 450 bp for serogroup B and 250 bp for serogroup C, 400 bp for serogroup A, 120 bp for serogroup Y and 120 bp for W-135.
Results: In category 1, 43 out 45 samples were positive by PCR yielding a sensitivity of 96%. In category 2, the overall sensitivity was 63% of the samples positive by PCR; 6/6 (100%) samples that were positive by direct smear were positive by PCR but only 1/3 (34%) positive by antigen detection were PCR-positive. Of the 18 samples in which Gram stain from skin lesions were positive, 10 (55.5%) were PCR-positive. For possible meningococcal disease, category 3, 170 (62%) specimens were confirmed by PCR. Two of the false positive results were obtained with blood samples from patients with septicemia due to *Escherichia coli* (category 4). For categories 5, 6, and 7 only 2 of 199 of samples were positive by PCR and the calculated specificity was 96.5%.
Conclusions: The sensitivity of the PCR method was 96% and the specificity was 96.7%. We conclude that the PCR method is an efficient approach in the surveillance of meningococcal disease when culture fails to isolate *N. meningitidis*.

P726 Prenatal diagnosis of toxoplasmosis: timing influences prediction

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Objective: The validity of prenatal diagnosis for congenital toxoplasmosis (CT) by PCR is still a vexed question. We enrolled 10 clinical centers in Italy to get established or supposed seroconversions to assess the validity of prenatal diagnosis on the basis of our standardized protocol for molecular and parasitological diagnosis on amniotic fluid. An accompanying card with all serological, therapeutic and ecographic data were requested for each diagnosis. Moreover, a serum sample was demanded to test serology at the moment of amniocentesis.
Methods: In this work, data concerning 660 prenatal diagnoses for CT in seroconverted pregnant women are analyzed. Diagnostic procedures have included PCR and *Toxoplasma* culture on amniotic fluid and maternal peripheral blood to assess both maternal and fetal infection. In a limited number of women, one or more amniocenteses were carried out and for a large amount of enrolled patients also IgG avidity test values were available.
Results: Results analysis showed that prenatal diagnosis carried out by molecular and parasitological methods can be indicative of vertical transmission when the molecular and parasitological results are correlated with time interval among maternal infection, avidity test, beginning of treatment and amniocentesis. Moreover, few false negatives were founded in our PCR analysis. PCR and cultural results were in agreement each other for positivity but positive data were obtained much more by molecular (37%) then by parasitological (7.5%) methods. If both tests are used for parasite detection in following amniocentesis a control therapy effectiveness can be achieved.
Conclusion: Our results suggest the following conclusions: (1) the predictive value for prenatal toxoplasmosis is high if both molecular and parasitological approaches are used; (2) positive prenatal tests are more associated to previous rather than to recent mother infections, serologically established; and (3) molecular and parasitological approaches are very good markers of therapy efficacy when two amniocenteses are carried out. Most serious danger could be caused by false-negative PCR, because the need for CT diagnosis is suggested by a serological maternal situation which could be too premature to check parasites in amniotic fluid. A later fetus infection could not be excluded in this situation and the need of specific preventive prolongation treatment must be emphasized.

P727 Identification by 16S rDNA sequence analyzes of *Ehrlichia* sp. and *Anaplasma marginale* in *Boophilus micropus* ticks in Tibet, China

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Objective: To identify ehrlichial pathogens in *Boophilus micropus* ticks in China.
Methods: The DNA samples (each sample was prepared from three ticks) of Boophilus micropus collected from Tibet and Sichuan of China were screened by a nested PCR analysis specific for amplification of 16S rRNA genes of tick-borne *Ehrlichia* spp. The 3′{!/~}-end and 5′{!/~}-end fragments of 16S rDNA were amplified from the positive samples in nested PCR by semi-nested PCRs, respectively, and the DNA fragments were cloned and sequenced.
Results: Sixteen of 43 DNA samples (37%) of B. micropus from Tibet were positive in nested PCR analysis, but 27 samples from Sichuan were all negative. Two kinds of 16S rDNA sequences were found by sequencing the 5′{!/~}-end fragments (~450-bp) of the positive samples, one sequence identical to that of *Anplasma marginale*, an etiological agent of cattle anaplasmosis, and other most similar to that of *E. chaffeensis*, an etiological agent of human monocytic ehrlichiosis. The almost complete 16S rDNA sequences (1501-bp) of the novel ehrlichial agent was obtained by linking its 5′{!/~}-end and 3′{!/~}-end sequences (1440-bp) based on their overlapping regions. In 16S rDNA sequence (1400-bp) comparisons with other members of the tribe Ehrlichieae and related species, the sequence of the novel ehrlichial agent was most similar to that of *E. chaffeensis* (Arkansas strain, GenBank: M73222), but they were different in 19 nucleotides (~1.4%).
Conclusion: Based on 16S rDNA analysis, the novel ehrlichial agent in B. micropus in Tibet of China is a new species of genus *Ehrlichia*, *Ehrlichia* sp. Tibet, that is most closely related to *E. chaffeensis*. *Ehrlichia* sp. Tibet is firstly identified in the *Boophilus micropus* ticks and whether it infects human or animals is well worth studying in future.

P728 Molecular diagnosis of *Legionella* infections using NASBA with 'real-time' detection

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Objective: Pneumonia caused by *Legionella* species has a poor prognosis unless it is diagnosed early. The objective of this study was the construction of a molecular assay for fast and reliable detection of *Legionella* species in clinical respiratory-tract specimens.
Methods: Oligonucleotide primers and a molecular beacon were derived from regions of the 16S ribosomal RNA that were conserved among the different *Legionella* species. For nucleic acid extraction and subsequent RNA amplification, reagents from the NucliSens® Basic Kit were used. To monitor amplicon formation throughout the amplification process, a molecular beacon was added to the reactions.
Results: Sensitivity of the 'real-time' NASBA assay was between 1 and 10 bacterial cells for most *Legionella* species. In simulated respiratory tract specimens, between 1 and 10 colony forming units (cfu) of *Legionella* pneumophila could be detected. Specificity of the primers and molecular beacon probe was demonstrated with 26 different microorganisms. Preliminary data on diagnosis of *Legionella* infections in clinical specimens ($n = 17$) indicated that positive results obtained by culture could be confirmed with this assay.
Conclusion: The NucliSens Basic Kit Legionella application with 'real-time' detection revealed a sensitive and specific method for the detection of *Legionella* pneumophila and other *Legionella* species.

P729 Improved detection of *Legionella pneumophila* in environmental water samples with a real-time PCR hybridization assay

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Objectives: *Legionella pneumophila* is the causative agent of Legionnaire's disease in more than 90% of cases. As culture-based identification of *L. pneumophila* is a lengthy process (3–10 days), DNA amplification techniques have been suggested as rapid, sensitive and specific alternatives for monitoring possible environmental reservoirs during routine surveillance and suspected outbreaks. This study evaluated an improved real-time LightCycler assay for the rapid detection of *L. pneumophila* in environmental water samples.
Methods: An existing real-time LightCycler assay based on detection of the macrophage infectivity potentiator (*mip*) gene was modified in an attempt to improve the sensitivity. The modified assay was used to test 100 environmental water samples received from a variety of sources for routine testing by culture. Also tested were 16 environmental water samples previously found to be culture positive for *L. pneumophila*.
Results: The improved assay had a detection limit of 10 μg (i.e. *c.* 2 cfu/assay), equivalent to 300 cfu/L in the original water sample. When compared with conventional culture, the assay failed to detect 10 samples containing *L. pneumophila* cell counts below this detection limit, but successfully detected samples with higher cell counts. One additional sample was repeatedly positive by the LightCycler assay but negative by culture. Of the 16 known culture-positive samples tested retrospectively, 14 were positive by LightCycler PCR. The two negative samples were estimated to contain 20 and 40 L pneumophila cfu/L by culture, i.e. again below the detection limit of the assay, although other samples also estimated to contain <50 cfu/L were detected successfully. No PCR inhibitors were detected in the two negative samples. A major advantage in comparison with culture was that the LightCycler assay generated results on the same day that a sample was received.
Conclusion: The LightCycler hybridization assay provides a promising rapid alternative to culture-based identification of *L. pneumophila* in environmental water samples. The assay seems to be most suitable for screening large numbers of samples during outbreak situations when there is an urgent need to identify possible heavily contaminated environmental sources of infection.

P730 Molecular diagnosis of *Chlamydia pneumoniae* infections by using NASBA with 'real-time' detection

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Objective: *Chlamydia pneumoniae* has been established as an important human pathogen causing acute respiratory illnesses such as pneumonia and bronchitis. Conventional diagnosis on the basis of serology or by isolation of the organism in cell culture is time consuming and laborious. An assay was developed for the detection of *C. pneumoniae* in respiratory tract specimens based on NASBA amplification and 'real-time' detection using a molecular beacon.
Methods: Oligonucleotide primers and a molecular beacon were derived from 16S ribosomal RNA sequences. For nucleic acid extraction and subsequent RNA amplification, reagents from the NucliSens Basic Kit were used. Amplicon formation was measured over time by a molecular beacon labeled with a fluorogenic reporter molecule.
Results: The sensitivity of the assay was determined at 10 molecules of synthetic RNA in the amplification reaction. Using simulated respiratory tract specimens, about 1000 molecules of synthetic RNA revealed a consistently positive test result. Cultured *C. pneumoniae* could be detected down to 0.01 inclusion forming unit (ifu). Specificity was demonstrated with 26 different microorganisms, including *C. trachomatis* and *C. psittaci*.
Conclusion: NASBA amplification using genus-specific primers could be combined with 'real-time' detection by a species-specific molecular beacon to reveal a sensitive and specific assay for the detection of *C. pneumoniae* in respiratory tract specimens.

P731 NASBA with 'real-time' detection for the molecular diagnosis of *Mycoplasma pneumoniae* infections

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Objective: *Mycoplasma pneumoniae* is a well-known causative agent of upper and lower respiratory tract infections. Culture-confirmed laboratory diagnosis is hindered by the slow growth kinetics of the organism and serology-based diagnosis often is retrospective owing to the requirement of both acute and convalescent-phase sera. Aim of this study was the construction of a molecular diagnostic assay for the detection of *M. pneumoniae* in clinical respiratory tract specimens.
Methods: Oligonucleotide primers and probes were derived from the 16S ribosomal RNA (rRNA). For nucleic acid extraction and subsequent RNA amplification, reagents from the NucliSens® Basic Kit were used. For the detection of amplicon formation during the NASBA process, a molecular beacon was added to the amplification reactions.
Results: Sensitivity of the assay was about 100 molecules of synthetic RNA in the amplification reaction. Simulated respiratory tract specimens spiked with cultured *M. pneumoniae* revealed a sensitivity of 5–50 color changing units (ccu). Specificity of the assay was demonstrated with 26 different micro-organisms. Analysis of clinical respiratory tract specimens for the presence of *Mycoplasma pneumoniae* confirmed results obtained by culture and was comparable to detection by an in-house PCR assay based on the P1 adhesin gene.
Conclusion: Sensitive and specific detection of *M. pneumoniae* could be achieved by NASBA amplification of 16S rRNA combined with 'real-time' detection using a molecular beacon.

P732 Development of real-time PCR assays for detection of *U. urealyticum, M. hominis, C. trachomatis, T. vaginalis* and *G. vaginalis*

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Objectives: There are many diagnostic methods for detection of *U. urealyticum, T. vaginalis, G. vagimalis, C. trachomatis* and *M. hominis* based on PCR. However, there is a little real-time PCR methods for detection of these bacteria. The aim of this study was to develop technologies for detection of these bacteria by means of real-time PCR; to develop software for automatic registration of received results. and to compare the results of the newly developed method with the results of routinely used PCR assay where the amplification products are analyzed on gel electrophoresis.
Methods: PCR, real-time PCR, gel electrophoresis.
Results: We developed strategies for detection of *U. urealyticum, T. vaginalis, G. vaginalis, M. hominis* and *C. trachomatis* DNAs based on the real-time PCR technique. SYBR Green I was chosen as fluorescent agent. The real-time PCR was performed on iQ iCycler (Bio-Rad). Specificity of PCR-amplified products was confirmed by melting curve analysis and comparison of melting temperatures of received products with known melting temperatures of specific products. These calculations were produced by special software developed in this study. This software allows to eliminate subjectivism in analysis of real-time PCR results, powerfully relieves and accelerates undertaking the analysis. To avoid false-negative results, we constructed specific internal controls for each newly developed assay. These internal controls were cloned into modified lambda phages. The length and melting temperature of internal control's amplification products significantly differed from analogous specific products. So, this approach allowed us to differentiate these products. The results of the developed assays were compared to the results of the routine PCR, which were developed and used in Central Research Institute for Epidemiology (see Table 1). The comparison of the results of two methods shows perfect agreement, which indicates that the developed assay is a reliable one.

Table 1 Resistance rates of *E. faecalis* and *E. faecium* to the antibiotics tested

	Number of samples	Positive sample (%) Real-time PCR method	Routine PCR method	Agreement (%)
U. urealyticum	3245	48	48	100
C. trachomatis	2970	7	7	100
M. hominis	1650	15	15	100
T. vaginalis	548	0.5	0.5	100
G. vaginalis	1247	34	34	100

Discussion: This study confirms that the real-time PCR method is powerful approach for scientific and diagnostic application. Absence of gel electrophoresis or hybridization steps totally eliminates risk of false-positive results owing to the contamination by amplification products. The developed software allows to simplify and accelerate analyzing the results. The real-time PCR can be used instead of routine PCR which is labor consuming and contamination dangerous.

P733 Detection of *Chlamydiae trachomatis* in cervical smear samples with determined HPV

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Objective: *Chlamydia trachomatis* causes infertility, ectopyc pregnancy, pelvic inflammatory disease/PID and conjunctivitis. Humane Papiloma virus/HPV/is a virus with broad spectrum effects. This particularly refers to HPV of high oncogenic potential because of its participation in cervical oncogenesis. The authors wanted to determine correlation between cervical HPV infection and isolation of *Chlamydia trachomatis* taken from female patients' smear of different levels of cervical intraepithelial neoplasia/CIN I–III/and ASCUS modifications.
Methods: Cytological analysis was used for 64 cervical samples – Papanicolau method. The samples were presented in accordance with valid Bethesda Classification. The samples were treated by HPV DNA/Digene Hybrid Capture II/.
Results: Out of 64 HPV positive samples, isolated *C. trachomatis* was found in 24/37 (5%) samples. Out of 50 HPV-positive group samples with high-degree risk/HR/, 18/36%/contained *C. trachomatis*. Frequency of *C. trachomatis* was registered in ASCUS modifications in 25%, CIN I 22%, CIN II 23.5% and CIN III 20.2% samples. We found 9% of isolated *C. trachomatis* in HPV-negative control group.
Conclusion: Research results showed correlation between HPV cervical infection and *C. trachomatis*. The higher the level of cell dedifferentiation/ASCUS CIN I-III/is, the higher number of cervical infections with *C. trachomatis*/is.

P734 APTIMA CT and APTIMA GC: discrete and confirmatory assays for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Objectives: *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infections are among the most common bacterial sexually transmitted diseases in the world. The Gen-Probe APTIMA Combo 2 Assay (AC2) is a commercially available amplified assay for the simultaneous detection and differentiation of CT and GC. Amplified assays like AC2 are extremely sensitive compared to commonly used methods such as culture and EIA. Because of this sensitivity and the large amount of amplicon these tests generate, some laboratories choose to confirm positive results by re-running the test. A better approach to validating positive results is to use a test that amplifies a different target sequence than originally targeted.
Methods: The APTIMA CT and APTIMA GC assays target different rRNA sequences from those of the AC2 assay. Because of the unique rRNA target molecules, APTIMA CT and APTIMA GC can be used to check positive specimens as well as direct, stand-alone tests for CT and GC.

Results: Analytical sensitivity of APTIMA CT for all 15 serovars was determined to be 1 ifu/reaction. Analytical sensitivity of APTIMA GC with 20 clinical isolates was determined to be 50 cells/assay. Analytical specificity was determined for both assays by evaluating 109 bacteria, fungi, yeast and viruses; no cross-reactions were detected. A performance qualification of the single APTIMA assays was run on endocervical and male urethral swabs and male and female urines with concordant positive and negative results (agreement of both reference methods) in AC2, LCx (CT, GC), PCR (CT), and GC culture. APTIMA CT and APTIMA GC detected 578/588 (98.3%) CT and 671/705 (95.2%) GC concordant specimens, respectively. These data demonstrate that the CT and GC individual assays accurately detect target rRNA. The assays also were used to check samples positive only in AC2 and negative with the reference tests. These could be false positive samples, samples with very low target levels subject to sampling errors, or samples positive because AC2 performs better than the reference tests. For CT and GC, respectively, 203/269 and 194/212 samples were positive with the APTIMA CT and/or APTIMA GC assays.
Conclusions: The data in this study show that the APTIMA CT and APTIMA GC Assays yield accurate detection of CT and GC and, as such, may have applications as both confirmatory and stand-alone diagnostic assays.

P735 PCR multiplex in the diagnosis of pulmonary opportunistic pathogens

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Udine, I

Objectives: the aim of this study is to evaluate the reliability of multiplex PCR for a rapid diagnosis of opportunistic pathogens in immunocompromised patients.
Methods: A total of 158 bronchoalveolar fluids were assayed. The patients were immunocompromised for AIDS, emathologic disorders, immunosuppressive therapy or intensive care. The samples were analyzed by optimized PCP–MPCR kit (Maxim Biotech Inc.) for the simultaneous detection of *Chlamydia pneumoniae, Legionella pneumophila Mycoplasma pneumoniae* and *Pneumocystis carinii*. One tube PCR was performed. The amplified fragments have a different size: 871 bp (*C. pneumoniae*), 700 bp (*L. pneumophila*), 375 bp (*M. pneumoniae*) and 300 bp (*P. carinii*). These fragments were easily distinguishable by electrophoresis in 2% agarose gel. All positive results were confirmed by a conventional PCR. The whole assay can be performed in a one-day routine.
Results: Sensitivity of PCP–MPCR for *P. carinii* seems to be greater than that of microscopy: 10 (6.3%) samples were positive by both immunofluorescence and PCP–MPCR but one patient submitted to liver transplant had a negative microscopy and a positive PCP–MPCR test. One positive assay for *L. pneumophila* was detected by PCP–MPCR and the same sample had a positive culture. *C. pneumoniae*, a common microorganism of the upper respiratory tract in absence of disease, is the most frequent pathogen (13.9%) in our study. Only six samples (3.8%) were positive for *M. pneumoniae*.
Conclusions: microbiological diagnosis of opportunistic pathogens in immunocompromised patients is very difficult. Immunofluorescent microscopy, cell culture or conventional cultures which take several days are required. PCP–MPCR assay is a more satisfactory diagnostic tool than traditional methods for an accurate, sensitive and rapid detection of the overcalled pathogens.

Methods in virology

P736 Comparative evaluation of three methods for serological diagnosis in EB-infection

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Objectives: To evaluate the following methods: indirect immunofluorescence assay, ELISA and Western blot for efficient diagnosis in acute EB-infection and to estimate sensitivity using WB-assay as the gold standard.
Methods: During 1-year period (12/99–11/00), 378 serum samples were obtained from children up to 14 years old—presented with clinical symptoms of acute mononucleosis. All samples were examined so as to detect IgM and IgG antibodies to VCA antigen by IFA (Stellar Biosystem) and ELISA (Gull Laboratories). WB assay (Blot 3.0 Genelabs Diagnostics) was performed on samples which were found positive for IgM antibodies by the other two methods. Both IgG and IgM antibodies to specific EBV antigens: VCA (p23), EA diffuse (p54), EA (p138), EBNA-1 (p72) were determined. Each method was carried out according to manufacturer's instructions.
Results: A total of 153 samples out of 378 were found positive for IgG antibodies by IFA and 130 by ELISA. WB detected 152 positive samples. ELISA sensitivity for IgG was 86%. IgM antibodies were positive detected by IFA in 40 samples, while ELISA detected 27 positive samples. Examining these serums by IgM-WB assay 34 out of 40 were positive. Sensitivity in IgM detection was 85% for IFA and 82% for ELISA. Furthermore, WB assay enables distinction among primary, past infection and reactivation of the virus. Thus, according to WB results the infection was primary in 76% of the patients, past in 18%, while in 6% of the patients the virus had been reactivated.
Conclusions: Both methods correlated well with WB. Indirect immunofluorescence assay proved to be slightly prevalent. These results indicate that IFA remains reference method for the everyday diagnostical practice. WB assay will be useful to resolve questionable cases and improve diagnostic potential of EB serology. However, its cost and time-consuming procedure doesn't permit yet its use as routine method.

P737 Novel markers facilitate the diagnosis of Borna disease virus infections in psychiatric patients and healthy carriers

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Objectives: Borna disease virus (BDV)—specific circulating immune complexes (CICs), their antigen part consisting of BDV N/P proteins, are detectable in blood plasma. They represent novel tools to monitor productive infection in psychiatric patients, and to identify healthy carriers.
Methods: Novel ELISA tests detect BDV-CICs, antigens (N/P) in plasma, or antibodies directed against N and P proteins, in the same blood sample. Their specificity is based on two monoclonal antibodies recognizing conserved conformational epitopes on either proteins (anti-N; anti-P).
Results: BDV, an unique enveloped RNA virus (Bornaviridae) with a broad host spectrum (e.g. horses, sheep, cats, cattle, and man), infects neurons of the limbic system, but also other cells of brain and blood. In animals, episodes of behavioral changes are a major pattern of overt disease, resembling recurrent mood disorders in humans. Infections persist, are dormant and productive in turns, and cause no structural damage. This matches with the hypothesis of a functional disturbance of the neurotransmitter network by viral components. Human infections and their possible association with major effective disorders raised world-wide interest, but inconstant findings of serum antibodies, and antigens and/or RNA in leukocytes weakened a linkage. Discrepancies can now be explained by CICs, the discovery of which was unexpected due to the low multiplicity of infection of BDV. In contrast to low amounts of infectious particles, N and P proteins, the major components of the viral core, were abundantly produced. Longitudinal studies of some thousand patients revealed that these proteins are released into the blood, followed by antibodies, and the formation of CICs. In acute major depression, almost 100% of the patients were CIC-positive, indicating a productive infection period, whereas previous serology missed the majority. Likewise, the CIC assay could detect 10-fold higher infection rates in healthy subjects (20–30%) than

previous antibody assays did, suggesting that the majority of infections may be harmless.
Conclusions: The discovery of novel infection markers (BDV-CICs) further supports an association of BDV infection and major mood disorders. These easy-to-use CIC tests are superior to antibody-only and RT-PCR approaches, and will considerably improve the identification of infected patients, as well as facilitate a monitoring of subclinical infections in the normal population.

P738 Evaluation of CMV IgG avidity on sera from patients with a primary CMV infection

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Objectives: Both primary and recurrent CMV infection during pregnancy can lead to congenital infection. Since congenital infection acquired after primary maternal CMV infection is more often associated with sequelae it is important to differentiate primary from recurrent CMV infection. The presence of CMV IgM antibodies is not always indicative of a primary infection. Additional tests to differentiate between primary and recurrent infection are necessary. In this study, we evaluate the evolution of CMV IgG avidity after primary infection.
Methods: Fifty-one sera from 30 patients, with a CMV seroconversion, and 27 sera from 27 patients, with a stable serology for at least 1 year, were analyzed. For each patient with a seroconversion, the time of infection was estimated. This estimation was based on the dates of the last negative serology, the first positive serum sample and the serological profile following seroconversion. All these sera were analyzed retrospectively with two commercial enzyme immunoassays, measuring CMV IgG avidity: BioMérieux: VIDAS CMV IgG AVIDITY and Bouty: BEIA CMV IgG AVIDITY. The results were interpreted according to the manufacturers' instructions. The cut-off values differentiating between an infection of less or more than 3 months were an index value of 0.8 for BioMérieux and a value of 25% for Bouty.
Results: Avidity above the cut-off value was not found during the first 12 weeks after CMV infection. Most samples obtained between 13 and 30 weeks after infection still show avidity values below the cut-off. Most samples from patients with a stable serology show avidity values above the cut-off.

	Weeks after CMV infection	*N*	Avidity		
			Index <0.8	Index ≥0.8	
BioMérieux	0–12	24	100%	0%	
	13–25	16	100%	0%	
	26–30	5	100%	0%	
	>50	6	17%	83%	
Control group		27	4%	96%	
			<25%	25–45%	>45%
Bouty	0–12	24	92%	8%	0%
	13–25	16	81%	13%	6%
	26–30	5	40%	40%	20%
	>50	6	0%	33%	67%
Control group		27	0%	4%	96%

Conclusion: This study demonstrates that avidity above the cut-off values 0.8 and 25% for BioMérieux and Bouty, respectively, excludes a recent infection, however, avidity values beneath these cut-off values do not necessarily indicate a recent infection.

P739 Evaluation of the ImmunoCard™ STAT ! RSV (IC-RSV) for the detection of RSV in pediatric nasopharyngeal aspirates

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Objective: To evaluate the sensitivity and specificity of the IC-RSV using the immunofluorescence (IF) on the specimens and culture by IF on inoculated shell vials as reference methods for the detection of RSV in nasopharyngeal aspirates (NPA).
Material and methods: During the 2000–2001 winter, 59 pediatric NPA collected in each of the Brussels and Antwerp Hospitals were investigated by three techniques: (1) the IC-RSV, a fast and simple immunochromatographic test, (2) IF with monoclonal antibodies on the specimen, and (3) culture by IF on inoculated, centrifuged shell vials with Hep-2 cells after 2 days incubation.
Results:

Table 1

	IF on specimen		Culture	
	+	−	+	−
IC-RSV+	48	2	37	14
IC-RSV−	12	56	8	59
Total	60	58	45	73
	se = 80.0%		se = 82.2%	
	sp = 96.5%		sp = 80.8%	

Conclusions: The sensitivity of IC-RSV compared with IF on the specimens and on shell vials is rather low: 80 and 82.2%, respectively. The specificity of IC-RSV is high (96.5%) compared with IF on the specimens but low compared with the shell vial assay. This, however, results from the well-known lower sensitivity of the latter. The ImmunoCard STAT ! RSV test cannot replace the IF reference for routine use but we could be helpful in special situations such as out of working hours to exclude a possible RSV infection, pending the results of the reference test.

P740 Comparison of three commercial methods for the characterization of the avidity of cytomegalovirus-specific IgG

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Objectives: Serological diagnosis of cytomegalovirus (CMV) infections is usually done by detection of specific IgM. However, this approach does not discriminate primary infections from reinfections or reactivations. Furthermore, cross-reactions and polyclonal stimulation of B lymphocytes can lead to CMV IgM false positive results. The assays for the characterization of the avidity of specific IgG allows the differentiation of primary and secondary infections. The aim of the present study is to compare the performance characteristics of three avidity assays for CMV-specific IgG.
Methods: Ninety-six samples were used for the comparison. The samples were selected on the basis of showing CMV-specific IgM (83 samples), or were taken from pregnant CMV seropositive women (13 samples). Three avidity assays were compared: Enzygnost anti-CMV Avidity (Dade Behring, Germany), Vidas CMV IgG Avidity (BioMérieux, France), and CMV IgG avidity EIA Well (Radim, Italy). The method from Dade Behring establish as cut off a reduction in the titer after treating the samples with the denaturating agent of 40%; in the case of BioMérieux the cut-off is a reduction of 80%. CMV IgM was tested by indirect ELISA (Dade Behring) and by a capture ELISA (Medac, Germany); CMV-specific IgG was measured by indirect ELISA (Dade Behring). The results obtained in Enzygnost and BioMérieux were compared to those obtained by Radim, since this method is currently used in our laboratory.
Results: In comparison with the method from Radim, Enzygnost recognize as low avidity samples 18 of 24 (sensitivity: 75%), and as high avidity 65/67 (specificity: 97%). Vidas recognize as low avidity 24/24 samples (sensitivity: 100%), but only 23/67 as high avidity (specificity: 34.3%). If a reduction of 50% is considered as cut off in this method, the figures are 23/24 (sensitivity: 95.8%) and 61/67 (specificity: 91%), respectively.
Conclusions: The methods studied are comparable tools for the characterization of the avidity of CMV-specific IgG. However, the cut off the method from BioMérieux must be redefined.

P741 Development of a CMV IgG avidity assay for the diagnosis of primary cytomegalovirus infection in pregnancy

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Background and objectives: Primary CMV infection during pregnancy is the commonest cause of congenital virus infection, resulting in severe fetal damage in 10% of cases. It is frequently asymptomatic. Unless a CMV-specific IgG seroconversion can be demonstrated, diagnosis is often difficult using CMV IgM alone as a marker of recent infection since it may persist for many months after acute infection. The maturation of CMV-specific IgG affinity over time, however, predicts that antibodies of high avidity will reflect a past infection, with acute infection presenting naïve antibodies of low avidity. We modified an existing CMV IgG assay and used serial samples from patients with acute CMV infection where the date of symptom onset was known to develop an in-house CMV IgG avidity test. We then compared our current CMV IgM testing method with this test in pregnant women attending the Fetal Medicine Unit for investigation of maternal illness or fetal/neonatal abnormalities.
Methods: The standard semiquantitative enzyme immunoassay (EIA) for CMV IgM (Eurogenetics) was performed and followed by the IgG avidity EIA (modified Biokit method). This involved an additional wash procedure using an 8 M urea solution to dissociate low avidity IgG antibodies. The avidity index (AI) was then calculated by comparing the binding of CMV IgG with normal and 8 M urea wash buffers.
Results: A total of 45 immunocompetent patients with symptoms and laboratory markers of acute CMV infection (increased liver enzymes/jaundice, blood lymphocytosis, malaise, lymphadenopathy) each provided a mean of two samples. These serial samples enabled us to estimate the rate of avidity development to be 4% (range 2–9%) per week during the primary infection. Over a period of 2.5 years a total of 16 women with a viral illness and/or fetal abnormalities had CMV IgM detected. Of the five women with high level CMV IgM, all had low avidity IgG antibodies consistent with current infection. Of the 11 who had low to moderate levels of CMV IgM, 5 (45%) had a high avidity index (AI), 6 (55%) having a low AI. Measurement of IgG avidity was found to be superior to the IgM result in defining an infection as acute or chronic. Out of the 13 pregnancies where fetal outcome was known, 6 were uninfected, 2 were terminated and 5 had signs of congenital infection.
Conclusion: The CMV IgG avidity index can be used to more accurately define the timing of CMV infection in pregnant women.

P742 Reduced need for HBsAg confirmation of Abbott Axsym HBsAg (V2) results

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Objectives: To assess if the S/N ratio from the Abbott Axsym HBsAg (V2) assay can reliably predict the results of confirmatory testing and thus, reduce the number of samples requiring confirmatory testing.
Methods: The Axsym assay uses a S/N cut-off >2.0 to define reactivity and the manufacturer suggests confirmation of all repeatedly reactive samples. Our present practice is to confirm samples with S/N < 10.0. Over a 8-month period, all serum samples submitted for HBsAg testing with initial HBsAg S/N from 1.6 to 10.0 underwent duplicate retesting after recentrifugation and then HBsAg confirmatory testing using the Abbott Auszyme Mono HBsAg confirmatory system on the Abbott Commander system. The initial and repeat S/N-values were noted and compared against the final confirmation result. Sensitivity, specificity, positive and negative predictive values for each S/N cut-off value were calculated and ROC curves constructed.
Results: Of 7324 HBsAg requests, 40 samples had S/N 1.6–10.0 of which eight were subsequently confirmed as positive. The areas under the ROC curves were: initial S/N 0.900 and mean of repeat duplicate S/N 0.895. At a S/N cut-off of 6.0, sensitivity was 50%, specificity 100%, positive predictive value 100%, negative predictive value 89%, test efficiency 90%.
Conclusions: By performing confirmatory testing only on samples with S/N 1.6–6.0, the number of samples requiring confirmation can be safely reduced by 10%. Such an approach can reduce cost and improve result turnaround time.

Quality of blood drawing

P743 Study to validate diversion and an improved donor arm disinfection procedure in reducing bacterial contamination in blood donation

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Introduction: Bacterial contamination of blood products is still the major cause of morbidity and mortality. The UK Serious Hazards of Transfusion (SHOT) surveillance system reported between 1995 and 2000, 15 incidents due to bacterial contamination with 5 associated fatalities. A study was therefore, undertaken to validate the effectiveness of diversion and an improved donor arm disinfection procedure in reducing bacterial contamination in whole blood donation.

Diversion is based on the concept that most bacterial contamination of blood components is skin derived: removal of the initial 20 mL containing these skin contaminants will reduce contamination.

An improved donor arm disinfection procedure consisting of a two-stage process of isopropyl alcohol followed by tincture of iodine has been shown to be 10 times more effective than the current procedure of an isopropyl alcohol wipe.
Methods: The study was performed in two stages, consisting of 1409 blood donations in each, one consisting of disinfection using the current procedure and the other the new improved technique.

Donations were collected using bags specifically manufactured for the validation of diversion. These bags incorporated two satellite pouches (in each of which 20 mL of blood was collected) welded to the normal collection system. Blood initially flowed into sample bag one (P1) representing the initial 20 mL of collection then into a second sampler bag (P2), which held a sample of the collection bag if diversion was in operation. Blood then flowed into the standard collection bag and the donation collected. The contents of the sample pouches were cultured anaerobically and aerobically.
Results: The intervention of diversion only gave a 52% reduction in contamination and improved donor arm disinfection only gave a 57% reduction. Diversion plus improved donor arm disinfection gave a 71% reduction in contamination.
Conclusion: In conclusion, the study validates diversion and an improved donor arm disinfection procedure. Combined these two interventions gave a substantial reduction in contamination, that should reduce bacterial transmission and result in a safer blood supply.

P744 Whole blood cytokine response as a measure of airborne microbial contaminations

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The pathogenic effects of inhaled environmental microorganisms as well as their pyrogens (especially endotoxins) for humans is increasingly recognized. Various syndromes are described after contact with air-borne microbial contaminations via the respiratory tract: Sick-building-syndrome, humidifier lung (a form of hypersensitive pneumonitis), 'monday sickness', among others.

Air-conditioning and ventilating systems intensify this problem, but storage of organic garbage in households also represents a considerable source of air-borne pollutants. In 1995, we described a new method for the detection of pyrogenic (fever-inducing) substances. This whole blood assay utilizes the natural reaction of the immune system in order to detect a broad spectrum of pyrogens (including Gram-positives and fungal spores) very sensitively in the relevant species.

Injectable drugs are the main area of application in which this test has already proven effective and is currently validated in a collaborative study in collaboration with European Pharmacopoeia. We adapted this whole blood system for different filter materials, which are used in air-control pump systems. We show that it is possible to measure the cytokine response after incubating the contaminated filters directly with whole blood. The measurement was performed with samples from different surroundings, stables of pigs, sheep and cows and in flats. Air-borne pyrogens up to 3×10^6 international units per square meter (300 µg) were found at such work places. Microbiological burden with fungal and bacterial colony forming units were roughly correlated with the whole blood response. With this integral attempt, it might be possible to correlate contaminated air and the resulting reaction of the immune system, indicating therefore, a potential risk for the person exposed.

P745 National monitoring of the bacterial contamination rate of blood products using the BacT/ALERT system

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Objective: Bacterial transmission is still the major cause of morbidity and mortality associated with transfusion transmitted infection. The UK Serious Hazards of Transfusion surveillance system reported 15 incidents due to bacterial contamination, 5 of which were fatal. Internal reorganisation of the National Blood Service (NBS) of England resulted in the establishment of a National Bacteriology Laboratory with the remit to provide a centralized bacteriological service. The laboratory was given the task to determine the bacterial contamination rate on a 'truly' national basis. Prior to the establishment of the National Bacteriology Laboratory, a collection of blood centers monitored the bacterial contamination rate of blood products, but for the majority this was unknown.
Methods: Time expired red cells and platelet concentrates are sent from the 10 blood processing sites within the NBS. The BacT/ALERT 3D automated culture system was utilized in the study. Aerobic and anaerobic culture was performed, 5–10 mL of blood product was inoculated into standard BacT/ALERT culture bottles and incubated for 7 days at 37 °C. Initial reactive samples were confirmed by retesting the indicated units. All isolates obtained were identified to genus and species level.
Results: From April 1999 to October 2001, 5679 platelet concentrates have been tested with a 0.5% (1 in 210) confirmed positive rate. Pooled platelet (3766 tested) and apheresis platelet concentrates (1913 tested) gave a confirmed positive rate of 0.6% (1 in 171) and 0.3% (1 in 383), respectively. The red cell confirmed positive was 0.1% (1 in 1394) from testing 4183 units. Skin flora were almost exclusively isolated.
Conclusion: The bacterial contamination rate was found to be extremely high particularly in comparison to viral markers screened for by the blood service. Interventions such as an improved donor arm disinfection technique and diversion of the initial 20 mL of donation from the collection bag are now planned to be implemented by the NBS to reduce bacterial-transfusion transmission. Monitoring will indicate the effectiveness of these interventions.

P746 Contaminated blood cultures: is it worthwhile to use alcoholic chlorhexidine in skin preparation?

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Introduction: Despite all the new blood culture methods, contamination of cultures continue to cause problems with the interpretation of test results. A proper skin preparation is one of the most important measures to reduce the rate of contamination.
Objective: To evaluate the impact of changing the bactericidal agent (povidone-iodine to alcoholic chlorhexidine) for skin antisepsis in the contamination rate of blood cultures.
Setting: General Intensive Care Unit (ICU) and General Ward of a private Community acute-care Hospital in Lisbon (SAMS).
Methods: From November 1997 to March 1998, an aqueous solution of 10% povidone-iodine was used for skin preparation before blood culture collection. After April 1998 antisepsis with an alcoholic solution of 0.5 chlorhexidine, prepared in the hospital pharmacy, was implemented.
Results: The contamination rate in UCI and General Wards was, respectively, 7% (3/41) and 13% (22/167) at our Institution between November 1997 and March 1998. The rate of contamination decrease was 5% (5/104) in ICU and 6% (15/232) in General Wards by the end of 1998. In 1999, the rate was 3% (6/219) in ICU and 7% (22/334) in General Wards. ICU maintained 3% (6/240) in 2000 and General Wards decrease was 3% (10/296).
Conclusions: The use of alcoholic chlorhexidine indeed contribute for our objective: decrease blood cultures' contamination rate. Motivation of the staff and feedback are other factors that also contribute to this purpose, and may be an explanation for the delay of General Ward in achieving the recommended contamination rate (2–3%).

New drugs I

P747 Oxapenem XOB (AM-114): in vitro activity alone and in combination with amoxycillin or cefaclor against community-acquired respiratory tract pathogens

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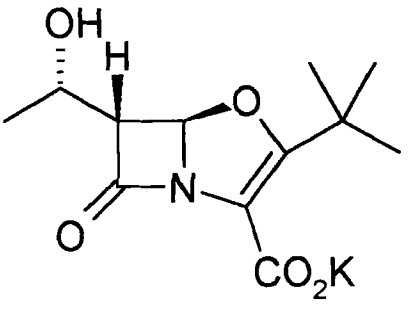
Background: Resistance to β-lactams is a rapidly emerging problem among common community-acquired respiratory tract (RT) pathogens. XOB is a novel, orally absorbed, oxapenem with both antibacterial and broad-spectrum β-lactamase inhibitory properties. The present study reports on the in vitro activity of XOB alone and in combination with amoxycillin (AMX) or cefaclor (CCL) against isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* with characterized resistance phenotypes.
Methods: MICs of each agent alone and combinations of amoxycillin or cefaclor with XOB, either 2:1 ratio or using sub-MIC concentrations of XOB, were determined (NCCLS guidelines). Amoxycillin/clavulanate (AMX/CLV) was included as a control.

Results: MICs for XOB alone and in combination with AMX or CCL are summarized here:

	MIC (mg/L)						
	S. pneumoniae			*H. influenzae*			*M. catarrhalis*
Phenotype	Pen-S	Pen-I	Pen-R	β-la−	β-la+	BLNAR	β-la+
N	5	6	4	5	5	4	6
Agent alone							
XOB	1–2	1–2	≥64	8–16	8–16	8–64	0.12–1
CCL	0.25–0.5	0.25–1	≥64	0.5–1	4–16	4–64	1–2
AMX	≤0.06	≤0.06	0.5–2	0.12–0.25	>64	1–16	4–16
Agents in 2 : 1 combination							
CCL + XOB	0.12–0.25	0.25–1	32–>64	1–2	1–2	4–16	0.12–0.25
AMX + XOB	≤0.06	≤0.06	0.5–2	0.12–0.25	2–4	1–8	≤0.06–0.25
AMX + CLV	≤0.06	≤0.06	0.25–2	0.12–0.25	0.5–1	1–16	≤0.06–0.12



Conclusion: XOB possesses similar activity to cefaclor against RT pathogens. Combinations of AMX and XOB are as potent as amoxycillin/clavulanate against RT pathogens.

P748 Oxapenems: unexpected synergistic activity with other β-lactams against *Enterococcus* spp.

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Background: Enterococci are responsible for an increasing number of nosocomial infections. Isolates are generally β-lactamase negative but resistant to cephalosporins. Oxapenems are broad-spectrum β-lactamase inhibitors, which exhibit little antibacterial activity except against *S. aureus* and common respiratory tract pathogens. We report an unexpected synergy between oxapenems and other β-lactams against *Enterococcus* spp.
Methods: MICs and bactericidal activity of agents alone and in combination, including checkerboard studies, with oxapenems against vancomycin sensitive (Van-S) and resistant (Van-R) *Enterococcus* spp. were determined to NCCLS guidelines. Affinity for penicillin binding proteins (PBPs) of *E. faecalis* SFZ was determined in a competition assay with 3H-propionylampicillin.
Results: Oxapenems AM-112 and AM-113 possessed variable activity against *Enterococcus* spp. (MICs 8 to >64 and 2 to >64 mg/L, respectively), but at 1:1 ratio or fixed 4 mg/L, exhibited synergy (>4-fold reduction in MIC of both components) in combination with ceftazidime. Their C1 prime stereoisomers, AM-114 and AM-115 and clavulanic acid were inactive. AM-112 exhibited synergy or additivity in combination with six other cephalosporins tested, including a 16-fold reduction in ceftazidime MIC against an *E. faecalis* Van-R (vanB) isolate. Synergy was more pronounced in viable count studies against Van-S and Van-R isolates. Little evidence of synergy was noted between AM-112 and imipenem or ampicillin. AM-112 exhibited significant binding to all enterococcal PBPs at concentrations of 0.095–2.6 mg/L but required higher concentrations to totally inhibit PBP3.
Conclusion: Some oxapenems show unexpected synergistic activity with cephalosporins against enterococci. The synergistic effect may be attributable to complementation at the PBP level.

P749 Comparison of in vitro oxidative action of Cu(II)-aminoglycosides complexes with their bactericidal efficacy

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Objectives: Aminoglycoside antibiotics are potent copper(II) ions chelators. They form stable complexes in physiological conditions and the resulting species are capable of hydrogen peroxide conversion to hydroxyl radicals. These complexes were screened for their antibacterial activity.
Methods: Nucleic base oxidation by Cu(II)-aminoglycosides was studied by HPLC assay. RNA and DNA damage was followed by polyacrylamide and agarose gel electrophoresis. Bactericidal action of kanamycin A and B, amikacin and sisomicin complexed with copper(II) ions was tested in time–kill assay, against bacterial strains of *Escherichia coli* (PCM 2427), *Staphylococcus aureus* (PCM 2054) and *Pseudomonas aeruginosa* (PCM 2058).
Results: The process of reactive oxygen species generation is responsible for nucleic base oxidation and plasmid DNA nicking, linearization and further degradation. Cu(II) complexes of aminoglycosides are also proved to cause highly specific cleavages in the anticodon loop of tRNA Phe. These complexes, in our studies, did not increase the bactericidal action of antibiotics.
Conclusions: A discovery of a very high catalytic activity of Cu(II) complexes of amikacin, kanamycin A and B, as well as sisomicin in the reaction of 2′-deoxyguanosine hydroxylation and nucleic acids cleavage were of particular importance. Oxygen activation by these complexes opens up new perspectives for their application. It may constitute the intracellular activity of these drugs, affecting therapeutic and toxic properties. Further studies are needed to estimate microbiological efficacy and clinical significance of the complexes tested.

P750 In vitro activity of the oxazolidinone AZD2563 against vancomycin-resistant enterococci determined by time–kill methods

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Objectives: We have previously shown that the new oxazolidinone, AZD2563, inhibits growth of a broad range of enterococcal isolates at concentrations less than or equal to 2 mg/L. In general, AZD2563 was two-fold more active than linezolid (LZD). In the present study, we examined the potential in vitro bactericidal activities of the two oxazolidinones against vancomycin-resistant enterococci (VRE) using time–kill methods.
Methods: Bactericidal activities of AZD2563 and LZD were determined in 20 mL volumes of Mueller–Hinton II broth against 10 strains of VRE: five *Enterococcus faecalis* (four vanB, one vanA) and five *E. faecium* (four vanA, one vanB). For each isolate, the antimicrobials were examined at 2 and 4× the MIC of each compound. Starting inocula were approximately 5×10^6 cfu/mL. Flasks were sampled at 4, 24 and 48 h of incubation at 35 °C; samples were serially diluted in saline and spotted in duplicate onto blood agar plates for colony counting.
Results: At concentrations representing 2 and 4× the MICs determined previously by standard broth microdilution methods, both AZD2563 and LZD inhibited the growth of VRE for up to 48 h of incubation at 35 °C. Neither agent demonstrated bactericidal activity (i.e. 3-log 10 reduction in cfu/mL) against any strain. The mean changes in log 10 cfu/mL relative to the inoculum at the given multiple of MIC are shown in table.

	Sample at 24 h		Sample at 48 h	
Agent	Mean	Range	Mean	Range
AZD2563 2 × MIC	−0.05	+0.27 to −0.58	+0.11	+1.22 to −0.73
AZD2563 4 × MIC	−0.25	+0.01 to −0.84	−0.32	+0.19 to −1.15
LZD 2 × MIC	−0.17	+0.20 to −1.26	−0.28	+0.31 to −1.80
LZD 4 × MIC	−0.34	+0.05 to −1.21	−0.71	−0.08 to −1.94

Conclusions: This study confirmed the bacteriostatic effect of AZD2563 against VRE. Neither AZD2563 nor LZD demonstrated bactericidal activity over a 48-h period of incubation.

P751 In vitro activity of AZD2563 in combination with various antibacterial agents

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Objectives: AZD2563 is a new oxazolidinone, with targeted activity against Gram-positive bacteria including multiresistant strains. Combinations of antibiotics may be used to enhance antibacterial activity. Combinations known to act synergistically may also be employed to enhance the antibacterial potency against known pathogens. It is therefore, important to test the synergistic potential of any new agent and identify any antagonistic interactions, which may jeopardize treatment.
Methods: The in vitro activity of AZD2563 alone and in combination with up to 19 antibiotics representing all major classes, including β-lactams (including meropenem), glycopeptides, aminoglycosides, macrolides and quinolones, was evaluated against multiple strains of staphylococci,

pneumococci and enterococci. The strains included multiresistant *Staphylococcus aureus*, vancomycin-resistant enterococci and penicillin-resistant *S. pneumoniae*. By using the checkerboard broth dilution method, drug interactions were determined and expressed as fractional inhibitory concentrations (FIC) indices.

Results: FIC indices in the range ≥ 0.5 to ≤ 4 denoting indifference/additive, were recorded for AZD2563 in combination with every antibiotic tested, against all 21 strains of *S. pneumoniae*, including 9 penicillin-resistant strains. This result was also obtained against 10 strains of *S. epidermidis*. AZD2563 combinations also demonstrated indifference against 23/24 strains of *S. aureus* and 19/23 strains of enterococci. Synergism (FIC ≤ 0.5) was seen in five instances with AZD2563 in combination with clarithromycin, against 1/24 of *S. aureus*, 3/13 *Enterococcus faecalis* and 1/10 *E. faecium* strains. One example of synergism was recorded against 1/13 strains of *E. faecalis* when AZD2563 was combined with meropenem.

Conclusion: These results demonstrate that AZD2563, combined with various antibacterial agents, produces interactions that are primarily indifferent/additive. No antagonistic responses were recorded against any of the strains tested.

P752 Activity of AZD2563, a new oxazolidinone, against worldwide Gram-positive isolates

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Objectives: The increasing number of drug-resistant Gram-positive organisms worldwide is well known and is severely limiting treatment options. This poster examines the activity of AZD2563, a new oxazolidinone, against worldwide Gram-positive isolates.

Methods: Bacterial isolates were obtained from worldwide sources. In vitro susceptibility against AZD2563 and comparators including linezolid, quinupristin/dalfopristin, erythromycin, vancomycin and levofloxacin was determined according to NCCLS criteria.

Results: A total of 5643 Gram-positive isolates were examined. All isolates were inhibited by AZD2563 at ≤ 4 mg/L and, all except two penicillin-resistant *Streptococcus pneumoniae*, were inhibited by linezolid at the same concentration. However, at ≤ 1 mg/L, AZD2563 inhibited 82% of all isolates whereas linezolid only inhibited 57% of isolates. Against *S. pneumoniae* ($n = 1337$ isolates) 41% were penicillin-resistant and MIC_{90} for AZD2563 and linezolid were 1 and 2 mg/L, respectively. This improved in vitro potency of AZD2563 against linezolid was also seen against *Enterococcus faecium* ($n = 626$ isolates; MIC_{90} 2 versus 4 mg/L, AZD2563 versus linezolid, respectively), while similar activity was demonstrated against *E. faecalis* ($n = 724$ isolates; MIC_{90} 2 mg/L for both compounds). The isolates tested also included methicillin-susceptible (MS) *Staphylococcus aureus*, methicillin-resistant (MR) *S. aureus*, MS *S. epidermidis*, and MR *S. epidermidis* (see table).

	AZD2563		Linezolid	
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
MS *S. aureus* ($n = 523$)	1	2	2	2
MR *S. aureus* ($n = 591$)	1	1	2	2
MS *S. epidermidis* ($n = 115$)	0.5	1	1	1
MR *S. epidermidis* ($n = 174$)	0.5	2	1	2

Conclusion: AZD2563 is a promising new oxazolidinone which in many instances shows a 1–2 dilution improvement over linezolid.

P753 Low in vitro selection frequencies of enterococcal and staphylococcal mutants resistant to novel oxazolidinone AZD2563

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Objectives: Oxazolidinones are a novel class of antibacterial agents active against most Gram-positive genera. This study aimed to determine the frequency with which mutants resistant to novel oxazolidinone AZD2563 could be derived in vitro from clinical isolates of major nosocomial Gram-positive pathogens.

Methods: Five clinical isolates each of *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) were studied. For each isolate, 10^8 cfu were plated onto Mueller–Hinton agar plates containing two-fold increasing concentrations of AZD2563. These plates were examined for the appearance of colonies daily for 4 days, which were subcultured on drug-free MH agar. In parallel experiments, 10^9 cfu of each isolate were inoculated into MH broth containing 0.5× MIC AZD2563. After overnight incubation, a sample of each turbid broth was subcultured in fresh broth containing a two-fold increased concentration of antibiotic. This was repeated daily, with further two-fold increases in AZD2563 concentration, until the broths failed to support visible bacterial growth. Growth from the highest concentration to show turbidity was then subcultured on drug-free MH agar. The identification of all suspected mutant colonies was checked, pulsed-field gel electrophoresis was used to confirm that mutants were derived from the parent strains, and MICs of AZD2563 and linezolid were determined.

Results: No mutants resistant to AZD2563 were generated from any species in the broth enrichment experiments. The plate selection protocol failed to generate mutants from *E. faecium*, *S. aureus*, or CNS. However, mutants were obtained from two *E. faecalis* strains, with MICs (AZD2563 MIC, 8 mg/L; linezolid MIC 4 mg/L) four-fold higher than those for the parent.

Conclusion: Mutants with raised MICs of oxazolidinones were selected from occasional isolates of *E. faecalis*, but at frequencies of $<10^{-8}$. Resistance was not selected in isolates of *E. faecium* or staphylococci.

P754 AZD2563, a new oxazolidinone: bactericidal activity and synergy studies with gentamicin and vancomycin against staphylococci and streptococci

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Background: Emerging resistances among Gram-positive organisms has limited treatment options and stimulated development of new antimicrobial classes such as oxazolidinones. This report summarizes comparative cidal activity and synergy studies of AZD2563 and linezolid (LZD) using non-pneumococcal streptococci and staphylococci.

Methods: Kill-curves were determined for AZD2563 at concentrations two-, four-, and eight-fold the measured MIC. Drug interactions (synergy) were evaluated also by kill-curve techniques using AZD2563 (4× MIC) combined with subinhibitory concentrations of either gentamicin (MIC/4) or vancomycin (MIC/4). Tested organisms were: *S. aureus* (one methicillin-susceptible [MS], two methicillin-resistant [MR]), CoNS (one MS, one MR), viridans gr. (three; penicillin-susceptible, -intermediate, -resistant) and β-hemolytic streptococci (two; gr. A and G; macrolide-susceptible and -resistant).

Results: Consistent patterns of cidal activity was observed for both organism groups and the two oxazolidinones (LZD by earlier experiments): (1) static action; and (2) 30% of strains showing a modest concentration-dependent inhibition. AZD2563 plus vancomycin combinations were indifferent and remained static versus both genus groups. Also static action and indifferent interactions were noted for AZD2563 plus gentamicin versus the five staphylococci tested. In contrast, all streptococci were rapidly killed (>3 log 10 cfu killing; cidal action) by AZD2563 plus gentamicin. No antagonism was observed and AZD2563 findings were the same as prior experience for LZD.

Conclusions: AZD2563, a long-acting oxazolidinone with once-daily dosing, was observed to be predominantly static in action, and generally was indifferent when combined with an aminoglycoside or glycopeptide. Cidal effects were only noted for streptococci when AZD2563 was combined with gentamicin. AZD2563 appears to possess oxazolidinone class characteristics (like LZD) and PK/PD features favorable to continued development.

P755 Concentrations of gemifloxacin in potential sites of respiratory infection in patients following once-daily 320 mg dosing for 4 days prior to diagnostic bronchoscopy

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Objectives: Gemifloxacin (GEM) is an advanced-generation, enhanced-affinity fluoroquinolone with excellent activity against Gram-positive cocci,

in particular *Streptococcus pneumoniae*. It has been shown to have higher affinity than other quinolones for topoisomerase IV, including isolates displaying resistant mutations. The aim of this study was to measure concentrations of GEM in respiratory samples following multiple once-daily dosing of 320 mg in patients undergoing elective fiber-optic bronchoscopy.

Methods: Twelve patients were enrolled into the study; however, data for two patients are not included in the analysis as one was shown to have an active lung infection and the other became distressed and the bronchoscopy procedure had to be halted. Samples were collected by standard lavage and biopsy procedures. A validated microbiological assay was used to measure concentrations of GEM in plasma, epithelial lining fluid (ELF), alveolar macrophages (AMs) and bronchial mucosa (BM).

Results: Data for the 10 evaluable patients are shown in the table below.

Trial no.	Time after last dose (h)	Plasma level (mg/L)	ELF		AMs		BM	
			Level (mg/L)	S:P	Level (mg/L)	S:P	Level (mg/kg)	S:P Level (mg/kg)
003	2.0	1.72	2.50	1.50	60.4	35.1	1.30	0.8
007	2.1	1.20	0.62	0.50	NDL	–	1.60	1.3
012	3.0	0.37	NDL	–	34.2	92.4	NS	–
010	3.3	1.40	0.90	0.60	57.5	41.1	1.50	1.1
009	3.9	0.70	0.90	1.30	46.0	65.4	1.30	1.9
004	4.2	0.80	1.00	1.30	24.0	30.0	1.30	1.6
002	4.8	0.83	0.71	0.86	10.4	12.5	1.47	1.8
005	11.4	0.50	0.20	0.40	NDL	–	NDL	–
011	12.4	0.38	0.20	0.50	77.9	205.0	NDL	–
006	12.8	0.70	0.50	0.70	29.7	42.4	0.80	1.1

S:P, site:plasma; NDL, no detectable level; NS, no sample.

Conclusion: Measurable concentrations of GEM in ELF, AMs and BM exceeded the GEM $MIC_{[90]}$ for the common respiratory pathogens *S. pneumoniae* (0.06 mg/L), *Haemophilus influenzae* (0.008 mg/L) and *Moraxella catarrhalis* (0.015 mg/L) in most samples for a 12-h period after dosing.

P756 In vitro activity of gemifloxacin against multiresistant and invasive clinical isolates of *Streptococcus pneumoniae* from diverse UK hospitals

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Objectives: Gemifloxacin is an advanced-generation, enhanced-affinity quinolone with potent in vitro antipneumococcal activity. We evaluated its comparative in vitro activity against 1082 recent clinical isolates of *Streptococcus pneumoniae* from 167 geographically diverse UK hospitals, comprising 201 isolates referred primarily for confirmation of resistance to first-line therapeutic agents and 881 isolates obtained during continuous national surveillance of invasive pneumococcal infections.

Methods: MICs were determined in air on Diagnostic Sensitivity Test agar supplemented with 5% lysed horse blood.

Results: Among referred isolates, the prevalence of resistance to penicillin, erythromycin and tetracycline was 87.1% (42.3% intermediate and 44.8% fully resistant), 48.8 and 40.8%, respectively. The corresponding prevalences among surveillance isolates were 5.1% (3.4% intermediate and 1.7% fully resistant), 14.4 and 5.8%, respectively. Seventy (34.8%) of the referred isolates and 12 (0.2%) of the surveillance isolates were resistant to penicillin, erythromycin and tetracycline. Modal MICs of gemifloxacin, moxifloxacin and ciprofloxacin were 0.06, 0.25 and 2 mg/L, respectively, for the referred isolates and 0.06, 0.25 and 4 mg/L for the surveillance isolates; corresponding $MIC_{[90]}$ were 0.125, 0.5 and 4 mg/L for both groups of isolates. Apart from one referred isolate with a gemifloxacin MIC of 1 mg/L, the MICs of the drug were $\leq$0.015–0.25 mg/L.

Conclusions: Gemifloxacin was the most active quinolone tested and generally retained full activity against multiresistant isolates.

P757 Activity of ABT 773 against *Staphylococcus epidermidis* biofilms on plastic biomaterials

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Objectives: Bacterial biofilms are commonly resistant to antimicrobial agents. The in vitro activity of a new ketolide, ABT 773, against *S. epidermidis* biofilms on siliconized latex catheters was evaluated. The effect of this agent on *S. epidermidis* adherence to this biomaterial was also assessed.

Material and methods: Two *S. epidermidis* strains (one slime producer and one non slime producer) were used. Bacterial biofilms were prepared by incubating 1-cm length catheter segments and bacteria (10^5 cfu/mL) in Mueller–Hinton broth (MHB) for 24 h at 35 °C. Catheter containing the bacterial biofilms were incubated in MHB for 24 h containing different concentrations of ABT 773 (1× MIC, 4× MIC and 8× MIC). At this time, catheter segments were washed and sonicated to remove adherent bacteria. Viable bacteria were counted by a colony counting method. In other series of experiments, the effect of subMIC of ABT 773 on the adherence of *S. epidermidis* to plastic biomaterials was evaluated. For these assays, either catheter segments or bacteria were preincubated in the presence of 1/4 and 1/8 MIC of ABT 773. Afterwards, bacterial adherence was performed as described above.

Results: ABT 773 at 1× MIC, 4× MIC and 8× MIC significantly decreased the bacterial viability of 24 h-biofilms ($2.5 \pm 0.6 \times 10^3$, $2.5 \pm 0.3 \times 10^3$ and $1.5 \pm 0.5 \times 10^3$ cfu/cm^2, respectively, compared to controls without antimicrobial agent: $6.7 \pm 1.9 \times 10^3$ cfu/cm^2) for the slime producing strain. Preincubation of slime producing *S. epidermidis* with subMIC of ABT 773 significantly decreased bacterial adherence to the catheter (281 ± 117 cfu/cm^2 for 1/4 of MIC, 316 ± 141 cfu/cm^2 for 1/8 of MIC and 493 ± 123 cfu/cm^2 for control). These effects were not observed with the slime non-producing strain.

Conclusions: The new ketolide, ABT 773, showed in vitro activity against a *S. epidermidis* strain (slime producer) biofilm. This agent also inhibited the adherence of this strain to a siliconized latex catheter.

P758 In vitro activity of linezolid against MRSA clinical isolates using E-test strip method

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Objective: The aim of this study was to investigate the in vitro activity of linezolid against methicillin-resistant *Staphylococcus aureus* (MRSA) from patients suffering community or nosocomial infections, as an alternative therapy in an 660-bed tertiary hospital where there is a wide spread use of vancomycin for treating this kind of infections.

Methods: Between April 2000 and September 2001, we isolated consecutively a total of 125 clinical strains of MRSA in our laboratory, from an equal number of patients. Identification and susceptibility testing to other antibiotics than linezolid was performed by the VITEK II (bioMèrieux) automatized system. Methicillin resistance was proved by standard oxacilin disk-diffusion test and gene *mec-A* detection. Linezolid activity was performed by E-test strip method.

Results: A total of 38.4% of the strains were isolated from wound infections, 33.6% from tracheal aspirates and sputum, 11.2% from blood, 11.2% from urine and 5.6% from other locations. These strains were found most frequently in ICU (24%) and medical wards (52.8%). 8% of the strains were sensible to clindamycin, 10.4% sensible to erythromycin, 4% sensible to tobramycin, 84% sensible to tetracyclin and 100% were sensible to trimethoprim sulfametoxazole, teicoplanin and vancomycin. Linezolid showed MIC ranged between 0.25 and 1.5 mg/L ($CMI_{50} = 1$, $CMI_{90} = 1$).

Conclusion: Based on our in vitro data linezolid appears to be very active against methicillin-resistant *S. aureus* and can be a viable alternative to vancomycin in treatment of this kind of patients.

P759 Superiority of linezolid over ceftriaxone/cefpodoxime for the treatment of hospitalized patients with pneumonia: analysis using a severity of illness system

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Objectives: A retrospective analysis was conducted using data from a phase III, double-blind, multinational trial in patients hospitalized with community-acquired pneumonia (CAP), comparing IV/oral linezolid (LZD) 600 mg q12 h ($N=381$), versus a regimen of IV ceftriaxone 1 g q12 h followed by oral cefpodoxime 200 mg q12 h ($N=366$). The purpose of this analysis was to further explore the prospective observation that linezolid was significantly more effective in the intent-to-treat (ITT) population (83% cure rate for LZD vs. 77% for the comparator group, $P=0.040$, 95% confidence interval 0.3, 12.8). The most frequently isolated baseline pathogen was *Streptococcus pneumoniae*.
Methods: Severity of illness was determined by the method of Fine et al. [1], using age, comorbid illnesses, selected physical and laboratory examination results, and the presence of concurrent bacteremia, multilobar pneumonia or pleural effusion. Distribution of baseline severity was similar in both groups. Step-wise logistic regression analysis confirmed a significantly higher cure rate in patients treated with linezolid (83.0% vs. 76.4%, $P=0.025$, odds ratio 1.580).
Results: Failure rates were significantly higher regardless of treatment in patients who had elevated BUN or glucose levels, decreased hematocrit levels, or pleural effusion. LZD had significantly higher cure rates in subgroup analyzes for diabetes (83% vs. 60%, $P=0.040$); cardiac comorbidities (90% vs. 62%, $P=0.009$); elevated WBC (87% vs. 78%, $P=0.029$), pleural effusion (86% vs. 49%, $P=0.001$); and bacteremia (91% vs. 59%, $P=0.004$). LZD also had a significantly higher cure rate (82% vs. 71%, $P=0.016$) in patients with a Fine score higher than the median (≥ 80). There were no variables in this model that showed a significantly better outcome for ceftriaxone/cefpodoxime compared to LZD.
Conclusions: These data support the efficacy of LZD for the treatment of patients with CAP, and show that LZD has a significantly higher cure rate than the cephalosporin comparators for the treatment of patients hospitalized with pneumonia, including patients with diabetes, cardiac conditions, pleural effusions, and bacteremia.

Reference

[1] Fine MJ, Auble TE, Yealy DM, *et al.*A prediction rule to identify low-risk patients with community-acquired pneumonia. *N Engl J Med* 1997; 336(4): 243–50.

P760 Linezolid shows efficacy in eradication of nasal MRSA carriage status

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Carriage with methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent and serious problem that has been shown to precede infection. Eradication of MRSA carriage is of interest because it may reduce MRSA infection rate. Mupirocin is only moderately effective in the eradication so new strategies are needed in this setting. Linezolid is a new oxazolidinone with enhanced activity against Gram-positive cocci, including MRSA.
Objectives: To analyze the effect of linezolid compared to glycopeptides in nasal MRSA carriage in terms of eradication.
Methods: We performed a retrospective analysis of MRSA nasal carriers with demonstrated infection of any location that were admitted to our hospital during a period of 3 years (1999–2001). All patients received combined treatment with intranasal mupirocin at standard doses plus either a course of intravenous glycopeptide (vancomicin or teicoplanin, standard doses) or linezolid (intravenous or oral) at a dose of 600 mg/12 h, for at least 7 days. Nasal swab cultures were performed at 1, 2 and 3 weeks after mupirocin treatment and 1 month after finishing systemic treatment. Patients with positive swab controls were considered persistent carriers.
Results: Of the 55 patients nasal MRSA carriers admitted for MRSA infection 29 were not included: 18 died prematurely and 11 were lost during the follow-up. Twenty-six patients were studied, median age was 79.5 years (57–93 years), 10 (38.5%) were female, and 84.5% had at least two underlying diseases (mainly heart disease, COPD or vasculopathy). Sixty-one percent presented soft-tissue infection, 15.4% surgical wound infection, 7.7% sepsis, 7.7% respiratory tract infection, and 7.7% other sites of infection. Seven patients received linezolid that was well tolerated. Adverse events with this drug included one case of thrombocytopenia, one constipation and one muguet. In no case was discontinuation of linezolid dosing required. All patients receiving linezolid responded clinically well to treatment. Linezolid treatment showed efficacy in eradication of MRSA nasal carriage (only 1/7 were persistent carriers with linezolid vs. 12/19 with glycopeptide treatment; $P=0.036$, χ^2-test).
Conclusion: Linezolid showed excellent activity both in terms of resolution of infections and in eradication of MRSA nasal carriage. Linezolid was well tolerated with a scarce number of adverse events.

P761 Effect of linezolid on the bactericidal activity of human polymorphonuclear leukocytes (PMNs)

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Objectives: To evaluate the effect of a new oxazolidinone, linezolid, on the phagocytic and bactericidal functions of human PMN. The intracellular activity of linezolid against *Staphylococcus aureus* was also assessed.
Materials and methods: Superoxide and peroxide hydrogen radicals production by PMA-stimulated PMN was measured using a modification of the ferrocytochrome *c* reduction assay and a modification of the phenol red method, respectively. The phagocytic activity of human PMN was determined by a radiometric assay. Radiolabeled opsonized bacteria and human PMN were incubated for 12 min at a ratio bacteria/PMN: 10/1. The effect of PMN exposed to different concentrations of linezolid (2, 10 and 20 mg/L) on the phagocytosis of opsonized bacteria (2 *S. aureus* strains: 1 methicillin-susceptible/1 methicillin-resistant; 2 *Enterococcus faecalis* strains: 1 vancomycin-susceptible/1 vancomycin-resistant) was evaluated. The phagocytosis of bacteria preincubated (18 h; 37 °C) with 1/4 MIC of linezolid was also evaluated. The intracellular activity of linezolid (extracellular concentrations: 2, 10 and 20 mg/L) against *S. aureus* in human PMN was assessed in a 3-h assay.
Results: Linezolid did not affect either superoxide and hydrogen peroxide production by human PMN. This antimicrobial agent did not modify the phagocytosis of opsonized bacteria by human PMN. Neither the exposure of *S. aureus* strains, nor the exposure of *E. faecalis* strains to 1/4 MIC of linezolid significantly affected the phagocytosis of these bacteria by human PMN. At the extracellular concentrations evaluated, linezolid did not affect the bactericidal activity of human PMN against intracellular *S. aureus*.
Conclusions: Linezolid (subMIC and therapeutical) did not affect the phagocytic and bactericidal functions of human PMN.

P762 Treatment of bone and prosthetic joint infections with linezolid

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Background: Methicillin-resistant *S. aureus* (MRSA) is frequently isolated from osteomyelitis or prosthetic joint infections. The treatment of this infection is problematic. For prosthetic infections complete removal is frequently needed to cure the infection.
Material and methods: Six patients, four with chronic osteomyelitis and two with hip prosthesis infections, were treated with linezolid. All MRSA isolated were nosocomial and susceptible to linezolid (range 0.25–1 mg/L). The patients received 1200 mg daily dose of linezolid per oral route, until the cure was reached or a severe adverse event was noted.

Results: One patient with hip prosthesis was cured as demonstrated by negative leukocyte scan after 3 months of therapy. The other patient with hip prosthesis infection stopped the drug after 2 months of therapy because of severe anemia that recovered after the suspension of the drug; the infection was improved as demonstrated by reduction of ESR, pain and intensity of leukocyte scan. Among patients with osteomyelitis, one diabetic patient with nosocomial sternal infection was completely cured. The second with the same type of infection is still on treatment with improvement of the symptoms and radiology evaluation. A third patient with nosocomial lumbar dyscitis was improved but unfortunately he stopped the drug due to a concomitant cerebral stroke not correlated to the drug. The fourth patient with a tibia osteomyelitis was lost at follow-up after 2 months of treatment with symptoms completely recovered.
Conclusions: Linezolid might be a safe and easy option in the treatment of bone and prosthetic infections due to difficult to treat MRSA. Most of these patients were treated for prolonged time without adverse events, except severe anemia in one case.

P763 Clinical experience with linezolid in patients with a variety of Gram-positive infections

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Background: Linezolid is the first oxazolidinone antibacterial agent with inhibitory activity against Gram-positive bacteria including MRSA and VRE. Clinical experience with the new antibiotic does not exist in Greece, since it has not received license to the market yet.
Objective: To present clinical data concerning the experience of linezolid in the therapy of documented or presumed Gram-positive infections.
Methods: We evaluated 27 patients with 32 episodes of various infections who participated in an expanded access protocol. Linezolid was administered i.v. or orally 600 mg bid for 1–4 weeks or longer if necessary and results concerning efficacy, safety, intolerance and follow-up were recorded.
Results: Fifteen patients with skin- or soft-tissue infections (SSTIs, mostly erysipelas, cellulitis and skin abscess) with mean age 56 years received linezolid for a mean of 29.5 days (range 18–45). The causative microorganism was recognized in seven cases (MRSA four, MRSE two, MSSE one). Twelve patients were cured (80%) and the rest showed a marked improvement. After a mean follow-up period of 45 days only one patient relapsed. Ten cases with chronic osteomyelitis (CO) of the lower limbs (mean age 46 years) due mostly to MRSA, received linezolid for a mean of 37 days (range 28–50) significantly longer than the patients with SSTIs ($P < 0.05$). Six of them (60%) had a successful clinical response and four a partial one. After a follow-up period of 15–180 days (mean 60 days), only two cases relapsed. Three cases of non-puerpual mastitis due to MSSA (2) and *S. hominis* received linezolid for 28–42 days with cure or marked improvement. Two cases of bacteremia due to MRSA and MRSE were clinically and microbiologically cured with no relapse. The most frequent adverse effect was anemia recorded in 10/27 (37%). The mean drop of the hematocrit was 7.4% (range 3–10%) and presented 14–48 days (mean 28.4) after starting therapy. None required transfusion or discontinued therapy and the condition was completely reversed in 15–48 days (mean 28) after the cessation of therapy. A mild and fully reversible thrombocytopenia occurred in three patients (11%).
Conclusions: Linezolid is a highly effective agent in the treatment of various Gram-positive infections and especially CO and/or SSTIs. Longer duration of treatment (>18 days) is associated with a higher incidence of reversible anemia and thrombocytopenia. Close monitoring of the patients is required.

P764 The concentrations and penetration of linezolid for bone, fat and muscle

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Objectives: Linezolid (LZD), the first clinically useful oxozolidinone antimicrobial, is characterized by a spectrum of activity which includes both methicillin-resistant staphylococci and glycopeptide-resistant enterococci. There are few publications describing the pharmacokinetics of LZD, especially relating to bone and tissue. In this study, we report the concentrations and penetration of LZD for bone, fat and muscle after a single dose of LZD.
Methods: A total of 12 patients undergoing hip arthroplasty received 600 mg LZD as a 20 min infusion immediately before surgery and again 12 h after surgery. Samples of bone, fat, muscle and blood were collected at 10, 20 and 30 min after the LZD infusion and samples of drainage from the operation site were collected at 6–8, 10–12 and 14–16 h after surgery. Samples were assayed by a validated high performance liquid chromatography method. Tissue samples were extracted into phosphate buffered saline and any bone or fat samples with visible blood contamination were discarded.
Results: The mean (S.D.) levels of LZD found in the tissues and penetration relative to serum are shown in the table above. Mean (S.D.) levels of LZD in drainage were 6–8 h: 8.2 (3.3) mg/L, 10–12 h: 5.6 (2.1) mg/L and 14–16 h: 7.0 (5.4) mg/L.

	Concentration (mg/L)				Penetration (%)		
	Bone	Fat	Muscle	Serum	Bone	Fat	Muscle
10 (min)	9.1 (2.6)	4.5 (2.7)	10.4 (4.0)	19.2 (6.5)	51.0	26.5	58.3
20 (min)	8.6 (5.3)	5.2 (2.1)	13.4 (5.6)	15.8 (5.8)	60.0	36.9	94.3
30 (min)	6.3 (4.0)	4.1 (1.2)	12.0 (4.7)	14.3 (5.0)	46.4	31.4	93.0

Conclusions: LZD exhibits rapid penetration in bone, fat and muscle of patients undergoing hip arthroplasty to achieve levels in excess of the MIC for sensitive organisms (MIC of <8 mg/L); with therapeutic levels maintained in the drainage which surrounds the operation site for more than 16 h. We conclude that LZD exhibits a pharmacokinetic profile which appears appropriate to investigate the treatment of bone and associated soft tissue infections.

P765 Single and multiple dose pharmacokinetics of linezolid and amoxicillin/clavulanic acid in healthy human volunteers

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In an open, randomized, two-period crossover study the ecological effects of linezolid versus amoxicillin/clavulanic acid on the normal intestinal microflora and the pharmacokinetics of two antibiotics were investigated after single and multiple dose in 12 healthy volunteers (six females and six males). Linezolid was given in tablets of 600 mg bid for 7 days and amoxicillin/clavulanic acid in tablets of 1000 mg od for 7 days. The wash-out period was 4 weeks between the administration of the two antibacterial agents. Blood and urine samples were collected on days 1 and 7 before and at different time points up to 48 h after medication. The concentrations of the three antibiotics in serum and urine were measured by validated high-performance liquid chromatography methods. Linezolid concentrations in serum and urine were also determined by a microbiological assay. The agreement between the results obtained by bioassay and those of HPLC was good. Linezolid exhibited a mean C_{max} of 14.5 ± 4.6 µg/mL after T_{max} of 47.5 ± 20.1 min on day 1, with a significant increase to 24.0 ± 6.9 µg/mL on day 7 ($P < 0.01$). The AUDtot revealed a highly significant increase from 140.5 ± 28.3 µg h/mL on day 1–220.2 ± 42.6 µg h/mL on day 7 ($P < 0.01$). There were no significant differences of terminal half-life between days 1 and 7 (9.53 ± 2.87 vs. 7.97 ± 3.08 µg h/mL) and of total clearance (71.6 ± 17.6 vs. 81.5 ± 14.7 mL/min 1.73 m^2). Results are in agreement with the assumption of cumulation of linezolid under the given dosage regimen. Serum concentrations of females were always higher than those of males. The volume of distribution Vss/f differed significantly between females and males (41.6 ± 4.2 vs. 52.2 ± 3.3 L/70 kg; $P < 0.01$). No accumulation was observed. No serious adverse event was observed in all three drugs during the study.

P766 The normal human intestinal microflora and BMS-284756

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Objective: BMS-284756 is a new des-F(6)-quinolone agent active against many aerobic and anaerobic bacteria. The ecological effect of BMS-284756 on the intestinal microflora was investigated in two trials.
Methods: Forty healthy subjects participated in the first trial. Eight subjects were assigned to each of five dose panels (100, 200, 400, 800, and 1200 mg BMS-284756) and received daily oral dosing with either BMS-284756 ($n=6$) or placebo ($n=2$) for 14 days. Fecal samples were collected before (days −2 and −1), during (days 7 and 14), and after completion of the administration period (days 21, 28, and 45). In the second trial, 16 healthy subjects were given 600 mg BMS-284756 for 7 days. Fecal samples were collected before (days −2 and −1), during (day 5), and after withdrawal of administration (days 8 and 26). The fecal samples were diluted in prereduced media and inoculated aerobically and anaerobically on nonselective and selective media. The different colony types were identified to genus level by morphological, biochemical and molecular analyses.
Results: In subjects receiving 100 or 200 mg BMS-284756, no significant changes in the intestinal aerobic and anaerobic microflora occurred. The number of enterococci, bacilli, corynebacteria, bifidobacteria, lactobacilli, clostridia, and bacteroides decreased in subjects receiving 400, 600 or 800 mg BMS-284756, whereas the number of eubacteria increased. Subjects who received 1200 mg BMS-284756 had significant changes in the microflora: enterococci, bacilli, corynebacteria, enterobacteria, bifidobacteria, lactobacilli, clostridia, and bacteroides were suppressed, whereas eubacteria and yeasts were increased. Regardless of dose, the microflora returned to normal levels at days 26–28 (2 weeks after the administration of BMS-284756 had stopped). Fecal concentrations of BMS-284756 increased with the higher doses, from 35.7 (100 mg) to 262.8 mg/kg (1200 mg).
Conclusion: During the administration of BMS-284756, enterococci, bacilli, corynebacteria, bifidobacteria, lactobacilli, clostridia and bacteroides were suppressed in the intestinal microflora, while eubacteria increased. The eubacteria may inactivate different drugs in the intestine.

P767 Antimicrobial activity of BMS-284756 against staphylococci and respiratory pathogens

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Objectives: BMS is a novel des-F(6)-quinolone that has shown excellent antimicrobial activity against a wide range of clinically important microorganisms, including Gram-positive and Gram-negative aerobes and anaerobes. In this study, the activity of BMS-284756 against Staphylococci (MSSA and MRSA) and respiratory pathogens (*S. pneumoniae, H. influenzae, M. catarrhalis* and *S. pyogenes*) was further examined in comparison with other antimicrobial agents [ciprofloxacin (cip), levofloxacin (lvx), moxifloxacin (mxf), penicillin (pen), amoxicillin, coamoxi-clav, cefuroxime, cefotaxime, ceftriaxone, imipenem, erythromycin (ery), clarithromycin]. In addition, the bactericidal activity of BMS-284756, mxf, lvx and cip was evaluated by time–kill analysis against four strains of staphylococci (two MSSA and two MRSA) four strains of *S. pneumoniae* (two pen-S and two pen-R) and four strains of *S. pyogenes* (two ery-S and two ery-R).
Methods: Overall, 100 clinical isolates were selected for in vitro testing. MICs were determined by standard microdilution method (staphylococci + streptococci + *H. influenzae*) and by agar dilution procedures for *M. catarrhalis*, in accordance with the NCCLS guidelines. Time–kill studies were performed in flasks according to standard procedures. Antibiotics (BMS-284756, mxf, lvx and cip) were tested at concentrations 1–8 times the MIC.
Results: The MIC_{90} of BMS-284756 for the MSSA and MRSA were 0.03 and 0.06 μg/mL, respectively. Among all the quinolones tested, BMS-284756 yielded the lowest MIC values against all the pneumococcal strains ($MIC_{90} \leq 0.06$ μg/mL) irrespective of penicillin and/or macrolide resistance; the rank order of activity was BMS-284756 > mxf > lvx > cip. Excellent activity was shown also against *H. influenzae* ($MIC_{90} \leq 0.03$ μg/mL) and *M. catarrhalis* ($MIC_{90} \leq 0.03$ μg/mL). A total of 90% of *S. pyogenes* were inhibited at BMS-284756 concentration equal to 0.25 μg/mL, its activity being not influenced by macrolide susceptibility. BMS-284756 was rapidly bactericidal against staphylococci, producing 3 log 10 decrease in viable counts (cfu/mL) within 3 h at 4× MIC whereas a moderate, slower killing rate was observed versus pneumococci and streptococci.
Conclusions: The enhanced activity of BMS-284756 against staphylococci, pneumococci and other respiratory pathogens would suggest its suitability for treatment of community acquired respiratory tract infections. Clinical trials are warranted to establish its clinical efficacy and safety.

Ertapenem

P768 Comparative in vitro activities of ertapenem against aerobic bacterial pathogens isolated from patients with complicated intra-abdominal infections

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Objectives: Determine the in vitro activity of ertapenem, a new β-lactam with the potential to eliminate the need for combination and multidose regimens for treatment of community-acquired and mixed infections, against bacterial pathogens recovered from patients with complicated intra-abdominal infections (IAI) and compare it with the activities of other agents commonly used to treat such infections.
Methods: In two prospective, double-blind, multicenter studies, adults with complicated IAI were randomized to receive ertapenem or comparator therapy. Specimens from the site of infection were cultured for aerobic and anaerobic bacteria. Aerobic bacteria were shipped to Merck Research Laboratories, where they were tested for susceptibility to ertapenem, ceftriaxone, amoxicillin–clavulanate, ampicillin–sulbactam, and piperacillin–tazobactam by microtiter dilution following NCCLS guidelines.
Results: A total of 1018 aerobic bacteria from 531 patients were tested. Enterobacteriaceae accounted for 66.3%; *Escherichia coli* was the most common isolate. The ertapenem MIC was ≤4 μg/mL for 78.1% of all aerobic isolates and ≥16 μg/mL for 14.3% (enterococci, methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter* spp.). Excluding enterococci (NCCLS breakpoints for carbapenems, ceftriaxone, and piperacillin–tazobactam are not defined), the proportion of all aerobic isolates susceptible to the drugs tested were: ertapenem, 91%; ceftriaxone, 86%; amoxicillin–clavulanate, 78%; ampicillin–sulbactam, 58%; piperacillin–tazobactam, 92%. Against Enterobacteriaceae, ertapenem was the most active (100% susceptible); ampicillin–sulbactam was least active (64% susceptible). Piperacillin–tazobactam was the only drug with clinically useful activity against *P. aeruginosa*.
Conclusions: Ertapenem was highly active against the aerobic bacterial pathogens recovered from patients with complicated IAI. Against Enterobacteriaceae, ertapenem was more active than ceftriaxone, amoxicillin–clavulanate, ampicillin–sulbactam, and piperacillin–tazobactam.

P769 Use of surrogate antimicrobial agents to predict susceptibility to ertapenem

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Objectives: Ertapenem (ETP) is a once a day β-lactam antimicrobial agent that recently was licensed for treatment of various community-acquired and mixed infections. It is not yet on panels for automated susceptibility test

systems. The aim of this study was to identify surrogate antimicrobial agents for evaluating susceptibility to ETP against the major organism groups for which ETP is indicated.
Methods: Susceptibility-test results for 10 992 Enterobacteriaceae, 2888 anaerobes, 2206 staphylococci, 840 *Haemophilus* spp., 709 *Streptococcus pneumoniae*, and 709 other streptococci were analyzed. Organisms included clinical trial isolates, 'challenge' strains with known resistance mechanisms, and Enterobacteriaceae from a 1999 ICU Susceptibility Surveillance Study. Testing was performed by broth or agar dilution, following NCCLS guidelines. Isolates were tested against ETP and imipenem (IPM); staphylococci also were tested against oxacillin (OX) and streptococci against penicillin (PEN). For an agent to be considered a reliable surrogate, the very major error (VME, false susceptible) rate should be 1.5% or less and the absolute categorical agreement, 90% or more.
Results: Of the Enterobacteriaceae, 10 663 (97%) were susceptible (S) and 17 (0.2%) were resistant (R) to ETP and IPM. Absolute agreement between the two drugs was 97% (10 681/10 992); there were 94 VME (0.9%) and 40 (0.4%) major errors (ME, false resistant). 95/104 (91%) IPM-intermediate (I) Enterobacteriaceae were ETP-S. For anaerobes, absolute agreement between ETP and IPM was 99% (2858/2888); VME = 6 (0.2%); ME = 0. All OX-S staphylococci, all *Haemophilus* spp., all PEN-S/I *S. pneumoniae*, all groups A and B streptococci, and all other PEN-S streptococci were ETP-S.
Conclusions: The following agents were excellent in predicting susceptibility to ETP: IPM for Enterobacteriaceae and anaerobes, OX for staphylococci, and PEN for streptococci. For confirmed IPM-R/I Enterobacteriaceae, PEN-R *S. pneumoniae*, and other streptococci that are PEN-I/R, the ETP MIC should be determined. All OX-R staphylococci should be reported as ETP-R. Testing *Haemophilus* or groups A or B streptococci against ETP is not necessary; resistance has not yet been identified.

P770 Ertapenem 1 g once a day is highly effective in treatment of severe community-acquired and mixed infections

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Objectives: Assess the efficacy of ertapenem, a new once a day parenteral β-lactam, in the treatment of severe community-acquired and mixed infections and compare it with piperacillin–tazobactam (P/T) and ceftriaxone (CRO).
Methods: The efficacy of ertapenem, 1 g once a day, in treating complicated intra-abdominal (IAI), acute pelvic (PI), complicated skin and skin structure (SSSI), and complicated urinary tract infections (UTI) and community-acquired pneumonia (CAP) was evaluated in seven randomized, double-blind trials at 425 sites worldwide. Comparator antimicrobials, considered standard of care, were P/T, 3.375 per every 6 h, for IAI, PI, and SSSI and CRO, 1 g once a day, for CAP and UTI. Each patient's infection was rated as mild-to-moderate or severe, based on predefined scoring criteria. For IAI generalized peritonitis was the marker for severe infection. Clinical and/or microbiologic efficacy was assessed in protocol-evaluable patients at pre-specified timepoints post-therapy, defined by indication.
Results: Infection was rated severe in 29% (347/1178), 23% (119/520), and 36% (189/518) of evaluable patients, who received ertapenem, P/T, and CRO, respectively. Cure rates (%) for severe infections in each indication are shown in the Table 1 below.

Table 1 Cure rates (%)

	IAI	PI	SSSI	Overall	CAP	UTI	Overall
Ertapenem	50/60 (83)	40/42 (95)	32/40 (80)	122/142 (86)	81/90 (90)	112/121 (93)	193/211 (91)
P/T	39/53 (74)	30/35 (86)	22/31 (71)	91/119 (76)			
CRO					74/85 (87)	97/104 (93)	171/189 (90)

[a]Clinical & microbiologic response for IAI; clinical for PI and CAP; microbiologic for UTI.

Cure rates (ertapenem vs. comparator) for all (mild-moderate + severe) infections were: IAI, 176/203 (87%) versus 157/193 (81%); PI, 153/163 (94%) versus 140/153 (92%); SSSI, 152/185 (82%) versus 147/174 (84%); CAP, 335/364 (92%) versus 270/294 (92%); UTI, 229/256 (89%) versus 204/224 (91%). Ertapenem and comparator agents were generally well tolerated.
Conclusions: Ertapenem, 1 g once a day, was highly effective for treatment of IAI, PI, SSSI, CAP, and UTI, including severe infections, and as effective as comparator therapy. The safety and tolerability profiles of ertapenem and comparator agents were similar.

P771 Efficacy and safety of ertapenem 1 g once a day: the European experience

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Objectives: Large clinical trials have recently been conducted worldwide to evaluate the efficacy and safety of ertapenem, a new once a day parenteral β-lactam, as monotherapy for treating serious community-acquired and mixed infections. We report here on the clinical experience of 371 patients from nine European countries treated for serious bacterial infections in these studies.
Methods: Ertapenem, 1 g once a day, was evaluated in six separate randomized double-blind trials in the treatment of complicated intra-abdominal infections (IAI), acute pelvic infections (PI), community-acquired pneumonia (CAP) and complicated urinary tract infections (UTI) versus either piperacillin/tazobactam (P/T) 3.375 g per 6 h (IAI and PI), or ceftriaxone (CRO) 1 g once a day (UTI and CAP). Clinical and/or microbiologic efficacy was assessed in evaluable patients at pre-specified post-treatment timepoints as defined for each indication.
Results: Of the 371 patients randomized to treatment in Europe, 68% (251/371) were protocol evaluable. Treatment groups for each indication were similar with respect to age, gender, and ethnicity. Favorable primary efficacy response rates overall and by indication are shown in the table below.

Drug	IAI	PI	Overall vs. P/T	CAP	UTI	Overall vs. CRO
Ertapenem	27/34 (79%)	9/10 (90%)	36/44 (82%)	53/58 (91%)	49/52 (94%)	102/110 (93%)
P/T	25/30 (83%)	4/4 (100%)	29/34 (85%)			
CRO				27/32 (84%)	26/31 (84%)	53/63 (84%)

[a]Clinical & microbiologic response for IAI, clinical for PI & CAP, microbiologic for UTI.

The most commonly treated pathogens in these patients were: *Escherichia coli* (IAI, PI, and UTI) and *Streptococcus pneumoniae* (CAP). Eradication rates, by pathogen, for these and other organisms were comparable for ertapenem versus comparator agents. The frequency and severity of drug-related adverse events were generally similar in ertapenem and comparator groups.
Conclusions: In this subgroup analysis of European patients treated for serious community-acquired and mixed bacterial infections, ertapenem, 1 g once a day, was highly effective in the treatment of IAI, PI, CAP, and UTI. Ertapenem was as effective as either ceftriaxone or piperacillin/tazobactam, and had a comparable safety profile.

P772 Superinfections in clinical trials with ertapenem, ceftriaxone, and piperacillin–tazobactam therapy

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Objectives: To identify organisms that cause superinfections during therapy with ertapenem, a new once a day parenteral β-lactam with the potential to eliminate the need for combination and multidose regimens for treating community-acquired and mixed infections, piperacillin–tazobactam (P/T), or ceftriaxone (CRO), and to compare superinfection rates associated with these therapies.
Methods: During seven randomized, double-blind clinical trials of ertapenem therapy, data were collected on emergent pathogens isolated during treatment (superinfection) or up to 6 weeks after study therapy was completed (new infection). In these trials the efficacy of ertapenem, 1 g once a day, was compared to P/T, 3.375 g/day, for complicated intra-abdominal (IAI), acute pelvic (PI), and complicated skin and skin structure (SSSI) infections and with

CRO, 1 g/day, for community-acquired pneumonia (CAP) and complicated urinary-tract infections (UTI).
Results: In the trials, 3301 adult patients (on ertapenem or comparator) were randomized at 425 sites worldwide. Superinfections occurred in 15/1217 (1.2%), 30/567 (5.3%), and 7/525 (1.3%) evaluable patients who received ertapenem, P/T, and CRO, respectively; 63 (5.2%), 12 (2.1%), and 30 (5.7%) developed a new infection. Super and new infections were infrequent, regardless of treatment; rates (in percentage) for each indication are shown in the Table 1 below.

Table 1

	IAI	PI	SSSI	CAP	UTI
Ertapenem	2.1/3.8	0.6/1.2	3.8/1.6	0.3/2.5	0.4/15.1
P/T	6.3/2.1	2.0/0.7	6.9/3.4		
CRO				1.0/1.0	1.7/11.7

Most new infections represented recurrent UTIs; enterococci and *Escherichia coli* were the most frequent pathogens. Superinfection was documented most often in SSSI and IAI; *Staphylococcus aureus* (SSSI) and enterococci and Gram-negative anaerobes (IAI) were isolated most frequently. Susceptibility profiles of the bacteria responsible for these infections were comparable to those of baseline pathogens.
Conclusions: Overall, super and new infections were infrequent and isolation of ertapenem-resistant organisms was rare in patients treated with ertapenem. Both the rates of occurrence and the pathogens recovered were generally similar between ertapenem and the comparator.

P773 Ethnicity does not effect the efficacy and safety of ertapenem therapy

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Objectives: To assess the impact of ethnicity on efficacy and safety of ertapenem, a new once a day β-lactam with the potential to eliminate the need for combination therapy and multidose regimens when treating community-acquired and mixed infections, compared with comparator therapy.
Methods: Eligible patients with complicated intra-abdominal (IAI), complicated skin, acute pelvic (PI), or complicated urinary tract (UTI) infections or community-acquired pneumonia (CAP) were enrolled in seven separate double-blind clinical trials conducted worldwide and randomized to ertapenem, 1 g once a day, or comparator (piperacillin–tazobactam [P/T], 3.375 g per every 6 h, for IAI, PI, skin; ceftriaxone [CRO] 1 g once a day for UTI, CAP). Clinical and/or microbiologic efficacy was assessed in evaluable patients at pre-specified timepoints post-therapy, defined by indication.
Results: Of the 3301 patients randomized to ertapenem or a comparator group, 2209 (67%) comprised the primary efficacy population. Overall response rates (%) are shown in the Table 1 below.

Table 1 Overall response rates (%)

Ertapenem vs.	Caucasian ($n = 1180$)	Black ($n = 266$)	Hispanic ($n = 549$)	Other ($n = 214$)
CRO	91 vs. 92	96 vs. 91	90 vs. 92	90 vs. 87
P/T	82 vs. 84	88 vs. 82	93 vs. 88	94 vs. 88
Comparator	87 vs. 89	91 vs. 86	91 vs. 90	92 vs. 87

[a]Clinical & microbiologic response for IAI; clinical for skin, PI, and CAP; microbiologic for UTI.

A total of 1747, 751, and 775 patients received at least one dose of ertapenem, CRO, and P/T, respectively. The most common clinical drug-related adverse experiences (AEs) were diarrhea, nausea, and headache for all drugs. The frequency of their occurrence was generally similar in all ethnic groups (T5%), although the rate of diarrhea associated with P/T was highest in Caucasians (9%). The most common laboratory drug-related AEs for all drugs were elevated liver transaminases, which occurred most frequently in Hispanics: 7–13% versus 2–4% in Blacks and Caucasians. Study therapy was discontinued owing to a drug-related AE in <2% of patients in all ethnic groups.
Conclusions: Ertapenem 1 g once a day was highly effective in treating IAI, skin, PI, CAP, and UTI in all ethnic groups and was as effective as P/T and CRO. Ertapenem was generally well tolerated in all ethnic groups and had a safety profile comparable to P/T and CRO.

P774 Efficacy of ertapenem (ETP) in treatment of *Escherichia coli* infections

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Objectives: *Escherichia coli* is a common serious pathogen in community-acquired and mixed infections. The activity of 1 g ETP, a new parenteral once a day β-lactam, against *E. coli*, was compared with piperacillin/tazobactam (P/T) 3.375 per every 6 h or ceftriaxone (CRO) 1 g once a day, in clinical studies of bacterial infections requiring parenteral antimicrobial therapy.
Methods: Appropriate cultures were obtained at baseline in 2435 adult patients (pts) enrolled in five multinational, randomized double-blind clinical trials evaluating the efficacy of ETP for treatment of intra-abdominal (IAI), skin (SI), pelvic (PI), and urinary tract (UTI) infections. The pts with baseline cultures identifying *E. coli* as a pathogen were included in this subgroup analysis. Clinical and/or microbiologic efficacy was assessed in evaluable pts at pre-specified times post-treatment, as defined by each indication.
Results: *E. coli* was a baseline pathogen in 35% (845/2435) of the randomized pts. Ninety-nine percent of the isolates tested were susceptible to ETP; 97% were susceptible to the comparator. Of pts with *E. coli*, 87% (735/845) were protocol evaluable. Evaluable treatment groups were similar with respect to age, gender, ethnicity, and infection diagnosis. Of evaluable pts with IAI, SI, or PI, 86% overall had polymicrobial infection; 97% with UTI had monomicrobial infection. 33, 6, and 17 pts treated with ETP, P/T, or CRO had *E. coli* bacteremia. Favorable primary efficacy response rates (RR) are shown in the Table 1 below.

Table 1 Favorable primary efficacy response rates (RR)

	ETP	P/T	CRO
vs. P/T			
IAI (one study)	86% (127/147)	80% (106/132)	
PI (one study)	88% (36/41)	92% (36/39)	
Skin (one study)	94% (15/16)	80% (12/15)	
Overall	87% (78/204)	83% (154/186)	
vs. CRO			
UTI (two studies)	92% (174/190)		95% (143/150)

[a]Clinical & microbiologic response for IAI, clinical for PI & SI, microbiologic for UTI.

In pts with bacteremia, the primary RR in UTI was 92% (22/24) for ETP and 82% (14/17) for CRO; in other indications, it was 100% (9/9) for ETP and 83% (5/6) for P/T. Persistent bacteremia was not documented in any patient. Overall bacterial eradication rates were 91% (196/216) and 86% (162/189) for ETP versus P/T and 92% (176/191) and 92% (143/155) for ETP versus CRO.
Conclusions: In this subgroup analysis, ETP was highly effective for treatment of community-acquired and mixed infections caused by *E. coli*, including bacteremia, and was as effective as P/T and CRO.

P775 Ertapenem 1 g once a day is highly effective for treatment of postpartum endomyometritis

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Objectives: Ertapenem is a new once a day β-lactam with the potential to eliminate the need for combination and multidose regimens when treating community-acquired polymicrobial infections. The purpose of this subgroup analysis was to determine the efficacy of ertapenem in the treatment of postpartum endomyometritis and compare it with piperacillin–tazobactam (P/T).
Methods: In a prospective, multicenter, double-blind study, females at least 16 years of age with acute pelvic infection requiring parenteral therapy were stratified by obstetric/postpartum infection or gynecologic/postoperative infection and randomized to ertapenem 1 g once a day or P/T 3.375 g per every 6 h. The underlying specific diagnosis was identified for each patient at entry. Patients with postpartum endomyometritis were included in this subgroup analysis. At enrollment, appropriate aerobic and anaerobic cultures were performed. Prior to unblinding, the severity of the infection (moderate

or severe) was rated based on predefined criteria. The primary efficacy endpoint was the proportion of clinically evaluable patients who were cured 2–4 weeks post-therapy (test of cure [TOC]).
Results: A total of 73% (299/412) of the randomized patients had postpartum endomyometritis, of whom 116 in the ertapenem group and 113 in the P/T group were clinically evaluable. Treatment groups were similar with respect to age (mean, 23–25 years), and duration of therapy (median, 4 days for both groups). The infection was rated severe in 31 (27%) of the clinically evaluable patients who received ertapenem and 23 (20%) who received P/T. The most common pathogen in both the groups was *Escherichia coli*. Five patients in the ertapenem group and two in the P/T group were bacteremic at baseline. Cure rates at TOC were 93% for ertapenem and 90% for P/T; 94 and 87%, respectively, for severe infection and 93 and 91% for moderate infection. Overall favorable microbiologic response rates by patient were 94% (ertapenem) and 94% (P/T). Both ertapenem and P/T were generally well tolerated.
Conclusions: In this subgroup analysis, ertapenem, 1 g once a day was highly effective for treatment of postpartum endomyometritis overall, and in severe and moderate infections, and was as effective as P/T. The safety and tolerability profiles of ertapenem and P/T were similar.

P776 Analysis of ertapenem (ETP) efficacy and safety by gender

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Objectives: To determine the effect of gender on the efficacy and safety of ETP, a new once a day β-lactam, in community-acquired and mixed infections as compared with ceftriaxone (CRO) or piperacillin–tazobactam (P/T), a multidose β-lactam/β-lactamase inhibitor combination.
Methods: Males and females with intra-abdominal (IAI), skin (SI), or urinary tract (UTI) infections or community-acquired pneumonia (CAP), and females with acute pelvic infection (PI), were randomized in seven double-blind studies at 425 sites worldwide. Therapy was ETP 1 g once a day or standard comparator agent: P/T 3.375 g per every 6 h (IAI, SI, PI) or CRO 1 g once a day (UTI, CAP). Clinical and/or microbiologic efficacy was assessed in evaluable patients at timepoints pre-specified for each indication. Safety was evaluated in all treated patients in all studies.
Results: Of the 3301 patients randomized, 1166 females and 1043 males comprised the primary efficacy population. Overall cure rates (%) are shown in the Table 1 below.

Table 1 Overall cure rates (%)

ETP vs.	ETP	Comparator	ETP	Comparator
CRO (UTI, CAP)	92 (299/324)	92 (251/274)	90 (265/296)	91 (223/244)
P/T (IAI, SI)	82 (108/132)	79 (95/120)	86 (220/256)	85 (209/247)
P/T (PI)	94 (153/163)	92 (140/153)	–	–
Comparators combined	90 (560/619)	89 (486/547)	88 (485/552)	88 (432/491)

[a]Clinical and microbiologic response for IAI, clinical for SI, PI, and CAP, microbiologic for UTI.

The most common clinical drug-related adverse experiences (DRAEs) for ETP, P/T, and CRO, respectively, in both genders (1736 females, 1537 males) were diarrhea (4, 7, and 3% in females; 5, 6, 5% in males) and nausea (4, 4, and 3% in females; 1, 2, 2% in males). The most frequent laboratory DRAEs for ETP, P/T, and CRO in both genders were elevated ALT (4, 3, and 5% of females; 8, 6, 7% of males) and elevated AST (3, 2, and 5% of females; 7, 7, 5% of males). ETP, P/T, and CRO were discontinued owing to a DRAE in 14 (2%), 6 (1%), and 1 (<1%) females and 11 (1%), 8 (2%), and 3 (1%) males.
Conclusions: ETP was highly effective for treatment of community-acquired and mixed infections and as effective as comparator therapy. ETP was generally well tolerated with a safety profile comparable to that of P/T and CRO. The efficacy and safety of ETP were generally similar in males and females.

P777 Complicated intra-abdominal infection (IAI): impact of microbiology on outcome in patients treated with ertapenem versus piperacillin–tazobactam

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Objectives: To assess the impact of the microbiology of complicated IAI on outcome in patients (pts) treated with 1 g ertapenem (ETP), a new once a day parenteral β-lactam, or piperacillin–tazobactam (P/T) 3.375 g every 6 h. ETP has minimal activity against enterococci or *Pseudomonas aeruginosa*; P/T has relatively good activity against both organisms.
Methods: In a prospective, randomized, double-blind (with sponsor blinding), multicenter trial, pts were stratified at enrollment by primary site of infection and APACHE II score. The primary analysis was clinical and microbiological efficacy in the microbiologically evaluable (micro evaluation) pts 4–6 weeks post-therapy. Additional analyzes were performed in this population to determine the impact of the following on clinical and microbiological efficacy: (1) one or more isolate resistant (R) or intermediate (I) to study therapy in addition to at least one susceptible isolate (2) isolation of *Enterococcus*, and (3) isolation of *P. aeruginosa*.
Results: A total of 203 pts in the ETP group and 193 in the P/T group were micro evaluated. Overall success rates were 87% for ETP and 81% for P/T. For pts with polymicrobial infections and 0, 1, or 2 or more R/I isolates, respectively, success rates were 86% (111/129), 85% (34/40), and 82% (9/11) for ETP and 82% (112/137), 87% (13/15), and 100% (3/3) for P/T. Success rates in pts with and without *Enterococcus* were 80% (45/56) and 89% (131/147) in the ETP group ($P = 0.101$) and 70% (23/33) and 84% (134/160) in the P/T group ($P = 0.060$). Irrespective of study therapy, pts with *Enterococcus* at baseline were less likely to have a favorable outcome than those without it ($P = 0.013$ in overall analysis accounting for treatment group). For pts with and without *P. aeruginosa*, success rates were 72% (18/25) and 89% (158/178) in the ETP group ($P = 0.021$) and 89% (23/26) and 80% (134/167) and in the P/T group.
Conclusions: The presence of R/I isolates with at least one susceptible isolate did not adversely effect outcome in either treatment group. Although pts in both treatment groups with baseline *Enterococcus* were less likely than pts without it, to have a favorable outcome, this did not appear related to specific anti-enterococcal activity of the study therapy, which suggests that routine enterococcal coverage is not required in the treatment of IAI.

P778 Efficacy of ertapenem in treatment of *Bacteroides fragilis* group infections

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Objectives: Members of the *B. fragilis* group (*B. fragilis*, *B. distasonis*, *B. uniformis*, *B. thetaiotaomacron*, *B. ovatus*, *B. vulgatus*) are the anaerobes most frequently isolated from clinical specimens and are more resistant to antimicrobial agents than most other anaerobes. The purpose of this subgroup analysis was to determine efficacy of ertapenem, a new parenteral once a day β-lactam, for treatment of complicated intra-abdominal (IAI), complicated skin and skin structure, and acute pelvic (PI) infections caused by *B. fragilis* group pathogens and compare it with piperacillin–tazobactam (P/T) 3.375 g per every 6 h.
Methods: The efficacy of ertapenem, 1 g once a day, in treating IAI, skin, and PI was evaluated in 1585 adult patients (on ertapenem or P/T) in the United States and internationally. In three randomized, double-blind trials, appropriate specimens were cultured for aerobic and anaerobic pathogens. Patients whose cultures grew *B. fragilis* group were included in this subgroup analysis. Clinical (skin, PI) or clinical and microbiologic efficacy (IAI) was assessed as primary efficacy response in protocol evaluable patients at post-therapy timepoints pre-specified for each indication.
Results: Of the 353 patients with *B. fragilis* group isolates, the majority (79%) of whom had IAI, 86% (303/353) were protocol evaluable. Treatment groups

were similar with respect to age, gender, and ethnicity. Of all isolates tested, 98% were susceptible to ertapenem and 99% were susceptible to P/T. Infection was polymicrobial in 99% of evaluable patients treated with ertapenem and 96% treated with P/T. Favorable primary efficacy response rates for ertapenem versus P/T were: IAI, 86% (107/124) versus 85% (94/111); skin, 100% (7/7) versus 90% (9/10); PI, 96% (24/25) versus 96% (25/26); overall, 88% (138/156) versus 87% (128/147). Bacterial eradication rates, by pathogen, for ertapenem versus P/T were: IAI, 90% versus 94%; skin, 100% versus 92%; PI, 100% versus 91%; overall, 91% versus 94%. Ertapenem and P/T were generally well tolerated.

Conclusions: In this subgroup analysis, ertapenem was highly effective for treatment of complicated intra-abdominal, complicated skin, and acute pelvic infections with *B. fragilis* group, almost all of which were polymicrobial, and was as effective as P/T. The safety and tolerability profile of ertapenem was similar to that of P/T.

P779 Comparative in vitro activities of ertapenem against aerobic bacterial pathogens isolated from patients with complicated skin infections

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Objectives: Determine the in vitro activity of ertapenem, a new β-lactam with the potential to eliminate the need for combination and multidose regimens for treatment of community-acquired and mixed infections, against bacterial pathogens recovered from patients with complicated skin infections and compare it with the activities of other agents commonly used to treat such infections.

Methods: In a prospective, double-blind, multicenter study, adults with complicated skin infections were randomized to receive ertapenem or piperacillin-tazobactam. Specimens from the site of infection were cultured for aerobic and anaerobic bacteria. Aerobic bacteria were shipped to Merck Research Laboratories, where they were tested for susceptibility to ertapenem, ceftriaxone, amoxicillin–clavulanate, ciprofloxacin, and piperacillin–tazobactam by microtiter dilution following NCCLS guidelines.

Results: A total of 518 aerobic bacteria from 340 patients were tested. Facultative Gram-positive cocci accounted for 68.1%; *Staphylococcus aureus* was the most common isolate (45.6%). The ertapenem MIC was 4 µg/mL or less for 83.8% of isolates and 16 µg/mL or greater for 8.7% (enterococci, methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, other non-fermentative Gram-negative bacilli). Excluding enterococci (NCCLS breakpoints for carbapenems, ceftriaxone, and piperacillin–tazobactam are not defined), the proportion of all isolates susceptible to the drugs tested were: ertapenem, 93%; ceftriaxone, 85%; amoxicillin–clavulanate, 81%; ciprofloxacin, 92%; piperacillin–tazobactam, 92%. Activity against *S. aureus* was similar for all agents. Ertapenem was the most active against Enterobacteriaceae (100% susceptible); amoxicillin–clavulanate was least active (78% susceptible). Against *P. aeruginosa*, piperacillin–tazobactam was most active (100% susceptible), followed by ciprofloxacin (87% susceptible); ertapenem and ceftriaxone had minimal activity.

Conclusions: Ertapenem was highly active against aerobic bacterial pathogens recovered from patients with complicated skin infections. Against *S. aureus*, ertapenem was as active as ceftriaxone, amoxicillin–clavulanate, ciprofloxacin, and piperacillin–tazobactam; against Enterobacteriaceae, it was the most active of these agents.

Meropenem

P780 A snapshot from Spain: antimicrobial resistance patterns from the MYSTIC Program during 1999 and 2000

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Objective: To determine antimicrobial resistance patterns of broad-spectrum antibiotics in seven Spanish hospital units over 2 years (1999 and 2000). The participating units are part of the global Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program, a longitudinal antimicrobial resistance surveillance study. MYSTIC monitors susceptibility patterns in hospital units that prescribe the carbapenem meropenem.

Methods: Three ICUs, two neutropenia units and two general wards provided a total of 1637 Gram-negative and Gram-positive isolates over the 2 years. National Committee For Clinical Laboratory Standards (NCCLS) methodology was used to determine the minimum inhibitory concentration (MIC) values (mg/L) and susceptibility breakpoints of MEM, imipenem (IPM), ceftazidime (CAZ), gentamicin (GM), piperacillin–tazobactam (TAZ), and ciprofloxacin (CIP).

Results: The most common species tested were methicillin-susceptible *Staphylococcus aureus* (11.2%), *Pseudomonas aeruginosa* (10.8%), *Escherichia coli* (10.0%), *Enterococcus faecalis* (8.8%), *Klebsiella pneumoniae* (6.5%) and *Enterobacter cloacae* (5.9%). MEM and IPM showed high activity against the most common organisms, and the highest activity against *S. aureus*, *E. coli*, *K. pneumoniae* and *E. cloacae* (all >99.5% susceptibility, $MIC_{90} = 0.064$–0.25 and 0.13–2 mg/L, respectively) compared with the other antimicrobial agents. CAZ and TAZ were the least active antimicrobial agents against *E. cloacae* (77.1 and 72.6% susceptibility, respectively; $MIC_{90} > 128$ for both). MEM and TAZ were the most active agents against *P. aeruginosa* (89.2 and 90.2% susceptibility, $MIC_{90} = 8$ and 64 mg/L, respectively), while IPM and TAZ demonstrated the highest activity for *E. faecalis* (both 98% susceptibility, $MIC_{90} = 4$ and 8 mg/L, respectively).

Conclusion: Carbapenems remain the most active antimicrobial agents tested against a broad range of Gram-positive and Gram-negative organisms from hospital centers in Spain.

P781 Ciprofloxacin-resistant Gram-negative bacteria from European MYSTIC Program centers for 1997–2001: trends in co-resistance with other antimicrobial agents

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Objectives: Increased use of fluoroquinolones (in particular ciprofloxacin [CIP]) has led to a progressive loss of susceptibility to this antibiotic class, predominantly among Gram-negative bacteria. The use of longitudinal surveillance studies to monitor these trends is essential to guide empirical therapy. MYSTIC (Meropenem [MEM] Yearly Susceptibility Test Information Collection) gathers antimicrobial susceptibility data from MEM-prescribing clinical units worldwide, 1997–present. Analysis of MYSTIC data in the UK has revealed a trend towards loss of susceptibility to CIP which is associated with a marked level of co-resistance to other commonly used antibiotics. The objective of this analysis was to examine the wider European MYSTIC data to determine whether similar trends exist outside the UK.

Methods: Isolates were tested using National Committee for Clinical Laboratory Standards methodology to determine the minimum inhibitory concentration (MIC) (mg/L) and breakpoint susceptibility (%S) for MEM as well as imipenem (IPM), ceftazidime (CAZ), piperacillin–tazobactam (TAZ), gentamicin (GM), and CIP.

Results: Over the study period 15 442 Gram-negative isolates from European units have been tested. A total of 12 195 (79%) were found to be CIP-susceptible/intermediate (CIP-S). Of the 3247 (21%) CIP-resistant (CIP-R) isolates, the most common were *Pseudomonas aeruginosa* (39.4%), *Acinetobacter baumannii* (14.3%), *Escherichia coli* (13.7%) and *Klebsiella pneumoniae* (5.7%). Overall, the carbapenems showed the greatest activity against the isolated CIP-S and CIP-R organisms. MEM was significantly superior to IPM against CIP-S and CIP-R *B. cepacia* (83%, 50% vs. 56%, 19%, respectively) and *P. aeruginosa* (85%, 58% vs. 73%, 47%, respectively). As in the UK, whilst all the antimicrobial agents demonstrated lower susceptibility rates with CIP-R organisms, carbapenems appeared least affected. The most marked differences in the susceptibility of CIP-S organisms compared with CIP-R were evident

in the CAZ, TAZ and GM activity against *A. baumannii* (71.2% vs. 17.4%, 63.7% vs. 15.3% and 81.8% vs. 12.9%, respectively). There is a trend shown to increasing CIP resistance over time.
Conclusions: As observed in the UK, loss of CIP-susceptibility in Europe is associated with trends towards co-resistance to other commonly used antimicrobials. Led by MEM, carbapenems remain useful first-line agents against both CIP-R and CIP-S Gram-negative bacteria.

P782 Susceptibility of bacterial isolates from Turkey – a report from the MYSTIC Program 2000

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Objective: To determine the in vitro activity of meropenem (MEM) and seven comparator antimicrobial agents against nosocomial isolates obtained in 2000 from units that use MEM in nine Turkish university hospitals. This is part of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) program.
Methods: Minimum inhibitory concentrations (MICs) for MEM, imipenem (IPM), ceftazidime (CAZ), cefotaxime (CTX), cefepime (CPE), piperacillin–tazobactam (TAZ), ciprofloxacin (CIP) and tobramycin (TM) were determined for 100 Gram-positive (G+ve) and 100 Gram-negative (G–ve) species at each center using E-test (AB BIODISK, Sweden) and NCCLS susceptibility breakpoints.
Results: A total of 373 G+ve strains and 993 G–ve strains were studied. Methicillin-susceptible *S. aureus* (MSSA; $n = 212$) and MS coagulase-negative staphylococci (CNS; $n = 85$) were the most often tested. *Pseudomonas aeruginosa* ($n = 214$), *Escherichia coli* ($n = 203$), *Klebsiella pneumoniae* ($n = 156$), *Acinetobacter baumannii* ($n = 116$) and *Enterobacter* spp. ($n = 57$) were the most common G –ve isolates. Of MSSA isolates, >93% were susceptible to all agents tested. MEM and IMP were the most active agents against *E. coli* (both 99% susceptibility); the other β-lactams tested inhibited only 78–85% of *E. coli* isolates. The carbapenems were also the most active agents against *K. pneumoniae* isolates (≥95% susceptibility); the remaining agents tested inhibited <71% of isolates. Of *K. pneumoniae* isolates, 41% had a phenotype consistent with ESBL production, and in 15% of *E. coli* isolates ESBL production was suspected. All the *Enterobacter* spp. strains were susceptible to carbapenems; other β-lactams tested were less active (88% with CPE, and <70% with others). Against *P. aeruginosa* isolates, TAZ (69%) was the most active agent followed by MEM (52%), IPM (48%), CAZ (46%), CIP (46%), and CPE (44%). MEM and IPM were the most active agents against *A. baumannii* (70 and 67% susceptibility, respectively).
Conclusion: Carbapenems (MEM > IPM) were the most potent agents overall against G+ve and G–ve isolates from Turkish hospitals in 2000. MEM and IPM are also highly active against G–ve isolates that include ESBL/AmpC producers. MIC_{90} results against *P. aeruginosa* and *A. baumannii* indicate that the use of carbapenems in combination therapy could be clinically beneficial.

P783 No evidence of increasing Gram-negative resistance to meropenem in Germany: data from the MYSTIC Program 1997–2001

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Objective: To monitor resistance trends to meropenem (MEM) and other broad-spectrum antibiotics among Gram-negative organisms isolated over a 5-year period in German hospital units where meropenem is prescribed.
Methods: Isolates were tested using National Committee for Clinical Laboratory Standards methodology to determine the minimum inhibitory concentration (MIC) values (mg/L) and susceptibility breakpoints of MEM and several other antimicrobial agents including imipenem (IPM), ceftazidime (CAZ), piperacillin-tazobactam (TAZ), ciprofloxacin (CIP) and gentamicin (GM). Isolates were collected from seven centers in Germany; four intensive-care units, one general ward, one neutropenia center and one cystic fibrosis (CF) unit.
Results: During the period 1997–2001, a total of 2696 Gram-negative strains were collected and tested at the non-CF hospital units in Germany, of which 2003 (74.3%) were members of the Enterobacteriaceae. The most common species tested were *Escherichia coli* (25.6%), *Pseudomonas aeruginosa* (19.7%) and *Klebsiella pneumoniae* (9.5%), followed by *Enterobacter cloacae* (8.2%), *Proteus mirabilis* (5.8%) and *Klebsiella oxytoca* (4.3%). The activity of MEM was higher than or equal to IPM against the most commonly isolated organisms. Against *E. coli* and *K. pneumoniae*, MEM and IPM were the most potent agents (98.5–100% susceptibility). Interestingly, the percentage susceptibility of *P. aeruginosa* isolates to MEM and IPM was much greater in 2001 (95.6 and 87.6%, respectively) compared with 1997 (78.5 and 69.8%, respectively). Overall, the 5-year data show no evidence of a trend to increased resistance to the carbapenems. Against *P. aeruginosa*, a higher proportion of isolates overall were susceptible to TAZ (95.1%) than to the carbapenems (81.0–86.5%), although MIC_{90} values were four-fold lower for both MEM and IPM than TAZ. Generally CIP demonstrated the lowest activity against the most commonly isolated organisms. In the CF center, *P. aeruginosa* was by far the most common organism ($n = 579$ strains; 88.4% of the total). MEM and TAZ were the most active antimicrobial agents against these isolates (85.3 and 89.5% cumulative susceptibility, respectively).
Conclusion: Data from this 5-year study period show no evidence of increasing resistance among Gram-negative organisms to meropenem in German hospital units where this carbapenem is prescribed. However, continued surveillance is needed, in order to monitor any future resistance trends.

P784 Analysis of 5-year antimicrobial surveillance data from the MYSTIC program in Italy (1997–2001)

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Objective: The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Program is a global longitudinal antimicrobial surveillance study that collects isolates from intensive care units (ICU), neutropenia units, cystic fibrosis (CF) centers and general wards where meropenem (MEM) is prescribed. The four participating Italian centers represent each of these four types of hospital unit. Here we present a summary of combined cumulative data from 1997 to 2001.
Methods: Organisms were tested using National Committee for Clinical Laboratory Standards (NCCLS) methodology. Minimum inhibitory concentration (MIC) values and percentage susceptibilities at NCCLS breakpoints (%S) were obtained for MEM and a range of comparators including imipenem (IPM), ceftazidime (CAZ), piperacillin + tazobactam (TAZ) ciprofloxacin (CIP) and gentamicin (GM).
Results: The four Italian centers contributed a total of 2654 Gram-negative and Gram-positive isolates between 1997 and 2001, the most common isolate of those collected and tested being *P. aeruginosa* ($n = 841$; 31.7%). Just under half of the *P. aeruginosa* isolates ($n = 411$) were obtained from the CF unit, while 235 were from the ICU. *E. coli* (14.5% overall) was the most common isolate obtained from neutropenia units and general wards. Other common species were methicillin-susceptible *S. aureus* (9.3%), *K. pneumoniae* (6.9%), *E. cloacae* (5.9%) and *P. mirabilis* (5.1%). In general, the carbapenems (MEM and IPM) were the most active antimicrobial agents tested against the common organisms (range of susceptibility: 100–76.3% and 100–60.2%, respectively). The percentages of MEM was the same as or higher than IPM against every organism tested. Like MEM, TAZ also showed good in vitro activity against *P. aeruginosa* (>76% susceptible), however, the activity of CIP and GM against *P. aeruginosa* was relatively poor (<40% susceptible). All the agents tested demonstrated a high potency against *E. coli* (84.6–100% susceptible) with the exception of CIP (67.7%). *K. pneumoniae* was 100% susceptible to MEM and IPM throughout the study period while the activity of the other agents was considerably lower (CAZ 78.1%, TAZ 75.4%, CIP 61.8%, GM 78.1%).
Conclusion: The carbapenems, in particular meropenem, remain the most active antimicrobials against the range of Gram-negative and Gram-positive bacteria isolated from various hospital units in Italy. There was no apparent increase in resistance to meropenem over the 5-year period.

P785 Antimicrobial resistance patterns from organisms causing bacteremia in European intensive-care units: cumulative data from the MYSTIC program

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Objective: To determine the antimicrobial resistance of bacteria causing bacteremia in European intensive care units (ICUs). Data were analyzed from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program. MYSTIC is a global, longitudinal surveillance study in which data are collected on antimicrobial resistance patterns from hospital centers where the carbapenem meropenem (MEM) is prescribed.
Methods: National Committee for Clinical Laboratory Standards methodology were used to determine the minimum inhibitory concentration (MIC) values (mg/L) and susceptibility breakpoints of MEM and a range of other antimicrobial agents including imipenem (IPM), ceftazidime (CAZ), gentamicin (GM) or tobramycin (TM), piperacillin–tazobactam (TAZ), and ciprofloxacin (CIP).
Results: A total of 2228 blood-culture isolates were obtained from ICUs in Europe during the period of 1997–2001. A wide range of organisms were collected and tested, the most common being methicillin-susceptible *S. aureus* (13.6%), *E. coli* (10.9%), *S. epidermidis* (8.8%), *E. faecalis* (8.3%), and *P. aeruginosa* (7.6%). MEM, IPM, GM and TAZ demonstrated high potency for methicillin-susceptible *S. aureus* (94–97% susceptibility, $MIC_{90} = 0.25$–4) and CAZ demonstrated the lowest activity (77% susceptibility, $MIC_{90} = 16$). MEM and IPM were the most active agents against *E. coli* (both 100% susceptibility, $MIC_{90} = 0.064$–0.5) compared with the other agents tested and CIP was the least active agent (75% susceptibility, $MIC_{90} = 32$). MEM, IPM, and TAZ were most active against *S. epidermidis* (94–97% susceptibility, $MIC_{90} = 2$–4). CAZ and CIP were the least potent agents against *S. epidermidis* (64 and 66% susceptibility, respectively, $MIC_{90} = 32$ for both). IPM and TAZ demonstrated the highest activity against *E. faecalis* (93 and 91% susceptibility, $MIC_{90} = 4$ and 16, respectively) and CIP demonstrated the lowest (75% susceptibility, $MIC_{90} = 32$). The antimicrobial agents that demonstrated the highest potency against *P. aeruginosa* were TAZ and MEM (79 and 75% susceptibility, $MIC_{90} \geq 128$ and 64, respectively).
Conclusion: Carbapenems remain the most active antimicrobial agents against a broad spectrum of Gram-positive and Gram-negative blood-culture isolates, with meropenem demonstrating the highest activity against the majority of organisms isolated. CAZ, GM, and CIP generally demonstrated the lowest activities against the commonly isolated organisms.

P786 Bacteremia in European neutropenic patients: a report from the MYSTIC Program

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Objective: The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program is a global, longitudinal surveillance study initiated in 1997 that collects data on antimicrobial resistance patterns from centers where meropenem (MEM) is prescribed. The objective of this study was to examine the MYSTIC Program data in relation to bacteremia from European units with neutropenic patients and to identify any trends in antimicrobial resistance.
Methods: Blood-culture isolates from neutropenic patients in Europe were speciated using standard methods. The minimum inhibitory concentrations (MICs) and breakpoint susceptibilities (%S) for MEM, and a range of comparator agents including imipenem (IPM), ceftazidime (CAZ), piperacillin–tazobactam (TAZ), ciprofloxacin (CIP) and gentamicin (GM) were determined using the National Committee for Clinical Laboratory Standards methodology.
Results: To date, a total of 567 bacteremias (Gram-positive and Gram-negative) have been identified and tested from European neutropenia units. The most commonly isolated and tested organisms were *Escherichia coli* (30.3%), *Pseudomonas aeruginosa* (9.7%), *Staphylococcus aureus* (7.9%), *Staphylococcus epidermidis* (7.4%) and *Klebsiella pneumoniae* (6.0%).

The carbapenems, MEM and IPM, were the most active antimicrobial agents against all the most commonly isolated and tested organisms (%S = 83.6–100%) with the exception of *S. epidermidis* against which TAZ (76.2%) and MEM (71.4%) were more active than IPM (66.7%). Activity against *S. epidermidis* was low for all antimicrobials with CAZ showing particularly decreased activity (51.3%). The activity of MEM was ≥IPM against all the most commonly isolated organisms and notably greater than IPM against *P. aeruginosa* (94.6% vs. 83.6%, respectively). MEM was particularly more potent than IPM against *E. coli*, *P. aeruginosa*, and *K. pneumoniae* ($MIC_{90} = 0.064$ vs. 1, 4 vs. 16 and 1 vs. 2, respectively). CIP generally exhibited poor activity against the most commonly isolated and tested organisms (42.1–86.7%).
Conclusions: The carbapenems MEM and IPM remain the most active antimicrobials against a range of Gram-negative and Gram-positive bacteremia in neutropenic patients. MEM in particular demonstrated higher activity against *P. aeruginosa* isolates compared with IPM (94.6 and 83.6%, respectively). Continued surveillance by the MYSTIC program appears prudent.

P787 Comparison of antimicrobial susceptibility of organisms isolated from blood cultures and other sources: data from the MYSTIC Program in Europe

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Objective: To evaluate the antimicrobial susceptibility of organisms isolated from blood cultures (BC) and other sources, using data from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program. MYSTIC is a global surveillance study, in which data are collected on antimicrobial resistance patterns from intensive care units (ICUs), hematology/oncology units, cystic fibrosis (CF) units and general wards, where the carbapenem meropenem (MEM) is prescribed. Evaluating the susceptibility patterns of invasive pathogens versus noninvasive pathogens is particularly important, due to the potentially greater threat of invasive pathogens to the patient.
Methods: National Committee for Clinical Laboratory Standards methodology were used to determine the minimum inhibitory concentration (MIC) values (mg/L) and susceptibility breakpoints of MEM and a range of other broad-spectrum antimicrobial agents including imipenem (IPM), ceftazidime (CAZ), gentamicin (GM), piperacillin-tazobactam (TAZ) and ciprofloxacin (CIP).
Results: A total of 3136 BC isolates and 17261 non-BC isolates were collected and tested from 1997 to 2000. The same three organisms were isolated and tested most frequently from BC and other sources: *E. coli* (16%), methicillin-susceptible *S. aureus* (12%), *P. aeruginosa* (8%) were the most common BC isolates and *P. aeruginosa* (21%), methicillin-susceptible *S. aureus* (14%), *E. coli* (11%) were the most common isolates from other sources. The higher proportion of *P. aeruginosa* in non-BC isolates was derived from respiratory samples from the CF units, which only provided non-BC data. MEM and IPM demonstrated the highest activity (greater percentage susceptibility) against the Gram-negative organism *E. coli* and against the Gram-positive organism methicillin-susceptible *S. aureus* in both BC and non-BC isolates (97–100% for both). MEM and TAZ demonstrated the highest activity against the Gram-negative organism *P. aeruginosa* from BC (81 and 82% susceptibility, respectively) and non-BC isolates (79 and 84% susceptibility, respectively).
Conclusion: We found no substantial difference in the antibiotic susceptibility between BC and non-BC isolates. Carbapenems remain the most widely active antimicrobial agents against a broad spectrum of isolates obtained from bacteremic and non-bacteremic patients.

P788 Antimicrobial resistance trends from intensive care units in Ostrava, Czech Republic: 5-year data from the MYSTIC program

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Objective: To evaluate antimicrobial susceptibility patterns in organisms isolated over a 5-year period (1997–2001) from the four intensive care units (ICUs) of Ostrava Teaching Hospital, Czech Republic, participating the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) program, a global longitudinal surveillance study evaluating resistance trends in units where meropenem (MEM) is prescribed.

Methods: Using National Committee for Clinical Laboratory Standards (NCCLS) methodology, minimum inhibitory concentration (MIC) values and susceptibility breakpoints were determined for 1025 Gram-negative and Gram-positive isolates obtained from patients in the ICUs. Organisms were tested against MEM and other antimicrobial agents including imipenem (IPM), ceftazidime (CAZ), gentamicin (GM), ciprofloxacin (CIP) and piperacillin/tazobactam (TAZ).
Results: The most common Gram-negative organisms tested were *Pseudomonas aeruginosa* (9.3%), *Escherichia coli* (8.8%) and *Klebsiella pneumoniae* (8.2%) while the most commonly isolated Gram-positives were *Enterococcus faecalis* (17.9%) and methicillin-susceptible *Staphylococcus aureus* (16.8%). Susceptibility rates to the carbapenems were greater or similar overall than those to the other antimicrobials for the most commonly isolated organisms. *E. coli* and *K. pneumoniae* were 100% susceptible to MEM and IPM. *K. pneumoniae* demonstrated relatively lower susceptibility to CIP (86.9%), CAZ (73.8%), GM (67.9%) and TAZ (67.7%). The susceptibility of *P. aeruginosa* to all agents was lower (52.6–71.6%), but particularly for CIP and GM (both 52.6%). The susceptibility of *S. aureus* to carbapenems was 100% during the surveillance period except in 2000 (both 93.3%). *E. faecalis* was more susceptible to the carbapenems (71.6–94.5%) and TAZ (100%) than to the other antimicrobials (CIP 58%, CAZ 15.3%, GM 16.9%). Resistance rates to CIP for *K. pneumoniae*, *E. faecalis*, and *P. aeruginosa* appeared to increase between 1997 and 2001. No trend towards resistance to the other antimicrobials was observed.
Conclusion: Carbapenems like MEM were consistently the most active of the antimicrobials tested overall against the commonly isolated ICU organisms with no obvious trend to increased resistance over the 5 years. In contrast, steadily decreased susceptibility of several organisms to the fluoroquinolone was demonstrated over the surveillance period.

P789 Antibiotic susceptibility of Gram-negative bacteria isolated in the intensive care unit: 5 years of the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study

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Objectives: To compare the in vitro activity of meropenem (MEM) and eight other antibiotics against Gram-negative bacteria isolated in the intensive care unit (ICU).
Methods: Gram-negative aerobes ($n = 500$) were isolated from specimens obtained from children hospitalized in an ICU during 1997–2001. The isolates were identified using conventional methods. Minimum inhibitory concentrations (MICs) of MEM, imipenem (IPM), piperacillin/tazobactam (TAZ), cefotaxime, ceftazidime, cefepime, ciprofloxacin (CIP), gentamicin (GM) and tobramycin (TM) were determined using the NCCLS agar dilution method.
Results: MEM and IPM were active against >90% of Gram-negative isolates (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *K. pneumoniae*, *Serratia marcescens* and *Acinetobacter baumannii*), except *Pseudomonas aeruginosa*. The carbapenems were highly active against the Enterobacteriaceae (MIC_{90} 0.03–0.25 mg/L MEM; 0.25–0.50 mg/L IPM) even though there was a high prevalence of β-lactamase or AmpC-producing strains (>40%). The MIC_{90} for both MEM and IPM was 1.0 mg/L for *A. baumannii*. For CIP, the majority of tested organisms had very low MIC_{90} values (0.015–0.5 mg/L), except *K. pneumoniae* (2 mg/L in 1998), *S. marcescens* (16 mg/L in 2000 and 2 mg/L in 2001) and *A. baumannii* (4 mg/L in 1997, 16 mg/L in 1998, 128 mg/L in 2000 and 1 mg/L in 2001). MIC_{90} values for TAZ ranged between 32 and >128 mg/L (44.0–77.3% susceptibility). GM and TM were active against 58.8 and 52.3% of isolates, respectively. The MIC_{90} (mg/L) for *P. aeruginosa* of MEM was 8 in 1997 and 2001, and for IPM the MIC_{90} was 16 in 1997 and 2001.
Conclusion: MEM and IPM demonstrated low MIC_{90} for tested strains in the children's ICU with no observed reduction in activity over 5 years. Carbapenem MICs for *P. aeruginosa* also appeared to remain stable during the review period.

P790 In vitro activity of meropenem, imipenem, piperacillin/tazobactam, ciprofloxacin, amikacin, and cefepime against *P. aeruginosa* and *A. baumannii* isolated from Brazilian intensive care units – MYSTIC Study Group (Brazil 2001)

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The selection of multiresistant bacteria has increased subsequent to the introduction of a large number of antibiotics with various mechanisms of action. The scale of this problem is best exemplified by the fact that most nosocomial infection, mainly in ICUs, involve multiresistant bacteria producing several types of enzymes such as extended spectrum β-lactamases, depression of chromosomal *ampC* β-lactamases, plasmid-coded *ampC* β-lactamases in members of Enterobacteriaceae, and multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In this study, the in vitro activities of meropenem (MEM), imipenem (IMP), piperacillin/tazobactam (PIP/TAZ), ciprofloxacin (CIP), amikacin (AMI), and cefepime (CPM) were monitored and compared against 135 ICU isolates of *Pseudomonas aeruginosa* ($n = 90$) and *Acinetobacter baumannii* ($n = 45$). Minimal Inhibitory Concentrations (MICs) were determined by E-test methodology, using standardized and controlled procedures. Overall susceptibility results, MIC_{50} and MIC_{90} values are shown in the table below. A high rate of resistance was noticed among the tested strains. Meropenem was the most active agent against both species evaluated. Additionally, the MICs for *P. aeruginosa* were steadily lower when compared to imipenem. Towards *Acinetobacter baumannii*, performances of meropenem and imipenem were similar, showing much superior efficacy comparing to other class of agents. Currently, as we can see, there are few alternative therapies for infections caused by these two species. Infection control measures, rational antibiotic policies and rapid laboratory detection of resistance are the key measures in preventing the spread of these strains.

	P. aeruginosa ($n = 90$)					*A. baumannii* ($n = 45$)				
	%S	%I	%R	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	%S	%I	%R	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
MEM	72.2	7.8	20	0.38	>32.0	93.3		6.7	1.0	2.0
IMP	66.7	3.3	30	1.0	>32.0	93.3		6.7	0.5	2.0
PIP/TAZ	76.7		23.3	4.0	>256.0	24.4	8.9	66.7	>256.0	>256.0
CIP	53.3	2.2	44.5	0.38	>32.0	22.2	2.2	75.6	>32.0	>32.0
AMI	60	4.4	35.6	8.0	>256.0	22.2	11.1	66.7	>256.0	>256.0
CPM	71.1	11.1	17.8	4.0	32.0	26.7	2.2	71.1	96.0	>256.0

P791 Emerging resistance among *Proteus mirabilis* isolates in Europe: report from the MYSTIC program (1997–2001)

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Objective: Resistance (R) patterns that are currently problematic in Europe (EU) can vary greatly within the same species over time, among various patient populations and among geographic regions on the same continent. The results from the MYSTIC Program which monitors meropenem (MEM; a carbapenem)-R in institutions using MEM, were used to determine R differences among *P. mirabilis* for EU from 1997 to 2001.
Methods: MYSTIC collected MIC results from 688 *P. mirabilis* strains that were classified into patient care groups: ICU ($n = 426$), neutropenia (NP; $n = 145$), general wards ($n = 97$) and cystic fibrosis patients (pts) (CF; $n = 20$). A total of 31 centers from 10 countries participated, divided into three regions (East, North, South). All testing was by reference methods and interpreted by NCCLS criteria, including screening of ESBL phenotypes (clavulanate inhibition). Six β-lactams, ciprofloxacin (CIP), gentamicin (GM) and tobramycin (TM) were tested.

Results: Over the monitored 5 years, the R rates varied for each agent without a clear trend toward greater R. Rank order of susceptibility was: MEM (99%) >piperacillin–tazobactam (TAZ; 96%) > cefepime (95%) > ceftazidime (CAZ; 94%) > imipenem (IPM; 92%); CIP was least active (MIC_{90}, 4 mg/L; 86% susceptible). Unexpectedly, 3.6% of *P. mirabilis* were IPM-R (MIC, ≥16 mg/L). Greater was found for strains from NP and CF, for example 40–77% susceptibility to CIP. *P. mirabilis* in East-EU sites were significantly more R to cephalosporins (ESBL rate, 24%), but CIP- and GM-R was greatest in South-EU centers. ESBL rates were 8% in North-EU, but >20% for the other geographic regions. Carbapenems (MEM > IPM) and the β-lactamase inhibitor combination (TAZ) remained most active overall.
Conclusions: Normally susceptible species such as *P. mirabilis* have emerged as therapeutic problems in EU, following R mutations compromising CIP, CAZ and aminoglycoside use. IPM also showed decreased susceptibility of nearly 7% compared to only 4% with MEM. Continued surveillance by the MYSTIC Program appears to be a prudent practice to guide effective empiric treatment regimens.

Immunomodulation by antibiotics

P792 Immunomodulating effects of antibiotics

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In addition to their well-known antimicrobial properties, antibiotics have also been shown to have a wide range of immunomodulatory effects. Isoniazid (INH) is an essential agent in the management of tuberculosis, while ciprofloxacin (CPF) is extensively used in treating many infective states. There is no published data with regards to INH, and only limited information on CPF regarding their possible effects on the inflammatory response. We have evaluated the effect of INH and CPF on reactive oxygen species (ROS) generation by human neutrophils with lucigenin-dependent chemiluminescence assay using *N*-formyl-L-leucyl-phenylalanine (FMLP), phorbol-12-myristate-13-acetate (PMA) stimulation or spontaneous lucigenin-enhanced chemiluminescence. INH at concentrations of 1.25 mg/L significantly inhibited chemiluminescence, while CPF had no inhibitory effects at concentrations achievable in man. We have also examined the effect of INH and CPF on cytokine expression by monocytes obtained from healthy human volunteers and stimulated with either lipopolysaccharide on heat-killed *Staphylococcus aureus* [Pansorbin]. INH at level achievable in man suppressed the in vitro synthesis of interleukin-1α (IL-1α), and IL-10. However, there was no significant in vitro effect on the synthesis of IL-6 and tumor necrosis factor alpha. In contrast, CPF exhibited significant inhibitory effect on all tested cytokines in a concentration-dependent manner at levels ranging from 1.25 to 10 mg/L. These effects were not due to any direct effect of the drugs on cellular viability as determined by ATP assay. These results reveal that INH and CPF possess significant immunomodulatory activity in vitro and suggest that suppression of acute-phase inflammatory responses may occur in vivo by modifying human monocyte's cytokine synthesis.

P793 Moxifloxacin inhibits staphylococcal superantigen-induced apoptosis in T lymphocytes

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Objectives: *Staphylococcus aureus* represents a potent threat to both healthy and immunocompromised individuals. In this regard, *S. aureus* is a major cause of respiratory infection, including patients with cystic fibrosis (CF). The clinical isolates release a wide array of toxins including superantigens, which cause extensive T-cell proliferation, induce the release of excessive quantities of cytokines and promote programmed cell death. Programmed cell death or apoptosis is shown to be of central importance in immune functions. As the number of immunosuppressed patients requiring antibiotic treatment is increasing, the impact of antibacterials on immune responses has become of increasing interest. Therefore, we attempted to determine the modulatory role of the new antistaphylococci fluoroquinolone moxifloxacin on superantigen-induced apoptosis.
Methods: Moxifloxacin was used at physiological concentrations, which can be found in the serum of moxifloxacin-treated patients. For this purpose, the effects of staphylococcal enterotoxin B (SEB) on regulatory molecules in apoptosis (TNF-RI, Fas, Fas-L) on human peripheral blood mononuclear cells and Jurkat cells were analyzed in the absence and presence of moxifloxacin.
Results: Moxifloxacin inhibits apoptosis as well using annexin V binding to Jurkat cells by FACS analysis. Furthermore, moxifloxacin suppressed the expression of Fas, FasL, TNF-RI mRNA in the absence and presence of SEB. The two death receptors, TNF-RI and Fas, promote apoptosis when activated by their ligands, e.g. TNF-α and FasL, respectively. Therefore, we further analyzed whether moxifloxacin modulates the apoptotic pathway induced via the TNF-RI and Fas molecule. For this purpose Jurkat cells, sensitive to TNF-α and anti-Fas mediated apoptosis were left untreated or were treated with TNF-α, with anti-Fas or with TNF-α plus anti-Fas in the presence and absence of moxifloxacin.
Conclusion: The results clearly show that moxifloxacin influences apoptosis as could be demonstrated using annexin V binding to Jurkat cells by FACS analysis. The inhibitory effects of moxifloxacin on apoptosis were at least due to the inhibition of caspase-3 activity shown by enzymatic assay. The findings support the theory that moxifloxacin might diminish cell injury by interference with apoptotic effector molecules.

P794 Moxifloxacin modulates effector functions of human neutrophils

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Objectives: Evidence has been obtained that moxifloxacin exerts immunomodulatory effects in human effector cells, which then improves host defense mechanisms. In the present studies, moxifloxacin, clindamycin, rifampicin and ampicillin were interacted with human neutrophils which were subsequently stimulated with the bacterial peptide fMLP or the phorbolmyristate acetate (PMA), a protein kinase C activator.
Methods: The following parameters were investigated: (1) the expression of CD62L, CD11b, chemotaxin receptor CXCR2, fMLP-receptor by FACS analysis, (2) by ELISA the release of cytokines IL8, TNF-α, of the prostanoids leukotriene-B4, prostaglandin-E_2, the release of elastase, bacterial killing for MSSA and *E. coli*, (3) by RT-PCR the expression of chemotaxin receptors CXCR1, CXCR2, Toll receptors 2 and 4, cyclooxygenase 2 and 5-lipoxygenase.
Results: Each compound exerts individual and different characteristics as to the above parameters. Moxifloxacin induces: (1) enhanced bacterial killing in neutrophils, (2) suppresses PMA and fMLP-induced leukotriene-B4 formation with regulatory activity on prostaglandin-E_2, (3) increased IL8 induction, (4) enhanced CXCR2 and fMLP receptor expression on the surface, and also (5) modulates the mRNA expression for chemotaxin receptors and Toll receptors.
Conclusions: The data clearly indicate that various compounds behave differently and also that moxifloxacin most likely via defined cell biological properties of the signal transduction cascade exerts potent immunomodulatory activities which are supportive in host defense and bacterial clearance beyond its effects on individual microorganisms alone.

P795 Ciprofloxacin versus ceftazidime in cytokine generation in severe sepsis

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Objectives: To determine the effect of ciprofloxacin and ceftazidime on cytokine production in patients with severe sepsis.

Patients and methods: Forty-two patients with severe sepsis (septic SIRS plus at least one organ dysfunction) were randomized to receive either ciprofloxacin 400 mg × 3/day i.v. ($n = 22$) or ceftazidime 2 g × 3/day ($n = 20$). Both groups were matched for age, sex and simplified acute physiology II score. Serum cytokine levels were determined by using Elisa (Quantikine, R & D Systems, Minneapolis) on admission and 48 h during treatment.
Results: No differences in tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-1 receptor antagonists (IL-1ra), soluble TNF receptors (sTNFR-I and II) were found between the two treatment groups on admission and 48 h during treatment. Although, baseline IL-10 levels in ciprofloxacin-treated group were not significantly different from the ceftazidime group (147.23 ± 70.06 vs. 139.81 ± 51.3 pg/mL), we detected a significant increase in serum IL-10 levels 48 h post-treatment in patients receiving ciprofloxacin compared to those under ceftazidime (154.00 ± 64.01 vs. 117.58 ± 44.04, $P = 0.042$). In order to investigate the effect of both antimicrobials on anti- versus pro-inflammatory response, we compared the ratio of IL-10 : TNF-α in both the groups. No significant differences were noted between the two groups on admission, while 48 h during treatment a significant decline of IL-10 : TNF-α ratio was observed in the ceftazidime compared to ciprofloxacin group (1.96 ± 1.26 vs. 3.14 ± 1.57, $P = 0.01$).
Conclusion: It seems that ciprofloxacin but not ceftazidime induces a significant anti-inflammatory response through an increase of IL-10 : TNF-α ratio in patients with severe sepsis. This immunomodulatory effect might be important for the outcome of such patients as the excess proinflammatory response could be deleterious.

P796 Quinolone antibiotics have selective effects on polymorphonuclear leukocyte functions

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Objectives: Polymorphonuclear leukocytes (PMN) have an important role in innate immune responses and inflammation, through phagocytosis and intracellular killing, and cytokine release. We have shown previously that moxifloxacin (Bay 12-8039, Bayer Plc) has profound inhibitory effects on interleukin-8 (IL-8) release from human PMN at therapeutic concentrations [1]. Ciprofloxacin has also been shown to modulate IL-8 and IL-6 release from PMN and endothelial cells via effects on transcription [2, 3]. The effect of moxifloxacin and ciprofloxacin on other aspects of PMN function are not known. We therefore investigated the effect of these antibiotics on PMN release of the enzyme myeloperoxidase from activated human PMN in vitro.
Methods: PMN were isolated from 15 healthy volunteers aged 20–47 years, following Ethical Committee approval and informed consent, using single density gradient centrifugation. Cells were incubated with 2 μg/mL lipopolysaccharide (LPS) plus 0–50 μg/mL moxifloxacin or 0–100 μg/mL ciprofloxacin at 37 °C in 95% air/5% CO_2 for 24 h. Myeloperoxidase was measured in cell culture supernatants using enzyme immunoassay. Data are expressed as median (range) and were analyzed using Friedman analysis of variance.
Results: Despite the marked effects of both ciprofloxacin and moxifloxacin on IL-8 release, there was no effect on the release of myeloperoxidase from LPS-stimulated PMN. Concentrations remained constant at all concentrations of both antibiotics (Fig. 1).

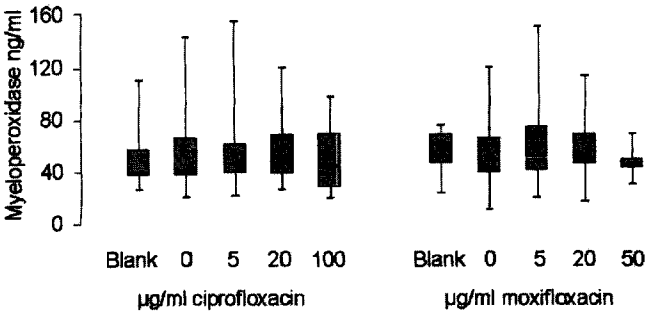

Figure 1

Conclusions: The immunomodulatory effects of moxifloxacin and ciprofloxacin in terms of PMN function are specific and do not represent generalized effects on cellular activity.

Acknowledgment: We are grateful to Bayer AG for financial support.

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P797 Impact of carbapenem administration on endotoxin and interleukin-6 blood levels of septic patients

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Objective: It has been proposed that α-lactams may elicit the in vitro release of endotoxins (LPS) by Gram-negative bacteria so as to perpetuate the inflammatory response. In the present study, endotoxemia was monitored following administration of imipenem and meropenem in critically ill patients.
Methods: Eleven septic patients aged 75.5 ± 8.7 years with 6.5 ± 4.3 SOFA score were enrolled in the study score. All had Gram-negative bacteremia (*Pseudomonas aeruginosa*: five; *Klebsiella pneumoniae*: three; others: three); seven were administered i.v. imipenem 1 g tid and four meropenem 2 g tid. Blood was collected by venipuncture at regular time intervals before and after the initiation of therapy. LPS was assayed by LAL QCL-1000, interleukin-6 (IL-6) by EIA and C-reactive protein (CRP) by nephelometry.
Results: Mean levels of LPS before administration of imipenem and 1, 2, 4, 6, 12, 24, 48 and 72 h after administration of imipenem were 5.22, 2.90, 4.97, 2.67, 3.10, 2.67, 18.82, 3.93 and 2.02 IU/mL, respectively. At the respective time intervals for meropenem, mean LPS were 3.37, 1.86, 3.00, 3.47, 3.23, 2.67, 3.42, 4.87 and 2.63 IU/mL. Mean levels of IL-6 before administration of imipenem and 1, 2, 4, 6, 12, 24, 48 and 72 h after administration of imipenem were 161.9, 140.9, 158.4, 149.3, 148.3, 148.5, 50.1, 76.2 and 72.1 pg/mL, respectively. At the respective time intervals for meropenem, mean IL-6 were 1029, 214.3, 170.9, 188.2, 160.6, 89.7, 157.1, 241.2 and 199.1 pg/mL. Mean levels of CRP before administration of imipenem and 24, 48 and 72 h after administration of imipenem were 135.9, 147.7, 133.6 and 120.6 mg/L, respectively. At the respective time intervals for meropenem, mean CRP were 133.1, 160.3, 148.9 and 78.7 mg/L.
Conclusions: Systemic endotoxemia does not differ considerably following intravenous administration of imipenem or meropenem so as to change the concentrations of IL-6 and CRP which are markers of systemic inflammation. Both carbapenems may be administered in critically ill patients without fear of antibiotic-induced endotoxemia.

P798 Effects of doxycycline on human polymorphonuclear leukocyte (PMN) function

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Objectives: Some in vitro studies have suggested that tetracyclines have depressive effects on polymorphonuclear leukocyte (PMN) function. We have previously found doxycycline in vivo and in vitro to be associated with a decrease in human PMN Fc-gamma-receptor expression. In the present study, we examined the effects of pure and commercial preparations of doxycycline on PMN chemiluminescence and random migration.
Methods: PMNs were incubated in dilutions of pure and commercial (Vibramycin (R), Pfizer, NY) doxycycline. Chemiluminescence following zymosan phagocytosis was recorded in an automatic photoluminometer. Random migration was measured in capillary tubes.
Results: Incubation in increasing (25–200 μg/mL) concentrations of doxycycline resulted in decreased chemiluminescence and tube migration (less than 50% of controls at 200 μg/mL). In contrast, incubation in Vibramycin® was associated with mean values of chemiluminescence and tube migration of between 80 and 95% of controls for all concentrations.
Conclusion: These results show that incubation in increasing concentrations of doxycycline is associated with decreasing PMN chemiluminescence and tube migration. However, this finding was absent with a commercial doxycycline preparation containing ascorbic acid as a preservative. This suggests caution in interpreting the results of in vitro studies of the effects of doxycycline on PMN function. Such studies with pure doxycycline may not be relevant in vivo when commercial solutions are used.

P799 Variation in the propensity to release endotoxin after cefuroxime exposure in different Gram-negative bacteria: uniform and dose-dependent reduction by the addition of tobramycin

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Gram-negative (G–) bacterial infections associated with shock still have a high mortality rate despite optimal treatment. Endotoxin (E), a major constituent of the outer cell membrane of the G– bacteria, is a potent stimulator of the systemic inflammatory response and has been considered to play an important role in the pathophysiology of septic shock. In a previous study, we have shown that the cefuroxime (CEF)-induced E from an *E. coli* strain could be considerably reduced by the addition of tobramycin (TM).

Objectives: The aim of this study was to investigate whether this response was generally applicable to other G– bacteria.

Methods: The release of E from different strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enteritidis* and *Neisseria meningitidis* was studied in vitro. The strains in logarithmic phase were exposed to 2, 10, and 50 × MIC of CEF, TM and a combination of CEF and TM, respectively. Samples for viable counts and E were drawn at 0, 2, and 4 h. All the experiments were made in triplicate. For the analysis of E, a chromogenic LAL assay was used. In an additional experiment with the *K. pneumoniae* strain exposed to 2 × MIC of CEF, TM, and the combination, the starting inoculum was varied from 103 to 107 bacteria/mL.

Results: The MICs of the strains varied from 0.05 to 8.0 mg/L for CEF and from 0.75 to 2.0 mg/L for TM. The experiment with varying inoculum sizes demonstrated that there was a proportional and significant linear relationship between the E release and the number of killed bacteria for each antibiotic ($P < 0.001$). Therefore, the propensity to liberate E was expressed as the E release per killed bacterium. After 4 h, the CEF-induced E release per killed bacterium (CEF 2 × MIC) was $3.7 + 0.9$ (+SE), $2.5 + 0.7$, $5.7 + 1.0$, $2.3 + 1.7$, $7.7 + 1.8$, and $0.4 + 1.43 \times 10^{-3}$ EU per killed bacterium for the three *E. coli* strains, *K. pneumoniae*, *S. enteritidis* and *N. meningitidis*, respectively. This E liberation was significantly and considerably lower for all strains when exposed to TM or the combination than to CEF alone ($P < 0.001$), except for *N. meningitidis* for which only a nonsignificant trend was seen. With increasing doses of TM or the combination, the E release was significantly reduced ($P < 0.05$).

Conclusion: There was a large variation between the tested strains in the propensity to release endotoxin. However, regardless of the strain, TM significantly reduced the CEF-induced endotoxin release despite a higher killing rate.

In vitro evaluation of antimicrobials in enterococci

P800 Evaluation of antimicrobial resistance in *Enterococcus* spp. isolated in Romania from January 1999 to December 2000

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Objectives: To study the antibiotic resistance in enterococci.

Methods: A total of 288 *Enterococcus* strains isolated between January 1999 and December 2000 were collected from: urine ($N = 144$), surgical wounds ($N = 76$), others ($N = 68$): drain, bile, blood, prosthesis, catheter, CSF, peritoneal fluid, sinus. The strains were characterized by using standard protocols and tested for β-lactamase. The enterococci were studied for susceptibility to nine antibiotics: penicillin (Pc), ciprofloxacin (Cip), erythromycin (Em), chloramphenicol (Cm), tetracycline (Te), gentamicin (Gn), streptomycin (Str), vancomycin (Va), teicoplanin (Tei) by two methods: screening agar plates and agar dilution according to NCCLS recommendations.

Results: The data were analyzed according to NCCLS 1999 and showed the following aspects: 78.1% strains were identified as *E. faecalis*, 15.9% as *E. faecium*, 2.08% as *E. durans*, 1.4% as *E. avium*, 1% as *E. casseliflavus*, 0.7% as *E. hirae*, 0.3% as *E. raffinosus*, and 0.3% as *E. gallinarum*. Penicillin resistance was seen in *E. faecium* only: 80% (MIC_{50}: 32 mg/L, MIC_{90}: 128 mg/L). No β-lactamase producers were detected. Ciprofloxacin resistance for *E. faecium* was 80% (MIC_{50}: 32 mg/L, MIC_{90}: 128 mg/L) and for *E. faecalis* was 12.5% (MIC_{50}: 1 mg/L, MIC_{90}: 4 mg/L). Em resistance for *E. faecium* revealed 67.5% (MIC_{50}: 128 mg/L, MIC_{90}: 256 mg/L)and for *E. faecalis* 40.6% (MIC_{50}: 1 mg/L, MIC_{90}: 256 mg/L). Cm resistance for *E. faecium* was 12.5% (MIC_{50}: 8 mg/L, MIC_{90}: 32 mg/L) and for *E. faecalis* 30.5% (MIC_{50}: 8 mg/L, MIC_{90}: 64 mg/L). Resistance to TC was observed for *E. faecium* 92.5% (MIC_{50}: 64 mg/L, MIC_{90}: 128 mg/L) and for *E. faecalis* 91.5% (MIC_{50}: 128 mg/L, MIC_{90}: 256 mg/L). Concerning *E. faecium* high level resistance (HLR) to Gn was 69.5% and to Str 43.4%. For *E. faecalis* HLR resistance to Gn was 14% and to Str 39%. No resistant strain to Va, Tei was found (vancomycin 8 mg/L was detected in three motile enterococci, only).

Conclusions: Enterococci phenotypes to Pc, Cip, Em, Cm, Gn and Str differed among species. HLR was increased in *E. faecium*, versus *E. faecalis*. In the future, more prudent policy for antibiotics, but preserving for seriously ill and immunosuppresed patients is needed.

P801 A multicentre European study on the prevalence of glycopeptide resistance among clinical isolates of enterococci

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On behalf of the European Ramoplanin Study Group

Objectives: To assess the prevalence of glycopeptide-resistance in clinical isolates of enterococci (GRE) from hospitalized patients in Europe and to determine their susceptibility to ramoplanin.

Methods: Between March and June 2001, 13 clinical microbiology laboratories in eight European countries prospectively collected all isolates of enterococci. A total of 1317 nonduplicate strains, identified as enterococci according to standard laboratory methods, were tested for glycopeptide resistance by plating on vancomycin screen agar (VSA) supplemented with vancomycin, 6 mg/L. For all strains that grew on VSA, the minimal inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined by E-test. Species confirmation and susceptibility testing by microdilution (NCCLS) were performed in a single laboratory.

Results: A total of 50 (3.8%) strains were confirmed to be GRE, with a prevalence varying widely between laboratories, and ranging from 0 to 50.0%. The mean country rates (%) were Austria (1 lab) 0, France (two labs) 1.1, Germany (one lab) 2.5, Italy (three labs) 20.6, Spain (two labs) 3.1, the Netherlands (one lab), UK (one lab) 10.4. The strains were identified as *E. faecium* 28 (56%), *E. faecalis* 12 (24%), *E. gallinarum* 6 (12%), *E. casseliflavus* 3 (6%) and *E. species* 1 (2%). A total of 36 out of 40 (90%) *E. faecalis* and *E. faecium* isolates were of VanA type of resistance. The sites of isolation were: blood or i.v. catheter 17 (34%), wound infection 11 (22%), urine 8 (16%), respiratory tract 4 (8%) and other 10 (20%). The wards involved were intensive care 19 (38%), hematology–oncology–bone marrow transplant unit 5 (10%), surgery 6 (12%), medicine 5 (10%), transplant units 3 (6%) and other 12 (24%). Ramoplanin was uniformly active against GRE regardless of species and phenotype, with a MIC_{90} of 0.5 mg/L.

Conclusions: Although a great variability was observed between participating centers and countries, GRE were isolated at significant rates in intensive care units (7.7% of all enterococci) and from clinically relevant sites, representing 10.2% of bloodstream and 6.4% of wound infection isolates. GRE epidemiology deserves careful surveillance at least in high-risk wards. Ramoplanin, which is effective in suppressing gastrointestinal carriage, may be useful in preventing GRE infection.

P802 Characterization of enterococci isolated from nosocomial infections in a university hospital in Greece

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Objective: Enterococcal infections are becoming an increasing concern, particularly due to the emergence and spread of resistance to antimicrobial agents. A total of 121 enterococci recovered from inpatients during the period of April 2000–October 2001 have been studied for their phenotypes.
Materials and methods: All isolates were characterized at species level by Gram stain, catalase production and by the Crystal ID Gram-Positive System (BBL). β-Lactamase production was tested by nitrocefin disks (Difco). Minimal inhibitory concentrations (MIC) to the antimicrobials: ampicillin (Amp), erythromycin (Em), chloramphenicol (Cl), gentamicin (Gm), ciprofloxacin (Cip), vancomycin (Va), teicoplanin (Tp), quinupristin/dalfopristin (Rp) and linezolid (Ln), were performed by the E-test (AB Biodisk) according to NCCLS recommendations.
Results: Fifty *E. faecalis*, 62 *E. faecium* and 9 other species (4 *E. hirae*, 2 *E. durans*, 2 *E. gallinarum* and 1 *E. avium* isolates) have been identified. No isolate was found to be β-lactamase producer. The clinical sources included blood: 17 isolates (7 *E. faecalis*, 9 *E. faecium*, 1 other spp.); urine: 29 isolates (17 *E. faecalis*, 11 *E. faecium*, 1 other spp.); catheter tips:13 isolates (7 *E. faecalis*, 6 *E. faecium*); wounds and intra-abdominal: 62 isolates (19 *E. faecalis*, 36 *E. faecium*, 7 other spp.). Fifty-eight (93.5%) *E. faecium* and 2 *E. faecalis* were resistant to ampicillin, while 55 (88.7%) and 29 (58%) to erythromycin, respectively. Forty-two (67.7%) *E. faecium* isolates were of the VanA phenotype. Thirty *E. faecium* (48.3%) and 16 *E. faecalis* (32%) showed a high-level resistance to gentamicin. Sixteen *E. faecium* (25.8%) and all the other enterococcal species (100%) were resistant to quinupristin/dalfopristin, while the MIC to linezolid ranged from 0.5 to 6 mg/L among all species.
Conclusions: Glycopeptide-resistant enterococcal infection is emerging as an important problem in our hospital. The identification of quinupristin/dalfopristin-resistant isolates is a threatening problem for hospitalized patients as well. Since these observations suggested a significant nosocomial problem, further infection control measures should be applied.

P803 Antimicrobial resistance of *Enterococcus* spp. from systemic and urinary tract infections (UTI) (1991–2000)

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Objective: To evaluate *Enterococcus* species distribution and antimicrobial resistance in different clinical forms of infection, in order to monitories etiological therapy.
Methods: A total of 454 strains of *Enterococcus* spp. from hemocultures (46) cerebrospinal fluid (19), different collections (100), i.v. catheters (18) and urine (217) were isolated and identified by conventional methods. Antimicrobial resistance and sensitivity were determined by disk diffusion test (NCCLS guidelines).
Results: Enterococcal infections (sepsis, meningitis, localized infections, UTI) were caused in 88% by *Enterococcus faecalis*. Resistance to penicillin and ampicillin was 55.5%, respectively, 44.3%. In contrast, only 1.5% strains were resistant to ampicillin/sulbactam and 14% to amoxicillin/clavulanic acid. 44.5, 31.6 and 64.8% enterococci were resistant to erythromycin, chloramphenicol and tetracycline. High-level resistance to gentamicin was of 26.2%. Sensitivity to imipenem was 100% and two strains (out 57 tested) were resistant to vancomycin. There were differences between systemic infections and UTI isolated enterococci, the last being more resistant to antimicrobials.
Conclusions: Enterococci resistance to common antimicrobials was relatively high (31–44%), the strains being pluriresistant. Resistance to β-lactams/inhibitor of β-lactams was 8% on average, depending on specie and infection localization. Vancomycin resistance was uncommon (<1%) and all strains were susceptible to imipenem.

P804 Prosthetic valve endocarditis due to vancomycin-resistant *Enterococcus faecium* (VREF) treated successfully in the setting of quinupristin/dalfopristin (QP/DP) failure

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Introduction: Early prosthetic valve infections are serious and associated with higher mortality, especially in the setting of multidrug resistant microorganisms. Optimal antimicrobial therapy for endovascular infections due to VREF remains uncertain.
Results: A 68-year-old Caucasian woman presented with low-grade fever, dyspnea, and fatigue 4 weeks following elective aortic valve replacement and coronary artery bypass surgery. Her past medical history was remarkable only for adult-onset diabetes mellitus, which was well controlled. Physical examination revealed her to be in mild respiratory distress, and a mid-systolic murmur with mechanical heart sounds. All surgical wounds were well healed and there were no cutaneous or mucosal stigmata of endocarditis. Laboratory studies showed the following: WBC 9.8 K/μL, platelet 210 K/μL, AST 29 U/L, ALT 15 U/L, and ESR 97 mm/h. Transesophageal echocardiogram showed an ill-defined, mobile mass measuring 0.5 cm at the proximal aortic root. All peripheral blood cultures (six sets) grew VREF with high-level resistance to penicillin G (>16 μg/mL), ampicillin (>256 μg/mL), gentamicin (>1024 μg/mL), and vancomycin (>256 μg/mL). Initial therapy with vancomycin was changed to QP/DP (7.5 mg/kg every 8 h). Blood cultures on day 5, day 8, and day 12 following QP/DP therapy remained positive. On day 12, treatment with chloramphenicol (2 g daily), and doxycycline (200 mg daily) was started and QP/DP was discontinued. Blood cultures on days 4 and 6 of new antimicrobial regimen were sterile. She was considered too high-risk for repeat aortic-valve replacement surgery. Medical treatment was continued for 8 weeks. On 18-month follow-up, no recurrence was observed.
Conclusions: In our patient, coadministration of chloramphenicol and doxycycline for persistent refractory bacteremia and probable prosthetic aortic-valve endocarditis had favorable outcome and therapy for 2 months was tolerated with no adverse events.

P805 Vancomycin-resistant enterococci are prevalent in urban sewage and in sewage from a hospital in the Stockholm area of Sweden

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Objectives: Enterococci are members of the normal gut flora of animals and humans, and are thus, released into the environment directly or via sewage outlets, where they can survive for long time periods. Their role in nosocomial infections has increased due to their ability to acquire high-level resistance to antimicrobial agents that make them difficult to treat. In Europe, the use of the growth promoter avoparcin is considered to have selected for vancomycin-resistant enterococci (VRE). Sweden ceased using avoparcin 1986 and only occasional cases of clinical VRE have been reported since 1995. Within the framework of a European study, samples from urban raw sewage, treated sewage, surface water and hospital sewage in Sweden were screened for VRE.
Methods: VRE were isolated both through enrichment in broth supplemented with vancomycin and through membrane filtration followed by growth on agar plates with vancomycin. The isolates were phenotyped with the PhenePlate™ rapid screening system and genotyped with PFGE. All isolates were subject to antimicrobial susceptibility testing. Detection of the resistance genotypes *vanA* and *vanB* and identification to the species level of *E. faecalis* and *E. faecium* were done by PCR.
Results: VRE were isolated from 21 of 35 (60%) untreated sewage samples, from 5 of 14 (36%) hospital sewage samples, from 6 of 32 (19%) treated sewage samples and from 1 of 37 surface water samples. Thirty-five isolates from 33 samples were characterized with the specified methods. Most isolates (30 of 35) carried the *vanA* gene and the majority (24 of 35) of the isolates were

Enterococcus faecium. Most of the VRE were multiresistant. The typing revealed a high diversity for the isolates. However, one major cluster with seven identical or similar isolates was found. These isolates came from three different sewage treatment plants and were collected at different occasions during 1 year. All VRE from hospital sewage originated from one of the two hospitals studied. That hospital also had a consumption of vancomycin 10-fold that of the other.
Conclusions: We conclude that VRE were commonly found in sewage samples in Sweden. The origin might be healthy individuals or individuals in hospitals. Alternatively, antimicrobial drugs or chemicals released into the sewage system may sustain VRE in the system.

P806 External quality control of a multicentre European study on the prevalence of glycopeptide resistance among clinical isolates of enterococci

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On behalf of the European Ramoplanin Study Group

Objectives: Estimation of the variability of the results of in vitro susceptibility tests among 13 clinical microbiology laboratories in eight European countries participating in a multicenter study on the prevalence of glycopeptide-resistance enterococci (GRE).
Methods: The minimal inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined by *E*-test against *E. faecium* UA210 (*VanA*-type of resistance), *E. faecalis* UA605 (*VanB*), *E. gallinarum* UA604 (*VanC*), and *E. faecium* UA392 (susceptible). Each laboratory proceeded from 4 to 10 independent determinations of MICs, with a total number of 904 measurements. One center was not included on the basis of a too low number (<5 per strain) of determinations. Variance analysis with two criteria (strain-center), by the method of the complete blocks with repetitions and the protected least significant different test (PLSD) of Fisher, were carried out with StatView™ software. MIC values were converted into clinical categories and analyzed by the χ^2-test.
Results: There was full concordance in terms of clinical categorization among all centers for vancomycin and teicoplanin resistance of *E. faecium* UA210 and for vancomycin resistance of *E. faecalis* UA605; however, three laboratories found the latter strain teicoplanin-resistant, which represents a discrepancy. *E. gallinarum* UA604 was correctly categorized as vancomycin intermediate and teicoplanin susceptible except by one center which displayed great variability (from 12 to 256 mg/L) among the different, MIC determinations for vancomycin. *E. faecium* UA392 was categorized as glycopeptide susceptible by all centers but two, which reported it as intermediate in two and four instances, respectively.
Conclusions: The overall concordance of data for clinical categorization of glycopeptide susceptibility of enterococci was high. Three out of 12 laboratories, however, failed in identifying correctly the VanB phenotype of resistance. There were more minor discrepancies for vancomycin than for teicoplanin.

P807 Prevalence and resistance phenotypes of glycopeptide-resistant enterococci from hospitalized patients during a 2-year period

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Objectives: To study the prevalence and resistance phenotypes of glycopeptide resistant enterococci (GRE) isolated from clinical specimens, obtained from hospitalized patients, during 2-year period (November 1999–November 2001).
Methods: Enterococcal strains were identified and tested for antimicrobial susceptibility with the automated Vitek-II system (bioMérieux, France). The susceptibility was also tested by disk-diffusion procedure according to NCCLS standards.
Results: During 2-year study period, 60 GRE strains were isolated from 51 patients. Most of them were hospitalized in the ICU (36/51) and the Hematology Unit (5/51). The isolated strains included 28 (46.7%) *Enterococcus faecalis*, 31 (51.7%) *E. faecium* and 1 (1.6%) *E. casseliflavus*. Thirty-one strains were recovered from blood cultures, 4 from peritoneal fluids, 10 from pus and 15 from venous catheters. Fifty-four (90%) GRE strains were resistant to vancomycin and to teicoplanin (MIC > 32 mg/L), which characterizes *VanA* glycopeptide resistance. Five (8.3%) GRE strains were resistant to vancomycin (MIC > 32 mg/L) and susceptible to teicoplanin (MIC < 0.5–1 mg/L), *VanB*. One strain was identified as *E. casseliflavus* with MIC = 8 mg/L to vancomycin and <0.5 mg/L to teicoplanin, *VanC*. All *E. faecalis/faecium* strains were resistant to quinolones (MIC > 8 mg/L), lincosamines (MIC > 8 mg/L) and streptomycin (high level), while they were sensitive to tetracycline and chloramphenicol. *E. faecalis* strains ($n = 28$) were sensitive to ampicillin (MIC < 2 mg/L) and 67.8% (19/28) of them were resistant to gentamicin (high level). *E. faecium* strains ($n = 31$) were resistant to ampicillin (MIC > 32 mg/L) and 32.3% (10/31) of them were resistant to gentamicin (high level). *E. casseliflavus* was resistant to clindamycin (MIC > 8 mg/L) and tetracycline (MIC > 16 mg/L).
Conclusion: Glycopeptide-resistant enterococci have become an increasing problem in our patients, especially in ICU and Haematology Unit. Most of them belong to VanA phenotype with high level aminoglycoside resistance. It is worthwhile to emphasize the isolation of VanB and VanC resistance phenotypes from our patients.

P808 First cases of vancomycin-resistant enterococci infection in a university hospital, Greece

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Objective: To study the first vancomycin-resistant enterococci (VRE) infections in our hospital.
Material and methods: On December 18, 2000, the first case of VRE infection was detected in our hospital. From December 18, 2000–January 19, 2001, four other VRE infections appeared. The clinical and epidemiological features of these cases were studied and the isolates were investigated by conventional and molecular methods.
Results: Case no. 1 was a 78-year-old woman in renal dialysis. She had developed respiratory infection and pleuritic fluid. VRE was isolated from pleuritic fluid on the 43rd day of hospitalization. She had received cefalosporins, aminoglycosides, imipenem, vancomycin and fluconazole. Case no. 2 was a 36-year-old man treated in the orthopedic ward and VRE was isolated from decubitus ulcer on the 11th day. He had received norfloxacin, ceftriaxone, and doxycycline. Case no. 3 was a 70-year-old woman who had undergone a surgical operation because of bowel rupture and was admitted in intensive care unit (ICU). VRE was isolated from peritoneal fluid on 10th day. She had received ciprofloxacin, vancomycin, and piperacillin/tazobactam. Case no. 4 was a 52-year-old man suffering from quadriplegia. He had been treated in ICU for 15 days, in neurological ward for 60 days, and in pathological ward for 3 days. VRE was isolated from decubitus ulcer on 78th day. He had received vancomycin, piperacillin/tazobactam, and imipenem. Case no. 5 was a 70-year-old man who was admitted in ICU because of crush syndrome. He had undergone a surgical treatment. VRE was isolated from peritoneal fluid on 23rd day. He had received vancomycin, ciprofloxacin, ticarcillin/clavulanic acid, and imipenem. One *VanB E. faecium* strain was isolated from each of the patients no. 1, 2, and 5. Each of these strains presented a different antibiotic susceptibility profile. Four *VanA E. faecium* strains with different antibiotic susceptibility profiles and 1 *VanC E. gallinarum* were isolated from patient no. 3. One *VanA E. faecium* and 1 *VanA E. faecalis* were isolated from the patient no. 4. The patients no. 1, 4, and 5 died, while the patients no. 2 and 3 were discharged from the hospital. During the next 6 months, no other case of VRE infection was determined in our hospital.
Conclusion: The first five cases of VRE infection occurred in our hospital during a short period (1 month), but a spread of a unique clone did not proved. VRE epidemiology needs more investigation.

P809 General characteristics and antimicrobial susceptibility of vancomycin-resistant enterococci isolated from clinical specimens

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Objectives: The aim of this study was to examine the characteristics and resistance profile of VRE isolated from clinical specimens in our hospital.

Methods: The study was performed on 18 strains of enterococci all vancomycin-resistant as determined by the disk-diffusion method. They were isolated from clinical specimens between January and October 2001 in our hospital. Sources of these specimens included blood (7), urine (4), central catheter tips (3), wound (2), sputum (1), drainage (1). Species identification was performed using the VITEK 2 system. Enterococcal strains were tested for the production of β-lactamase. Disk-diffusion test results were compared with those of VITEK 2 and E-test methods. Antimicrobial agents tested included ampicillin, penicillin, ciprofloxacin, vancomycin, teicoplanin, quinopristin/dalfopristin (VITEK 2, E-test) and linezolid (E-test). The strains were also tested for the presence of high level gentamycin (HLGR) and streptomycin (HLSR) resistance.
Results: *E. faecium* was the most frequently isolated species (13/18 strains) followed by *E. faecalis* (5/18 strains). No organism was found to produce β-lactamase. Two *E. faecalis* isolates reported as vancomycin resistant by disk-diffusion method were susceptible to the antibiotic by VITEK 2 and *E*-test methods. All other isolates were tested highly resistant to vancomycin (≥256 μg/mL) and teicoplanin (≥16 μg/mL) suggesting for the presence of a vanA phenotype. All *E. faecium* isolates were uniformly resistant to ampicillin and penicillin and all *E. faecalis* isolates sensitive to those two antibiotics. All enterococcal isolates were resistant to ciprofloxacin. All *E. faecalis* and all but one *E. faecium* isolates were tested positive for the presence of HLGR and HLSR. All *E. faecalis* isolates were uniformly resistant to quinopristin/dalfopristin but the antibiotic showed relatively high activity against *E. faecium* isolates with only 2/13 strains tested resistant. All VRE were tested sensitive to linezolid. There were no discrepancies between results obtained by the VITEK 2 system and *E*-test method.
Conclusions: *E. faecium* was the most frequently isolated VRE with high level of resistance to many antibiotics (multiple drug resistant). Newer antibiotics such as quinopristin/dalfopristin and linezolid show good promise. Identification of VRE to the species level and knowledge of the type of resistance is critical for infection control purposes in the hospital environment.

P810 Vancomycin-resistant enterococci: a prospective microbiological and clinical study

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Objectives: To evaluate the incidence and clinical significance of vancomycin-resistant enterococci (VRE) in a teaching general hospital over a 13-year period.
Methods: From July 1988 (when the first VRE was isolated in our hospital) to December 2000, all VRE recovered in our microbiology laboratory were studied. The isolates were identified by standard methods and susceptibility testing was performed by the broth microdilution method following the NCCLS guidelines. All patients from whom VRE was isolated were prospectively followed up.
Results: Over the study period a total of 18 277 enterococci were isolated. Among those, 130 isolates (0.7%) were VRE. The isolates were 71 *E. faecium*, 49 *E. faecalis*, 8 *E. casseliflavus* and 2 *E. gallinarum*. From now on we will refer only to *E. faecalis* and *E. faecium* (0.65%). Among those, 65 presented the VanA phenotype and 55 the VanB; 15% were ampicillin-resistant (all *E. faecium*), 20% presented high-level gentamicin resistance and 35% presented high-level streptomycin resistance. VRE were isolated from wounds (32), urine (30), blood (15), abscesses (12), peritoneal fluid (11), other sterile fluids (8) and miscellaneous (12), and corresponded to 108 patients hospitalized in 15 different wards. Seventy-two patients (66.6%) were infected and 36 were colonized. Among the infected patients (53% males, median age 65 years, 89% adults), 68 (95%) presented underlying diseases (45 organic, 21 tumoral and 2 HIV positive) and 20 (28%) received prior glycopeptide therapy. In all cases, VRE was nosocomially acquired. Infections were: surgical wound (20), UTI (19), bacteremia (12), abdominal (12), abscesses (6), other (3). The infection was polymicrobial in 41 cases (57%). Eighty percent of the patients recovered despite inadequate treatment or no treatment, and 15% died, with a 5.5% mortality directly attributable to ERV infection.
Conclusions: In our hospital, the rate of VRE is low, representing 0.65% of all enterococcal isolates. Only 66% of VRE have clinical significance and in general, infections due to ERV present good evolution despite inadequate antimicrobial treatment.

P811 Vancomycin-resistant enterococci (VRE): a point prevalence study of colonization in hospitalized patients and healthy hospital workers

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Kocaeli, TR

Objectives: Following the first VRE infection in our hospital, we investigate the prevalence of fecal carriage of VRE among patients and healthy hospital workers in a university hospital, in Turkey.
Methods: We carried out a point prevalence culture survey in a single day by culturing fecal samples of all hospitalized patients in intensive care unit and surgical wards. Rectal swabs were taken from 49 patients, 32 volunteer nurse/doctors and cultured on bile esculin agar supplemented with 10 μg/mL of vancomycin and 60 μg/mL aztreonam. Esculin-positive colonies were verified with Gram staining, pyrrolidonyl peptidase (PYR) tests and the Vitec system. Vancomycin resistance was detected by *E*-test.
Results: Enterococci were isolated from 5 of 49 patients from all intensive care unit and surgical wards, 2 of 32 healthy hospital workers. All of the isolates were *Enterococcus faecium*. None of the strains exhibited resistance to vancomycin.
Conclusion: After the first VRE infection observed in our hospital, our study on determination the rate of fecal colonization showed no carriage of VRE in our hospital.

P812 In vitro activity of daptomycin against a prospective collection of European enterococci

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Objectives: Treatment of patients with vancomycin-resistant (VAN-R) and multidrug-resistant (MDR; resistant to ≥3 antimicrobial classes) enterococci infections, especially *E. faecium* (EM), is a common challenge faced by physicians worldwide. Daptomycin (DAP), a novel cyclic lipopeptide currently in Phase III clinical trials, has shown in vitro activity against both VAN-R and MDR enterococci. This study examined the activity of DAP against a current European collection of enterococci to serve as a benchmark for future studies.
Methods: During 2000–2001, 1840 *E. faecalis* (EF), 454 EM, and 160 other *Enterococcus* spp. (Esp; non-EF, non-EM) were collected from 40 European laboratories in 15 countries. Isolates were centrally tested by NCCLS broth microdilution against ampicillin (AMP), VAN, teicoplanin (TEI), ciprofloxacin (CIP), quinupristin/dalfopristin (QD), linezolid (LZD), and DAP.
Results: The VAN-R rate among EF, EM, and Esp was 2.2, 25.1, and 10.6%, respectively. Nationally, EM VAN-R rates ranged from 0% in Switzerland and Scandinavia to 60.6% in Italy. The percent resistance to other agents for EF, EM, and Esp, respectively, was AMP (0, 69.8, 37.5), TEI (1.9, 19.6, 8.8), CIP (30.0, 67.8, 31.3), and QD (82.1, 3.5, 11.9). DAP showed equivalent activity against both VAN-susceptible (S) and VAN-R EF (MIC_{90}, 2 mg/L), EM (MIC_{90}, 4 mg/L), and Esp (MIC_{90}, 4 mg/L). LZD also showed consistent activity against VAN-S and VAN-R isolates with MIC_{90} of 2 mg/L for all species. As expected, QD was not active against EF (MIC_{90}, 8 mg/L; 82.1% R) but was consistently active against VAN-S and VAN-R EM (MIC_{90}, 2 mg/L) and Esp (MIC_{90}, 4 mg/L). Among the EM tested, 89 (19.6%) were MDR with resistance to AMP, CIP, and VAN being the most common phenotype (95.5% of MDR isolates).
Conclusions: Overall, DAP demonstrated consistent MIC_{90} against all enterococci tested, regardless of VAN-R or MDR phenotypes. Based on these data, DAP may represent a potential therapy for the treatment of enterococcal infections.

P813 In vitro activity of quinupristin/dalfopristin against vancomycin-resistant *Enterococcus faeciun* isolated from blood

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Objectives: To determine the in vitro activities of quinupristin/dalfopristin (Q/D) for clinical isolates of vancomycin-resistant *VanA Enterococcus faecium* (VREM). Quinupristin/dalfopristin is a new parenteral antibiotic

combination belonging to the streptogramin group, leading to an irreversible inhibition of bacterial protein synthesis.
Methods: The activity of Q/D was assessed against 12 clinical isolates of *VanA* VREM isolated from blood. The minimal inhibitory concentrations (MIC) Q/D was determined by the *E*-test method.
Results: All isolates of VREM were found susceptible to Q/D. These isolates were characterized by MIC values of 4–8 μL/mL.
Conclusion: The study revealed a very good activity of Q/D against VREM. The antibiotic may be useful in treatment of serious infections caused by multiresistant *E. faecium*.

P814 Linezolid—little biological variation in wild type populations of Gram-positive bacteria

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Introduction: Already, linezolid resistance has been described in vancomycin-resistant *Enterococcus* spp. and in methicillin-resistant *Staphylococcus aureus*. Representing the first drug of its class to come on the market and exhibiting no mechanistic cross resistance with other classes of drugs, linezolid offers the first real chance of successfully counteracting resistance development through strategic measures such as limiting the use of the drug and combating the spread of resistant bacteria through infection control. The present study was performed to elucidate the biological variation within wild type strains of target microorganisms.
Material and methods: A representative prospective selection of micro-organisms (see below) from 25 different geographical areas of Sweden was investigated with *E*-test (AB Biodisk, Sweden) and disc-diffusion (linezolid 10 μg disc from Oxoid, UK) by 25 laboratories. All laboratories simultaneously analyzed three type strains for comparison.
Results: The inhibitory concentrations (mg/L) obtained for 125 isolates of each of *S. aureus*, coagulase-negative staphylococci, *Enterococcus faecalis* and *E. faecium* and *Streptococcus pneumoniae* were between 0.125 and 2 mg/L. The degree of biological variation among the wild-type strains was exactly of the same magnitude as the variation obtained when three representative type strains were analyzed under the exact same conditions.
Discussion: The national breakpoint committees in Europe have decided on the following brekpoints: BSAC and SRGA $S \leq 4$, $R \geq 8$, CRG $S \leq 2$, $R > 8$, SFM $S \leq 2$, $R > 4$ mg/L and the ESCMID breakpoint committee EUCAST $S \leq 4$, $R > 4$ mg/L. All the breakpoints seem appropriate; neither of them will split homogenous biological wild-type populations and all of them will allow the early detection of resistance development. The linezolid resistance that has so far been described in VRE and MRSA have generated MICs of ≥ 32 mg/L and should be easy to detect with a reasonably standardized MIC- and/or agar disc-diffusion methods. The fact that the ranges of MICs and zone diameters were identical for the clinical isolates and the type strains shows a lack of biological variation within the wild-type population of a species and emphasizes the importance of not splitting wild-type populations with breakpoints.

P815 Fecal carriage of vancomycin-resistant enterococci in patients of the university hospital, Crete, Greece

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Objective: To investigate fecal colonization of vancomycin-resistant enterococci (VRE) among the patients of the hospital, 1 month after the appearance of first cases of VRE infection.
Material and methods: The study was performed on 1 February 2001. All patients (98) cared in high-risk departments were tested, from which four in intensive care unit (ICU), 7 in cardiological intensive care unit (CICU), 72 in renal unit, and 15 in neonatal unit. In parallel, a representative number of 120 (25.8%) out of the 465 patients treated in all other wards of the hospital were also tested. Fecal samples or rectal swabs were inoculated into enterococci broth with vancomycin (6 mg/L) and subcultured onto bile-esculin agar plates with vancomycin (6 mg/L). Enterococci were identified in genus and species level by conventional methods and specific PCR. Glycopeptides susceptibility testing was performed by disk-diffusion method, *E*-test, and MIC (by agar dilution method). Glycopeptides resistance types were confirmed by multiplex PCR. Sex, age, days of hospitalization, surgical and antibiotic treatment, hospitalization in ICU, and renal dialysis were studied as risk factors.
Results: A total of 218 samples were tested and 42 VRE strains were isolated from 41 patients. Glycopeptides resistance types were as follow: *VanA E. faecium*, 4; *VanB E. faecalis*, 1; *VanB E. faecium*, 7; *VanC1 E. gallinarum*, 24; *VanC2/C3 E. casseliflavus/flavescens*, 6. VRE were detected in 25.5% of all patients treated in high-risk departments: 50% in ICU, 14.3% in CICU, 6.7% in neonatal unit, and 29.2% in renal unit. VRE were also detected in 16 (13.3%) of 120 patients tested. *VanA* strains were detected only in high-risk departments. No significant difference was found regarding sex, age, duration of hospitalization, surgical and antibiotic treatment, except glycopeptides (14.6% in VRE colonized patients vs. 5% in no VRE). Hospitalization in ICU was in 17% of VRE carriers versus 9.6% of no VRE and renal dialysis in 51.2% versus 28.8%, respectively.
Conclusions: Different VRE strains were isolated from patients in the hospital. Higher prevalence of VRE colonization was found in ICU and renal unit. Treatment with glycopeptides, admission in ICU and renal dialysis were detected as risk factors.

P816 Prevalence of gastrointestinal carriage of glycopeptide-resistant enterococci in Europe

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On behalf of the European Ramoplanin Study Group

Objectives: To assess the prevalence of gastrointestinal carriage of glycopeptide-resistant enterococci (GRE) among hospitalized patients in Europe and to determine the ramoplanin susceptibility of the isolates.
Methods: During the second quarter of 2001, 3499 stool samples were processed for GRE by 13 laboratories in eight countries. Samples were plated on vancomycin–colistin–nalidixic acid Agar (VCNA) containing vancomycin 6 mg/L and for all strains identified as enterococci the minimal inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined by E-test. Species confirmation and susceptibility testing by microdilution (NCCLS) were performed in a single laboratory.
Results: A total of 388 (11.1%) samples were confirmed to yield GRE, prevalence, however, varied widely among laboratories, ranging from 1.7 to 39.1%. The mean country rates (%) were Austria (one lab) 5.9, Belgium (two labs) 4.4, France (two labs) 7.0, Germany (one lab) 4.6, Italy (three labs) 20.0, Spain (two labs) 8.5, UK (one lab) 39.0. The strains were identified as *E. gallinarum* 178 (46%), *E. faecium* 117 (30%), *E. casseliflavus* 75 (19%), *E. faecalis* 14 (4%) and *E. species* 4 (1%). One hundred and fourteen out of 131 (87%) *E. faecalis* and *E. faecium* isolates were of the *VanA* type of resistance. The wards that contributed the majority of strains were intensive care 127 (32%), hematology–oncology–bone marrow transplant unit 91 (23%) and medicine 74 (19%). Ramoplanin was active against all GRE regardless of the resistance phenotype, with a MIC_{90} of 0.5 mg/L.
Conclusions: Despite the fact that ward or patient selection and local epidemiology may have influenced the results, intestinal GRE carriage appears to be increasing in Europe, involving mainly high risk patients admitted in intensive care or oncohematology wards. Ramoplanin, that has been shown to be effective in suppressing gastrointestinal GRE carriage in the USA, is also highly effective in vitro on European isolates.

P817 The comparison of high-level aminoglycosides resistance in vancomycin-sensitive and vancomycin-resistant enterococci strains

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Objectives: Today, enterococci are among common causes of nosocomial morbidity and mortality. Recent attention has been focused on enterococci

because of their importance not only in nosocomial infections but also their increasing resistance to antibiotics. One of the most important antibiotic resistance is high level aminoglycoside resistance (HLAR). The aim of this study was to investigate whether there was a significant distinction in high level aminoglycoside resistance between vancomycin-sensitive enterococci (VSE) and vancomycin-resistant enterococci (VRE).

Methods: In the study, 116 enterococci strains from rectal swabs of 163 hospitalized patients were investigated. Vancomycin resistance was determined by screening test using brain–heart infusion agar (BHI) containing 6 μg/mL vancomycin. The HLAR was determined by two different methods, the standard agar screening and the disk-diffusion screening method. Standard agar screening method was applied in two BHI agar, one of which contained 500 μg/mL gentamicin and the other 2000 μg/mL. The disk-diffusion method was performed on Mueller Hinton Agar by using two different antibiotic disks, one of which contained 120 μg/mL gentamicin and the other 300 μg/mL streptomycin. *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212 were included in the test as reference strains.

Results: Vancomycin resistance was determined in 17 of 116 *Enterococcus* strains tested. HLAR in VRE was found significantly higher than that of VSE ($P < 0.001$). There was no significant difference between the two methods with respect to HLAR determining rates of *Enterococcus* strains. Among the *Enterococcus* strains, 1 was found to be resistant only to gentamicin, the 11 were to only streptomycin and the 29 were to both. In the study, HLAR was determined in 41 of 116 strains (35.3%).

Conclusion: HLAR rate in VRE was found two-fold higher than in VSE. But, clinical importance of HLAR in VSE strains is much more than the others. Because, the synergism among β-lactam antibiotics and aminoglycosides will not be seen in the treatment of infections due to VSE strains with HLAR. The causative agent should be examined for HLAR before administering of β-lactam antibiotic plus aminoglycoside combination.

P818 Antibiotic resistance and molecular identification of vancomycin-resistant enterococci isolated from patients and fecal carriers in a Greek hospital

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Objective: To determine the susceptibilities of vancomycin-resistant enterococci (VRE) to 11 antibiotics in correlation with vancomycin resistance type detected by molecular methods.

Material and methods: The first VRE strain was isolated in the laboratory in December 2000. From December 2000 to August 2001, a total of 60 strains were isolated from 6 patients and 45 fecal carriers. Enterococci identification was performed by conventional methods and confirmed by molecular assays. Susceptibilities to vancomycin (VA), teicoplanin (TEC), penicillin (PEN), streptomycin (STR), gentamicin (GM), tetracycline (TE), rifampicin (RA), chloramphenicol (CHL) erythromycin (ER), norfloxacin (NOR), and imipenem (IP) were detected by disk-diffusion method, *E*-test and MICs by agar dilution method according to NCCLS guidelines. Glycopeptide resistance genotypes (*VanA*, *VanB*, *VanC1*, *VanC2/C3*) and species level identification of *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. casseliflavus/flavescens* were detected by multiplex PCR.

Results: Vancomycin resistance genotypes and species distribution were as follow: *VanA E. faecium*, 8; *VanB E. faecium*, 16; *VanA E. faecalis*, 1; *VanB E. faecalis*, 1; *VanC1 E. gallinarum*, 27; *VanC2/C3 E. casseliflavus/flavescens*, 7. Ranges of MICs to VA were: *VanA* strains 256–1024 mg/L, *VanB* 16–512 mg/L, and *VanC* 8–16 mg/L; to TEC: 64–256 mg/L, 0.125–1 mg/L, and 1.5–1 mg/L, respectively. High-level resistance (HLR) to STR was detected in 67% of *VanA* strains, 70% of *VanB*, and 3% of *VanC*. HLR to GM was detected in 44% of *VanA*, and 47% of *VanB*. Resistance rates of *VanA* were as follow: PEN, 78%; TE, 67%; RA, 55%; CHL, 44%; ER, 100%; NOR, 55%; and IP, 89%. *VanB* presented the following resistance rates: PEN, 94%; TE, 59%; RA, 94%; CHL, 35%; ER, 100%; NOR, 94%; and IP, 100%. All *VanC* strains were sensitive to PEN, CHL, and IP. Among *VanC* 20% were resistant to TE, 35% resistant and 6% intermediate resistant (IR) to RA, 23% resistant and 9% IR to ER, and 50% IR to NOR.

Conclusion: VRE presented high resistance rates to the antibiotics tested. Multi-resistant strains were detected among *VanA* and *VanB* types. *VanC* strains presented lower resistance rates compared to *VanA* and *VanB*.

P819 Genetic relationship between *vanA*-containing *Enterococcus* spp. isolated from human and poultry in Korea

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Background: The number of vancomycin-resistant enterococci (VRE) isolated in tertiary hospitals have been rapidly increasing since firstly reported in 1992 in Korea. But the epidemiology on widespread dissemination of VRE in Korea was not fully elucidated. To investigate the possibility of horizontal transfer from poultry to human, we compared the phenotypic and genotypic characteristics of human isolates with poultry ones.

Methods: Total 148 isolates, including 58 *E. faecium*, 12 *E. feacalis*, 3 *E. casseliflavus*, and 4 *E. gallinarum* from humans and 71 *E. faecium* from poultry, were studied. Antimicrobial susceptibility tests were done by disk-diffusion or agar dilution methods. Pulsed-field gel electrophoresis (PFGE) was performed. The internal and structural regions of *vanA* gene cluster were analyzed by PCR fragment length polymorphism and RFLP.

Results: The antibiotic resistance patterns of human isolates were different from those of poultry ones. PFGE patterns revealed high heterogeneity. Three types of PCR fragment length patterns were found, as follows; (¥°) PCR amplicons of the same size as prototype (*E. faecium* BM4147) for all genes in the *vanA* cluster (13% of human isolates, 100% of poultry ones) (¥±) insertion in *vanX–vanY* intergenic region (3%, 0%) (¥^{2}) insertion in *vanX–vanY* intergenic and *Orf2* regions (84, 0%). IS1216V and IS1542 were located within *vanX–vanY* intergenic and *Orf2* regions, respectively. The base pair variation in *vanX* gene of human and poultry isolates was not found.

Conclusion: Despite of the diverse PFGE patterns, 84% of human isolates and all of poultry ones belonged to *vanA* gene cluster type ¥^{2} and ¥°, respectively. These data suggest that vancomycin resistance is spread through horizontal transfer of the *vanA* genes within each groups, and there was low genetic relationship between the *vanA* cluster of human and poultry isolates. Most of human isolates belonged to *vanA* gene cluster type is different from poultry ones. These data supported the low possibility that VRE isolates from poultry contribute to widespread dissemination in tertiary hospitals in Korea, although vancomycin resistance would be horizontally transferred within each group.

P820 Expression of proteins in enterococcal strains regulated by iron deficiency

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Objectives: Enterococci as facultative anaerobes have an obligate iron requirement. The most productive iron assimilation system consist of a siderophore and the protein transport apparatus. We have shown that enterococci produce hydroxamate type of siderophore. The aim of this study was to estimate the expression of iron-regulated proteins.

Methods: Four strains of the genus *Enterococcus*, isolated from the clinical material, were used in the study. Two of them belonged to the species *E. faecalis*—BD 123 and BD 181, and the remaining two to *E. faecium*—EN 5 and EF 5. The strains were cultured in the iron-deficient medium (0.35 mM) and in medium containing excess iron (100 mM) for 24 h. Total siderophore amount in the culture supernatants was determined with the universal Chrome Azurol S method. The whole cell lysates obtained with lysozyme and by differential centrifugation were subjected to electrophoresis in 12% polyacrylamide gel (SDS–PAGE) under reductive conditions.

Results: The investigated strains differed in the quantity of the produced iron chelators. The most active producer was the *E. faecalis* BD 123 strain, which produced them in 8.45 mg/mL quantity. The smallest quantity of siderophores −2.6 mg/mL, was produced by *E. faecium* EN 5. The *E. faecalis* BD 181 and *E. faecium* EN 6 strains produced 6.76 and 5.85 mg/mL, respectively. None of the strains produced siderophores in the excess of iron medium. The comparison of electrophorograms of the whole cell lysates harvested from deficient and excess iron media showed that under iron deficiency additional proteins appear in all the investigated strains. The *E. faecium* EN 5 strain synthesized only one additional protein of 31 kDa molecular weight, similarly as *E. faecium* EN 6, synthesizing one more protein of 24 kDa molecular weight. A protein of 38 kDa molecular weight occurred in both *E. faecalis* strains—BD 123 and BD 181. The *E. faecalis* BD 181 strain synthesized one more protein—of 35 kDa molecular weight, and *E. faecalis* BD 123—of 17 kDa.

Conclusions: Under conditions of iron deprivation all tested enterococci produce additional proteins. Their significance as the potential iron transport or iron receptor proteins has not be elucidated yet.

P821 Genetically related isolates among high-level gentamicin-resistant *Enterococcus faecalis* in Swedish intensive care units

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Objectives: To investigate genetic relatedness among *Enterococcus faecalis* isolated from patients in Swedish intensive care units (ICUs) with specific focus on high-level gentamicin and ciprofloxacin-resistant isolates.
Methods: Three hundred twenty-two (322) clinical enterococcal isolates were collected during 1996–1998 from ICUs at eight Swedish hospitals. MICs for 14 antibiotics were determined with *E*-test (AB Biodisk, Solna, Sweden). Isolates with high-level gentamicin-resistance (HLGR) and/or resistance to ciprofloxacin were selected for detection of genetic relatedness. Detection of related clones using pulsed-field gel electrophoresis of *Sma*I DNA macrorestriction fragments.
Results: No HLGR was found among the *E. faecium* isolates. Among the 244 *E. faecalis* isolates, 48 showed HLGR, and all but one isolate were at the same time resistant to ciprofloxacin. Another 25 *E. faecalis* isolates showed ciprofloxacin resistance, but no HLGR. These 73 isolates were tested for genetic relatedness. Three clusters of PFGE patterns (I, II and III) were found and several unique patterns. The isolates belonging to a cluster showed similarities in their antibiograms. Among isolates with HLGR almost 90% of the isolates belonged to cluster I or cluster II, and only a few had unique PFGE patterns. Among the isolates with ciprofloxacin resistance but no HLGR most isolates had unique PFGE patterns, and 20% belonged to cluster III. Cluster I was found in five hospitals, located in three geographically separated cities in the south-east of Sweden. Cluster II was predominantly found in one city in the south-west of Sweden.
Conclusion: The results show genetic relatedness among *E. faecalis* with HLGR in Swedish ICUs and suggest a spread of resistant clones within and between Swedish hospitals.

P822 Molecular epidemiology of *vanA*-containing *Enterococcus faecium* in a Korean hospital

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Objectives: Vancomycin-resistant enterococci(VRE) has been isolated from hospitalized patients worldwide. According to recent reports, molecular epidemiological studies of nosocomial spread of VRE indicate that the horizontal gene transfer plays an important role in the dissemination of vancomycin resistance. To better understand the spread of *vanA*-containing enterococci in Korea, a series of *vanA* VRE collected over a 15-month period from Ajou University Hospital in Korea were analyzed.
Methods: Seventeen isolates of *vanA*-containing enterococci were obtained from the patients of the Ajou University Hospital from January 1998 to April 1999. PFGE was performed with *Sma*I for bacterial genomic heterogeneity. Restriction analysis of the long distance PCR amplicon by *Eae*I was performed for structural analysis of Tn1546. All isolates were typed by ORF-, vanS-vanH-, vanX-, vanY-vanZ-, vanZ-, IS1216-, and IS1251-specific PCRs. Filter mating was performed to investigate transfer frequency.
Results: PFGE revealed coexistence of sporadic unrelated strains and a predominant clone suspected of intrainstitutional spread. Restriction fragment length polymorphism (RFLP) and PCR mapping of Tn1546 revealed two different patterns. The difference between the two of PCR mapping is whether the isolates contain larger vanS-vanH amplicons and IS1251 or not. IS1251 represents 24% (4/17) of the isolates. Transfer efficiency of AJ22 and AJ43 were 8.7×10^{6} and 9.0×10^{-5}, respectively.
Conclusions: Molecular typing of the *vanA*-containing VRE in Ajou University Hospital reveals a mixed pattern with clonal dissemination of strains and horizontal transfer of Tn1546. However, the horizontal transfer of Tn1546 dominates the dissemination of vancomycin resistance. Therefore, epidemiologic investigation to target infection control of VRE should include structural analysis of resistance gene and typing of bacterial genomic heterogeneity.

P823 Fingerprinting of enterococci colonizing the respiratory tract of mechanically ventilated patients at a Swedish intensive care unit

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Objectives: To investigate colonization of enterococci in the respiratory tract within and between patients treated with mechanical ventilation at an intensive care unit (ICU) and to determine the antibiotic susceptibility pattern for the isolated enterococci.
Methods: Twenty consecutive patients undergoing incubation at an ICU in a Swedish hospital were included. Samples were collected from the oropharynx, the subglottic space, the stomach and the trachea within 24 h of incubation and then every third day until day 18, thereafter every fifth day until day 33. Enterococcal isolates were genotyped with pulsed-field gel electrophoresis. Minimal inhibitory concentration was determined against ampicillin, imipenem, vancomycin, gentamicin and moxifloxacin with agar dilution method.
Results: Enterococci were isolated from the respiratory tract in 17 patients, in 12 of these from the lower respiratory tract. In 16 of the subjects were enterococci recovered from the respiratory tract already at the onset of incubation. Ten patients harbored only one enterococcal genotype. In three of five patients incubated 12 days or more the number of genotypes increased with time. Genotyping analyses suggested that seven different strains were shared by two or more patients. Thereby seemed 13 of the 20 patients to be involved in an enterococcal transmission event. *Enterococcus faecium* was more resistant than *E. faecalis* most commonly against ampicillin (67%) and imipenem (58%). No vancomycin-resistant enterococci were isolated and high-level gentamicin resistance was rare. Moxifloxacin resistance occurred frequently.
Conclusions: Occurrence of enterococci in the respiratory tract of this patient group was common. Transmission of enterococci between incubated patients appears to be a frequent event since 13 of the 20 patients shared one genotypically related strain with at least one other patient.

P824 Comparison of random-amplified polymorphic DNA analysis, pulsed-field gel electrophoresis analysis, and amplified fragment length polymorphism for typing of vancomycin-resistant enterococci

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Fifty-seven vancomycin-resistant enterococci (VRE), including 28 vanA *Enterococcus faecalis* and 29 vanB *E. faecium*, were isolated from nine hospitals in Taiwan during a period of 29 months. These isolates were genotyped using random-amplified polymorphic DNA (RAPD) analysis and amplified fragment length polymorphism (AFLP). Results obtained from these tests were compared with those derived from pulsed-field gel electrophoresis (PFGE), which is currently considered as the gold standard for VRE typing. The 28 *E. faecalis* isolates were classified into 15 types by RAPD, 23 types by PFGE, and 24 types by AFLP. The 29 *E. faecium* were classified into three types by RAPD, eight types by PFGE, and nine types by AFLP. In general, data generated by PFGE and AFLP were consistent, whereas RAPD showed a lower discriminatory power than PFGE and AFLP for differentiating VRE isolates. We conclude that AFLP is a useful alternative to PFGE for VRE typing.

P825 Iron supply of enterococci by carboxylate compounds

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Objectives: Enterococci have an obligate iron requirement but little is known about its assimilation. The aim of this study was the investigation of the utilization iron by enterococci with α-hydroxy or α-ketocarboxylate as siderophores.
Methods: The 79 strains of the genus *Enterococcus* belonged to 16 species of diverse origin were used. Twelve α-keto and α-hydroxy acids iron complexes serve as iron carriers. The solid medium containing ferric ions

chelator—o-phenanthroline to inhibit bacterial growth was used. The test of iron utilization was performed with disc-diffusion technique. In the presence of iron complexes, growth inhibition was reversed to provide a zone of growth stimulation. Uptake of ^{55}Fe by cells mediated by α-hydroxy and α-ketoacids was also estimated. α-Keto acids released from cells to medium were determined as of 2-quinolinol derivatives by means of HPLC.
Results: All tested strains utilized iron complex of citric acid. The iron complexes of α-keto and α-hydroxy acids have stimulated growth of 9 from 79 enterococcal strains only. All this strains utilized iron complexes of pyruvic acid, α-ketobutyric acid, DL-α-β-methylvaleric acid, α-ketoadipic acid and α-ketoisocaproic acid providing the best growth response. Eight of those nine strains utilized additionally γ-aminolevulenic acid and oxaloacetic acid, consecutive six strains α-ketomalonic acid and α-ketoglutaric acid, four strains β-phenylpyruvic acid, three strains α-ketoisovaleric acid and two strains α-hydroksyphenylpyruvic acid. α-Keto acids in bacterial culture supernatant of these nine strains were detected. Increasing iron stress resulted in the extraction of greater amounts of α-ketoacids.
Conclusion: Enterococci commonly infect the urinary tract. This infection are often associated with underlying metabolic disorders which can lead to increased concentration of hydroxy and keto acids in urine. Enterococci are able to utilize iron from complexes with those acids. This ability may be of great importance for survival in urine and enterococcal pathogenicity.

P826 Gastrointestinal and skin colonization of enterococci in hospitalized patients in an university hospital in Turkey

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Izmir, TR

Objective: To evaluate colonization of *Enterococcus* spp. in antibiotic administrated hospitalized patients.
Methods: We collected rectal and axillar swab specimens from 133 patients who were hospitalized and given an antibiotic during at least past 2 days in Dokuz Eylul University Hospital, on September 26, 2001. Bacteria grown on culture media were identified with Vitek system (bioMérieux), and disk-diffusion method was used for determining antibiotic susceptibility.
Results: Of 133 patients investigated, 62 (46.6%) were colonized with *Enterococcus* species. Of 68 isolates from 62 patients, 58 (85.2%) were isolated from rectal swab specimens and 10 (14.8%) were isolated from axillar samples. Point prevalence was calculated as 42.1% for gastrointestinal tract and 7.5% for axillar region skin colonization of enterococci. All isolates obtained from axillar swab samples were *E. faecalis*, but isolates from rectal swab specimens were *E. faecalis*, *E. faecium*, *E. flavescens* and *E. gallinarum* (67.3, 27.6, 2.4, 1.7%, respectively). Three *E. faecium* strains were found to be resistant to vancomycin with MICs >32 mg/L. *E. flavescens* and *E. gallinarum* strains were found to be susceptible to all antibiotics tested (Table 1).

Table 1 Resistance rates of *E. faecalis* and *E. faecium* to the antibiotics tested (%)

	Vancomycin	Teicoplanin	Ampicillin	HLGR*	HLSR*
E. faecalis	0	0	2.0	24.5	14.3
E. faecium	18.8	18.8	0	62.5	50.0

*HLGR: High level gentamicin resistance, *HLSR: High level streptomycin resistance (%).

Conclusion: No statistically significant difference was found between patient groups which enterococci were isolated or not isolated, in terms of age, gender, duration of hospitalization and antibiotic administration. But in the patients in intensive care units and department of oncology, axillar colonization was found to be significantly higher than the patients in other wards of hospital.

P827 Human Fe-transferrin and Fe-lactoferrin as iron sources in enterococci

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Objectives: Iron is not largely available in human tissues. It is complexed with high-affinity iron-binding proteins transferring (TR) and lactoferrin (LF). In this study, we have tried to estimate the ability of enterococci to growth in serum and iron acquisition from human TR and LF.
Methods: The study was carried out on 20 strains of the genus *Enterococcus* belonging to the species *E. faecalis*, *E. faecium*, *E. sulfureus*, *E. mundtii* and *E. durans* isolated from clinical material, animals and environment. The preliminary experiments involved checking the ability to grow in native human serum (containing Fe-TR and Fe-LF) and in serum where these Fe(II) carriers had been maximally saturated with iron. The proper investigations involved utilization of ^{59}Fe-labeled TR and LF.
Results: Eleven of the 20 investigated strains grew in the native serum, whereas the iron-saturated serum stimulated the growth of only two strains. Seventeen of the investigated strains assimilated iron from ^{59}Fe-TR, whereas none of them utilized the iron from ^{59}Fe-LF. It is in accordance to the physiology of iron assimilation by bacteria from systemic sources. All the strains growing in the serum assimilated iron from ^{59}Fe-TR.
Conclusions: Over the half of the investigation strains have grown in serum with physiological 30% iron saturation of TR. Maximal saturation of TR did not stimulate either the growth of strains failing to grow in the native serum of those which grew in it. To lack of LF utilization has corresponded with better iron availability in normal habitats of enterococci found—gastrointestinal tract and oral cavity. The ability to utilize iron bounding to TF may be of importance for promoting host colonization.

Fluoroquinolone resistance

P828 Genetic background of resistance to fluoroquinolones in epidemiologically defined clinical isolates of *Acinetobacter baumannii*

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To investigate the correlation between mutations in the *gyrA* and *parC* genes and decreased susceptibility to fluoroquinolones, 147 clinical isolates of *Acinetobacter baumannii* (outbreak related as well as sporadic isolates) from Europe ($n = 79$) and the United States ($n = 68$) were examined. Organisms were selected on the basis of exhibiting a unique fingerprint pattern as determined by randomly amplified polymorphic DNA (RAPD) analysis and pulsed-field gel electrophoresis (PFGE). The in vitro activities of ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin were determined using agar dilution. Quinolone resistance-determining regions (QRDR) of both *gyrA* and *parC* genes in all isolates were sequenced. Mutations in the *gyrA* gene were found in isolates with a ciprofloxacin MIC from ≥4 to >128 mg/L, additional mutations in the *parC* gene in isolates with a ciprofloxacin MIC of ≥64 mg/L. Elevation in MICs of novel quinolones was similarly correlated. In outbreak-related strains, mutations were significantly more frequent than in sporadic strains. Amino acid exchanges in GyrA were Ser83 → Leu and Glu87 → Gly conferred by a novel mutation. Other previously described mutations in the *gyrA* gene could not be detected. In ParC, Ser80 → Leu and Glu84 → Lys were seen as well as Ser80 → Phe. Amino acid exchanges at Ser83 of GyrA were detected in 42 strains followed by substitutions at Ser80 of ParC in 20 strains. Novel quinolone compounds, especially clinafloxacin, were up to five times less affected by these mutations. Due to MIC-variations in strains with identical mutations, other mechanisms are likely to contribute to resistance to fluoroquinolones in *A. baumannii*.

P829 In vitro selection of quinolone-resistant mutants of *Escherichia coli* ATCC 25922 by nalidixic acid, ciprofloxacin, clinafloxacin, levofloxacin, ofloxacin, sparfloxacin and trovafloxacin at different growth conditions

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Due to spontaneous mutations, quinolone-susceptible bacterial populations contain mutants resistant to quinolones. The mutant prevention concentration (MPC) is the concentration of an antimicrobial agent necessary to prevent the growth of resistant mutants among 10E10 cfu, and can be employed to compare different antimicrobial agents. We investigated in vitro, how different temperatures (37 and 20 °C) in aerobic and anaerobic atmosphere affected the number of mutants of *Escherichia coli* ATCC 25922. Nalidixic acid (NA), norfloxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFX), levofloxacin (LVX), trovafloxacin (TVA), and clinafloxacin (CLX) were compared for the selection rate of resistant mutants and MPC of ~10E10 cfu of *E. coli* ATCC 25922. The 2 × MIC (TVA), 4 × MIC (CIP, NOR, OFX), 8 × MIC (CLX, LVX), 16 × MIC (SPX), or 32 × MIC (NAL) were required to suppress the growth of resistant mutants among ~10E10 cfu of *E. coli* ATCC 25922 at aerobic conditions. The MPC of CIP at anaerobic conditions was 16 × MIC. The MIC of mutants was one out of the 256 × wild-type MIC, and thus higher than the respective MPC, irrespective of the quinolone or growth conditions. Calculated from the serum half-life and the MPC, respectively, the selection period of each quinolone, in which resistant mutants might be selected, was calculated to be 14 h for NAL, 16 h for NOR and CIP, 28 h for OFX, 30 h for TVA, 40 h for CLX, and 120 h for SPX. These in vitro data indicate that compounds with long serum half-lives and high MPC might favor the development of quinolone resistance.

P830 The role of gyrB and dnaA proteins in bacterial conjugation

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Objectives: Quinolones have been demonstrated to eliminate plasmid and to inhibit conjugal transfer. To assess whether the inhibition of plasmid Flac transfer by quinolone requires a functional DNA gyrase, different mating experiments were performed.
Methods: *Escherichia coli* K 12 N4177 (Flac) and E177 (Flac) which encode for a termolable gyrB and dnaA protein, respectively, were used as donor strains. J-53 rifampicin resistant was employed as recipient organism. Conjugation was carried out by standard methods employing 2×10^8 cells/mL in rich broth.
Result: Mating experiments were performed at the permissive and nonpermissive temperatures at the presence of 100 mg/mL of nalidixic acid for 1 h. Under permissive conditions (32 °C), Flac was transferred from the donor to the recipient with about the same frequency irrespective of the bacterial host considered. As expected, nalidixic acid reduced the number of recombinants of about 99% in comparison to the control. When the same experiments were performed at the nonpermissive temperature (43 °C), nalidixic acid inhibited the transfer of Flac of about 99% from dnaA (Ts) mutant but not from gyrB (Ts) host.
Conclusions: The present finding supports the hypothesis that Flac transfers does not require a functional DNA gyrase activity in F-conjugation and suggests the presence of an unknown target of the quinolone in the bacterial cell. The role of gyrB and the dnaA proteins in bacterial conjugation are under investigation.

P831 Use of moxifloxacin to examine development of quinolone resistance in clinical *Streptococcus pneumoniae* strains showing resistance or susceptibility to penicillin

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Introduction: *Streptococcus pneumoniae* is the major causative pathogen in community-acquired pneumonia. In recent years, pneumococci resistant to penicillins (PEN) and other β-lactam antibiotics have emerged, posing a serious medical challenge. The novel fluoroquinolone moxifloxacin (MXF, Bayer) shows enhanced antipneumococcal activity, and is recommended for the treatment of community respiratory tract infections.
Objectives: We have used MXF to examine the development of quinolone resistance in penicillin-resistant (PENR) and -sensitive (PENS) *S. pneumoniae*.
Material and methods: The MXF susceptibilities of 10 clinical *S. pneumoniae* isolates (five PENR and five PENS) were assessed by agar dilution, and mutation frequencies determined using $\sim 10^{10}$ cfu. Quinolone-resistant mutants were subjected to PCR and sequencing of the quinolone-resistance determining regions (QRDR) of gyrA, gyrB, parC and parE by previously described methods. Relative fitness was evaluated by growing mutants in direct competition with their susceptible, otherwise isogenic counterparts in mixed batch culture.
Results: MICs for MXF were in the range 0.06–0.12 mg/L. Mutation frequencies to resistance to MXF ranged from $<10^{-1}$ to 10^{-10}. No differences were observed between PENR and PENS strains, either in terms of MIC distribution, or in the frequency with which resistance to MXF emerged. Sequencing of the QRDR regions of the four target genes revealed no differences between PENR and PENS strains, and confirmed gyrA as the primary target of MXF in this organism. Fitness costs of ~20% were apparent in all drug-resistant strains, relative to wild-type.
Conclusions: PENR *S. pneumoniae* strains do not appear to exhibit an elevated MIC, or propensity to develop resistance to MXF, compared to PENS *S. pneumoniae*. Quinolones are a valuable class of antimicrobial for treatment of *S. pneumoniae* that do not appear to be compromised by PEN resistance.

P832 Nalidixic acid does not inhibit DNA transfer in a quinolone-susceptible derivative

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Objective: Quinolones exhibit a rapid bactericidal activity interfering with DNA gyrase activity. In addition, these compounds promote loss of plasmids from their hosts and block bacterial conjugation. The mode of killing of these drugs is not fully elucidated. To acquire more information about this matter, a mutant strain able to transfer its entire genome at the presence of nalidixic acid was selected.
Methods: A HfrH (*Eshcerichia coli* 3300) with Tn10 inserted near the gene *serB* on the *E. coli* chromosome was used on a donor, while a J-53 rifampicin-resistant derivative and AB1157 were employed as recipients. Conjugation was carried out by standard methods employing 2×10^8 cells/mL in rich broth. In this HfrH strain, Tn10 (which confers tetracycline resistance) is transferred as last marker, so the recipient, virtually, may acquire the entire chromosome of the donor including the F episome. Thus the recombinants obtained at the presence of nalidixic acid might become Hfr and, acquire the genetic information for the property to donate genetic material even if nalidixic acid is present at a level known to inhibit the growth of the strain.
Results: When HfrH was mated for 2 h at 37 °C with J-53 rif in the presence of 25 mg/mL of nalidixic acid, the number of recombinants was reduced by about 99% in comparison to the control. Twelve colonies obtained in the conjugation in the presence of the drug were used as donor in further crosses, carried out as above, and employing AB1157 as recipient. One strain was able to transfer genetic material in the presence of nalidix acid at the same frequency noted with the control. This last experiment was again repeated and results were confirmed. Susceptibility test carried out with all strains obtained in the mating experiments showed that they maintained the susceptibility to nalidixic acid as the original strain.
Conclusions: The results obtained in this study suggest that quinolones have a target in the cell that affect DNA transfer without interfering with DNA gyrase activity. Experiments are underway in order to detect this unknown function.

P833 Isolation and characterization of a quinolone-tolerant *Escherichia coli* strain

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Objectives: Quinolone antimicrobial agents exhibit a rapid bactericidal action against most pathogenic bacteria and their use has increased markedly worldwide in recent years. DNA gyrase, required for bacterial DNA replication is the primary target of quinolones. However, the mechanism of quinolone killing, apart from the involvement of DNA gyrase remains largely unknown. To gain more knowledge about quinolone killing, we have devised a

technique that makes possible the isolation of strain of *Escherichia coli* tolerant to quinolones.
Methods: A strain defective in the *lac* gene and carrying the Flac plasmid (10^9 cfu/mL in 20 mL of Muller–Hinton broth) was allowed to grow in the presence of graded concentrations of nalidixic acid at 37 °C overnight. The culture tubes with no detectable bacterial growth were harvested and the cells were collected and transferred in fresh broth at 37 °C for 3 h in order to stop all antibiotic effects. The cultures were concentrated to 1 mL and, after dilution, plated on of Mac Conkey agar containing nalidixic acid (2 mg/L). Since a tolerant mutant is as susceptible to the antibiotic as the wild-type parent strain, but undergoes only a slow loss of viability even in the presence of high doses of antibiotic, such strain may be able to survive drug concentrations that exceed the MIC but may be affected by sub-MICs. To test this hypothesis, colonies with *lac*-negative papillae were studied to evaluate their susceptibility to nalidixic acid.
Results: Six such colonies were isolated showing MIC values ranging between 16 and 64 mg/L and minimal bactericidal concentration (MBC) up to 2048 mg/L. Time–kill curves showed only a 1-log reduction for the mutant strain despite a 3-log reduction observed with the parent strain after 24 h.
Conclusion: Tolerance showed by these strains is not related to a decrease in permeability for nalidixic acid, because the MIC values are no affected if EDTA (0.25×10^{-3} M) is added to the antibiotic. Ongoing experiments are currently carried out in order to characterize the mutation responsible for tolerance.

Resistance in *S. pyogenes*

P834 *Streptococcus pyogenes* susceptibility to macrolides from inpatients at a clinical hospital Mostar

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Since February 2000 till September 2001, a survey of antimicrobial resistance of *Streptococcus pyogenes* was performed. Six hundred and thirteen (613) strains of *S. pyogenes* were tested against penicillin and macrolides–erythromycin, clarithromycin and azithromycin. The strains were isolated from throat swabs (552), nose swabs (50), ear swabs (4), vagina swabs (2), wound swabs (4) and broncho aspirat (1). Disk-diffusion tests were used for susceptibility testing. In Clinical Hospital Mostar, the resistance of *S. pyogenes* to erythromycin, azithromycin and clarithromycin was 7.99, 8.48, 8.68%, respectively. All strains were susceptible to penicillin. We had also intermediate strains to erythromycin (5.22%), azithromycin (5.54%) and clarithromycin (11.39%). The average resistance rates of *S. pyogenes* to erythromycin, azithromycin and clarithromycin in strains isolated in clinical hospital mostar were not higher than 9%. This percentage of macrolide resistance is similar to other neighbor countries. We did not detect any significant difference of resistance to tested macrolides. We detected only difference at intermediate strains; to clarithromycin is significantly higher than to erythromycin and azithromycin. Our results confirm the importance of global resistance data, but also regional resistance data in order to create an antibiotic policy.

P835 Antibiotic susceptibility of *Streptococcus pyogenes* and other β-hemolytic streptococci isolated from respiratory tract infections in Istanbul

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Objectives: The present study was performed to investigate the susceptibility to penicillin (PEN), erythromycin (ER), azithromycin (AZ), chlarithromycin (CL), ciprofloxacin (CIP) and levofloxacin (LEV) in β-hemolytic streptococci isolated from upper and lower respiratory tract infections between 1997 and 2000.
Methods: In total 103 (34 children, 69 adults) β-hemolytic streptococci strains were isolated from throat swabs ($n = 81$) and sputum ($n = 22$). Among these isolates classified by latex agglutination test (Dry Spot Streptococcal Grouping Kit-Oxoid) 62, 5, 6, 4, 14 and 12 were detected to be strains of groups A, B, C, D, F and G, respectively. Antibiotic susceptibility were determined by standard E-test methodology according to the recommendations of the manufacturer.
Results: All strains were found to be susceptible (MIC ≤0.12 mg/L) to PEN (one moderately susceptible in group D), but three (2.91%) to ER (one moderately susceptible), 2 (1.94%) to CL, 11 (10.67%) to AZ (eight moderately susceptible), five (4.85%) to CIP (one moderately susceptible) were detected to be resistant and two(1.94%) moderately susceptible to LEV.
Conclusions: Our results suggest that there is no change in the susceptibility to penicillin in *S. pyogenes*, and resistance to ER remains at a low level (2.91%). Sixteen of 103 β-hemolytic streptococci (15.53%) and 13 of 62 *S. pyogenes* (20.96%) strains were detected to be resistant or moderately susceptible to one or more than one antibiotic, whereas only 2 of the 62 *S. pyogenes* strains were found resistant or moderately susceptible to two or three antibiotics.

P836 Macrolide resistance in *Streptococcus pyogenes*: genes, frequencies and types in Denmark

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Objective: To set up a multiplex PCR, which detects macrolide resistance genes in group A streptococci (GAS). To collect macrolide-resistant GAS, analyze and report the frequencies of the different macrolide resistance genes and T-types in macrolide-resistant GAS
Methods: We developed a multiplex PCR for detection of *ermA*, *ermB* and *mefA* macrolide resistance genes in GAS. All clinical microbiological departments in Denmark were asked to send in all isolates of macrolide-resistant GAS, and from selected departments we received resistance data. The collection started in November 2000 and is still going on. The strains were phenotyped and resistance were determined by tablet diffusion (Rosco, Denmark) on Danish blood agar (Statens Serum Institut, Denmark), T-typing was done at the National Reference Laboratory at Statens Serum Institut.
Results: Among the first 53 strains send to us, we found that 44 were macrolide-resistant in our laboratory. All resistant strains harbored a resistance gene; none harbored more than one gene. A total of 32% were *mefA* positive, 20% were *ermA* positive and 48% had the *ermB* gene. In the *ermB* group 38% were inducible and 62% were constitutive. Phenotypes and genotypes matched 100%. Eleven different T-types were present, the two most frequent types were T-28 (27%; includes *ermA* and *ermB*) and T-1 (18%; all *mefA*) (Table 1). In the greater Copenhagen area, the frequency of macrolide resistance among GAS was 3.8% (99 of 2598).

Table 1 Gene frequency, inducibility and T-types among Danish macrolide-resistant group A streptococci

Gene	Frequency (%)	Inducible	T-type (no.)
mefA	32	No	T1 (8); T4 (1); T3, 13, B3264 (2); NT (3)
ermA	20	Yes	T28 (6); T8 (1); T13, 28 (1); NT (1)
ermB	48	38% Yes	Yes: T14, 49 (5); T3 (1); T4 (1); T9 (1)
	48	62% No	No: T28 (6); T12 (3); T3, 13, B3264 (2); T8 (2)

Conclusion: The three macrolide resistance genes are all frequently occurring in Denmark. Of all macrolide-resistant GAS 68% are resistant to clindamycin either inducible or constitutive. Eleven different T-types were present, and macrolide-resistant GAS, therefore, is not exclusively a clonal problem. Macrolide resistance in Denmark is still low in Denmark (<4%) but should be monitored.

P837 Phenotypic characterization of macrolide resistance in 362 *Streptococcus pyogenes* isolates coming from acute pharyngotonsillitis

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Objectives: We estimated the phenotypes of macrolide-resistant of *S. pyogenes* isolates among patients with upper respiratory tract infections in Reggio Emilia (Italy).

Methods: The study was conducted over a 9-month period and included patients with symptoms of acute pharyngotonsillitis. The 362 *S. pyogenes* isolates were identified according to standard laboratory techniques. Catalasi negative and β-emolitic colonies isolated from 5% sheep blood agar were identified with using latex agglutination test and api 20 S system. The phenotypes of the erythromycin resistance strains were determined by double-discs test with erythromycin and clindamycin. Antimicrobial sensitivity to penicillin, erythromycin, clindamicyn, rokitamycin, cefaclor and amoxicillin/clavulanate was determined by the disk-diffusion Kirby–Bauer method according to NCCLS recommendations.
Results: All the 362 isolates were uniformly susceptible to penicillin. Overall, 45 isolates (12.43%) showed resistance to erythromycin and 6 (1.68%) were also resistant to clindamycin (constitutive resistance) erythromycin-resistant but clindamycin-susceptible with no blunting of the erythromycin zone was found amongst 39 *S. pyogenes* isolates. These strains had the M phenotype of resistance. Only two isolates (0.56%) showed resistance to rokitamycin, these were resistant also erythromycin (cMLSb phenotype).
Conclusion: Tonsillitis is a complex infection that can involve streptococci as well as other bacteria and viruses. Although streptococci may be the most virulent organism, causing septic and nonseptic complications. Failure to eradicate streptococci from patients can occasionally lead to rheumatic fever rarely to glomerulonephritis. With the emergence of increased treatment failures, it has been necessary to consider alternative therapies for patients who cannot tolerate or who do not respond to penicillin. Erythromycin resistance in our area is 12.43% and among these strains was found to be lower than in previous studies (30% in 1999 and 24.63 in 2000). The prevalence of rokitamycin-resistant isolates in acute pharyngitis is 0.56% so rokitamycin can be an alternative for the treatment of particular patients.

P838 Resistance trends in *Streptococcus pyogenes* to erythromycin in Slovakia: is there a correlation with consumption?

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Objectives: There is an increasing trend of macrolide resistance in *Streptococcus pyogenes* and *S. pneumoniae* worldwide. Probably one of the risk factors for development of macrolide resistance (MR) is increasing consumption of macrolides.
Methods: We investigated consumption of 'older' macrolides erythromycin (ERY), spiramycin (SPI) and roxitromycin (ROX) and 'newer' macrolides azithromycin (AZI), clarithromycin (CLA) from the state institute of Drug Control Consumption Database and compared it with the level of resistance of *S. pyogenes* and *S. pneumoniae* to ERY from a yearly report of Antibiotic Resistance Surveillance in 1995–1998 and 1998–2000.
Results: Significant increase of consumption of macrolides has been observed in 1995–2000, from 835 DDD/1000 in 1995–1382 DDD/1000 in 1999 and 1265 DDD/1000 in 2000. The proportion of macrolides among all antibiotics increased from 12.7 to 17.2% (1995–1999) to 16.4% (2000) because of increasing consumption of 'newer' macrolides—AZI from 15 DDD/1000 in 1995 to 290 DDD/1000 in 2000 and CLA from 0 to 14 DDD/1000 within last 5 years. Concerning the level of ERY resistance in *S. pyogenes* (14.9%) and in *S. pneumoniae* (12.1%) in 2000, we found that resistance in both pathogens in Austria (11.3 and 10.5%) and in Czech Republic (6.5%) is lower than in Slovakia, however, in Poland and in Italy—about 40% in *S. pyogenes* is much higher than in Slovakia. However, the data from ESAR Project testing 3249 strains from five countries in UK and Germany found only 1.5% ERY resistance in *S. pyogenes*.
Conclusions: Increasing consumption of 'newer' macrolides including ERY, SPI and ROX, may led to increasing ERY resistance in *S. pyogenes* (5.5–14.9% during 6 years). However, if consumption only is responsible for an increase of ERY resistance, is not clear and would be too simple explanation for this complicated issue. Since 2000, consumption of macrolides starts to decrease (in first quarter of 2001 – 20% decrease has been noted in addition to 2000) and if resistance is consumption related, reversibility of ERY resistance should be noticed in 2001–2003.

P839 Erythromycin-resistance phenotype/genotype and number of RD2 repeats of the internalization-associated gene *prtF1* in Italian group A streptococci (GAS)

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Objectives: Following the recent demonstration by our group of a close and unsuspected association between erythromycin resistance and cell invasiveness in GAS isolated in Italy, suggesting that strains combining the two features may be able to escape β-lactams by virtue of intracellular location and macrolides by virtue of resistance, the present study was undertaken to characterize the Italian erythromycin-resistant cell-invasive isolates with a view to understanding the biological basis of this association.
Methods: A total of 77 (66 erythromycin-resistant and 11 erythromycin-susceptible) independent clinical strains of GAS recently isolated in Italy (all carrying the internalization-associated *prtF1* gene) were studied. The macrolide-susceptibility/resistance phenotype was determined by conventional methods. Erythromycin-resistance determinants were detected by PCR using specific primer pairs. The *prtF1* gene was detected by PCR using primers complementary to the flanking region of RD2; the expected PCR product depended on the number of RD2 repeats, one repeat being 111 bp.
Results: Amplicon size ranged from ca. 110 to ca. 550 bp, suggesting that the number of RD2 repeats ranged from 1 to 5. Most frequently, amplicons consisted of 4 or 3 repeats (32 and 27 strains, respectively) or of a single repeat (16 strains); 2 and 5 repeats were found in one strain, respectively. Among inducibly erythromycin-resistant isolates ($n = 34$), all those carrying the *ermB* methylase ($n = 13$) had four RD2 repeats, whereas all those carrying the *ermTR* methylase ($n = 21$) had three repeats. The picture was more varied among constitutively resistant strains ($n = 10$), the majority of which had four ($n = 5$) or three ($n = 3$) repeats; among the strains of the M phenotype ($n = 22$), all carrying the efflux *mefA* gene), most of which had one ($n = 13$) or four ($n = 7$) repeats; and among the erythromycin-susceptible strains ($n = 11$), most of which ($n = 7$) had four repeats.
Conclusions: A further issue in the context of the association between erythromycin resistance and cell invasiveness in GAS is the association between the resistance-phenotype/genotype and the number of RD2 repeats of *prtF1*.

P840 Nationwide surveillance of antimicrobial resistance in *Streptococcus pyogenes* in Slovenia

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Objectives: To determine rates of resistance to selected antimicrobial agents and phenotypes of resistance to macrolides and lincosamides.
Methods: A total of 1032 nonreplicated strains of *Streptococcus pyogenes* were isolated by eight microbiology laboratories from all regions of Slovenia between January and September 2001. Laboratories are part of public health institutes. Strains were isolated from outpatients and patients from general, but not from tertiary care hospitals. Most strains were isolated from throat swabs. β-Hemolytic colonies were identified as *S. pyogenes* by positive PYR test and group A latex agglutination or bacitracin test. Susceptibility to five antibiotics (penicillin, vancomycin, tetracycline, erythromycin and clindamycin) was determined by standard disk-diffusion procedure according to NCCLS. Intermediate and resistant categories were interpreted as resistant. Phenotype of resistance to macrolides and lincosamides was determined by double-disk induction method with disks of erythromycin and clindamycin.
Results: All strains were susceptible to penicillin and vancomycin. Resistance rate to tetracycline was 12%, to erythromycin 6% and to clindamycin 2%. There were wide differences among regions: the lowest rate of erythromycin resistance was in the eastern part of Slovenia, around Maribor (2%) and the highest rate in the western part of Slovenia, around Koper (24%). Three phenotypes were found in 64 erythromycin-resistant isolates: 22% M phenotype, 47% inducible MLSB phenotype and 31% constitutive MLSB phenotype.

Conclusions: Current rate of resistance to erythromycin in *S. pyogenes* in Slovenia is not high, except in one region. Resistance monitoring will be continued. Erythromycin-resistant strains were frozen and should be studied further. Detailed epidemiological analysis of collected data is also necessary.

P841 Antimicrobial resistance in *Streptococcus pyogenes* isolates in a town in northern Italy from 1998 to 2000

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Objectives: The aim of this study was to know the evolution of *Streptococcus pyogenes* susceptibility to erythromycin, rokitamycin, clindamycin and tetracycline in a period between 1998 and 2000.
Methods: One hundred and ninety-three isolates of *S. pyogenes* were collected in the Laboratory of Microbiology of A. Gallino Hospital, in Genoa, in 3 years (67 in 1998, 58 in 1999 and 68 in 2000). The strains were recovered from throat swabs of outpatients affected by pharyngitis and/or tonsillitis. In 1998, we started this study using only three antibiotics (erythromycin, clindamycin and tetracycline). In the following year, we added another macrolide (rokitamycin). The susceptibilities tests were determined by the disk-diffusion method on Mueller–Hinton agar supplemented with 5% sheep blood (Becton Dickinson). Besides, in 1999 and 2000, we evaluated the macrolide-resistance phenotypes using a double-disk test with erythromycin and clindamycin.
Results: The observed resistance data are reported as follows:
- In 1998: 26.8% to erythromycin, 20.9% to clindamycin and 35.8% to tetracycline.
- In 1999: 51.7% to erythromycin, 3.4% to rokitamycin, 27.6% to clindamycin and 17.2% to tetracycline.
- In 2000: 23.5% to erythromycin, 4.4% to rokitamycin, 14.7% to clindamycin and 4.4% to tetracycline.
- In the last 2 years, we observed a larger prevalence of constitutive resistance (42.1% in 1999 and 60% in 2000) compared to the other phenotypes.

Conclusions: While the resistance to tetracycline decreased during 3 years (from 35.8 to 4.4%), the resistance to erythromycin increased significantly during 1999 (from 26.8 to 51.7%) and it decreased in the following year (23.5%). This trend indicates that erythromycin resistance is still a problem and it emphasizes the need to screen for resistance to macrolides in *S. pyogenes*. Besides, the evaluation of these data indicates that rokitamycin is a good alternative to the other antibiotics under study.

P842 Colonization by *Streptococcus pyogenes* and susceptibility patterns against clinical relevant antimicrobials

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Objectives: To evaluate trends of colonization by *Streptococcus pyogenes* (GAS) and compare antimicrobial resistance profiles of colonizing and disease causing isolates.
Methods: Bacterial identification was carried out by standard methods. Antimicrobial susceptibility testing was performed by disk-diffusion (NCCLS) against penicillin (P), erythromycin (E), clindamycin (Da), clarithromicin (Cl), azithromycin (Az), josamycin (J), tetracycline (Te), chloramphenicol (C), ciprofloxacin (Cip). Macrolides resistance phenotype was based on a triple-disc test with E, Da and J.
Results: During four periods in 2000–2001 oropharyngeal samples were taken from different populations: 1351 from children (0–6 years) in day-care centers (DCC), 442 from school-aged children (7–15 years) and 273 from adults (226 school staff and 47 family members). Carriage in younger children was higher (13%) than among older than 7 years (6.7%), however, carriage rates varied with DCC and during the year. Colonization was higher among family members (15%) than among school staff (4%). A total of 295 GAS were isolated: 218 from carriers, 42 from patients with tonsillitis attending primary care settings and 35 from hospital origin (19 invasive; 26 abscess), collected during March 1999–January 2001. Resistance to one or more antimicrobials was higher in strains colonizing older children (41% vs. 10%). Resistance to macrolides was 10% for carriage, 17% for tonsillitis and 6% for hospital isolates. The M phenotype was prevalent in carriers (20/23 strains) whereas the MLSB phenotype was dominant among infection isolates (5/9 strains). Resistance to Te was 6% for colonizing strains (2% among the youngest and 17% among oldest children), 10% for tonsillitis and 47% for hospital strains, being most of the isolates susceptible to E (29/38). Resistance to C and Cip was found in two disease strains.
Conclusions: Resistance to E was higher for tonsillitis isolates than for carriage or other disease strains. In addition, differences in the antimicrobial resistance profiles of colonizing and disease-causing isolates were observed, namely for macrolides resistance phenotypes, suggesting that different strains may be involved in colonization and disease. Of interest was also the finding of resistance to tetracycline among carrier children since tetracycline in contrast to macrolides it is rarely used in pediatrics.

P843 Prevalence of erythromycin A resistance and activity of telithromycin against isolates of *S. pyogenes* from pediatric patients collected during 1999–2000 from the PROTEKT surveillance study

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Background: *Streptococcus pyogenes* is responsible for various acute supportive conditions, including the majority of cases of tonsillitis/pharyngitis in children. Whereas macrolide antibacterials have proved a valuable alternative to penicillin G in treating *S. pyogenes* respiratory tract infections (RTIs) in penicillin-allergic patients, increasing resistance to these agents has been observed recently.
Objective: A subanalysis of the PROTEKT antibacterial resistance surveillance study was performed to assess the prevalence of erythromycin A resistance (EryR) among *S. pyogenes* isolated from pediatric patients with community-acquired RTIs (CARTIs), and to measure the activity of the new ketolide, telithromycin, against these isolates.
Methods: During the winter of 1999/2000, *S. pyogenes* isolates were collected from children (aged <12 years) with community-acquired RTIs attending centers ($n = 69$) across Europe, Asia, Australia, North and South America (25 countries). MICs were determined centrally for a panel of antibacterials using an NCCLS broth microdilution method. Susceptibility data were analyzed by culture source.
Results: In total, 676 isolates of *S. pyogenes* were tested, comprising 14 sputum, 30 nasopharyngeal, 50 ear and 563 throat cultures, plus a small number of 'other' sources. The prevalence of EryR among these isolates is shown by source in Table 1.

Table 1 Prevalence of EryR among isolates of *S. pyogenes* from pediatrics

Culture source	*N*	% EryR
Sputum	14	21.4
Nasopharyngeal	30	20.0
Ear/middle ear fluid	50	12.0
Throat	563	9.6
All sources combined	676	10.2

Analysis by culture source showed sputum and nasopharyngeal sources to have the highest prevalence of EryR. Isolates resistant to erythromycin A were also resistant to clarithromycin and azithromycin. Telithromycin showed potent in vitro activity against *S. pyogenes* (MIC_{50}, 0.015 mg/L; MIC_{90}, 0.03 mg/L).
Conclusions: The prevalence of EryR among *S. pyogenes* is high in children, averaging 21% in sputum, 20% in nasopharyngeal, 12% in ear and 10% in throat isolates collected in this 1999–2000 surveillance study. The ketolide telithromycin showed excellent activity against isolates of *S. pyogenes* from pediatric patients, including EryR strains, and appears a promising alternative for management of CARTIs caused by Group A streptococci in children.

P844 Macrolide and ketolide susceptibility of *S. pyogenes* isolates collected in Austria during 2000–2001

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Background: An increase in macrolide resistance of *S. pyogenes* strains was recently reported from many European countries. Telithromycin is the first member of the ketolide family of antimicrobials with activity against Gram-positive bacteria including those with acquired resistance to erythromycin.
Objective: To investigate the activity of telithromycin and various macrolides on *S. pyogenes* strains collected in Austria.
Methods: A total of 296 strains of *S. pyogenes* isolated during the last year from children (222 strains) and adults (72 strains) from different regions of Austria were tested for susceptibility to telithromycin, erythromycin (E), roxithromycin (R), clarithromycin (C), azithromycin (A), clindamycin (Cl) and tetracycline (T) using an agar dilution method. E-resistant strains were further investigated for the presence of macrolide resistance genes (*mef*, *erm*, and *ermTR*) using a PCR-based method.
Results: Overall, the E, Cl, T resistance was 11, 1, 16%, respectively, with lower prevalence in children (11, 0.4, 11%) and higher prevalence in adults (13, 3, 33%). (In a similar investigation on strains collected during 1999 and 2000, the overall E and T resistance was 11 and 24%.)

The respective MIC_{50} and MIC_{90} of the antibiotics tested were: telithromycin $\leq$0.015 and $\leq$0.015 mg/L; E 0.03 and 1 mg/L; R 0.12 and 4 mg/L; C $\leq$0.03 and 0.5 mg/L; A 0.12 and 4 mg/L; Cl $\leq$0.06 and $\leq$0.06 mg/L. Resistance to E was exhibited by 33 strains, 25 of them harboring *mef*, 3 *erm*, 4 *ermTR* and 1 *mef*+ *erm* genes. Telithromycin showed the lowest MICs and retained a good activity also on strains containing E resistance genes; the range of MICs for those strains was $\leq$0.015–0.5 mg/L.
Conclusions: The macrolide resistance prevalence of *S. pyogenes* strains collected in 2000–2001 was similar to that found in strains collected in 1999–2000. Telithromycin was highly active against isolates of *S. pyogenes* collected during the last 2 years in Austria.

P845 A 7-year study of prevalence of resistance to erythromycin and penicillin among group A streptococci in Brazil

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Since the late of 1980s, a resurgence of severe Group A streptococci (GAS) infections has been noted through out the world, including the streptococcal toxic shock syndrome, necrotizing fasciitis, sepsis and rheumatic fever. As the most common bacterial cause of pharyngitis/tonsillitis in children, prevention of acute rheumatic fever is the principal goal of treatment with antibiotic therapy. Although penicillin is the drug of choice, several drug failure episodes have been reported during the past 20 years. Erythromycin has remained as the recommended alternative to penicillin clinical resistance since then. The aim of this study was to determine MICs of penicillin and erythromycin among *Streptococcus pyogenes* strains isolated from acute infections in children over a period of 7 years at Santa Casa Hospital in São Paulo, Brazil. The MICs of the1047 strains studied were determined by the E-test (AB Biodisk, Sweden). All strains were susceptible to penicillin G: MICs ranged from 0.002 to 0.094 µg/mL and MIC_{90} varied from 0.032 to 0.047 µg/mL over the period. For erythromycin MICs varied from 0.016 to 32 µg/mL and MIC_{90} changed from 0.5 to 1.0 µg/mL over the 7-year period study. The results showed a not statistically significant erythromycin resistance that varied from 6.25 to 10.95% during the study period, indicating that the prevalence of resistant strains were almost the same at the first an the last year of study. The intermediate susceptibility to erythromycin was observed varying between 14.29 and 21.74% during study period. Our conclusion was that no progressive increase of resistance to penicillin and erythromycin was observed among our GAS strains over a period of 7 years.

Staphylococci

P846 *Staphylococcus warneri, S. hominis* and *S. haemolyticus* are emerging nosocomial bloodstream pathogens in neonatal and pediatric intensive care units

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Background: Lacks data on epidemiology of coagulase-negative Staphylococci (CoNS) other than *Staphylococcus epidermidis* (SE) involved in nosocomial bloodstream infections.
Objective: To assess the isolation rate, species distribution and antibiotic susceptibility pattern of CoNS recovered from blood cultures (BCs) in neonatal intensive care unit (NICU) and pediatric PICU.
Methods: We reviewed for the period 1 January to 31 December 2000 microbiological data referred to 1428 BCs of 493 patients (pts) hospitalized in a 30-bed-NICU and a 25-bed-PICU. BCs were performed in BactAlert Pediatric bottles (Organon Teknika), species identification was obtained by coagualse test and API Staph System (BioMerieux), antibiotics susceptibility by WIDER MIC Gram-positive panels (Soria Melguizo – DADE manufactured).
Results: A total of 144 pts had pos BCs, 86 pts (59.7%) with CoNS: 46 Se, 16 *S. warneri* (SW), 13 *S. hominis* (SHO) and 11 *S. haemolyticus* (SHAE). 14/86 (16%) pts had $\geq$2 consecutive pos BCs for SW, 12/86 (14%) for SHO and 11/86 (13%) for SHAE. In pts with microbiological documented bloodstream infection risks factors present were: CVC and/or total parental nutrition and/or arterial catheter. On a total of 177 isolated, 106 strains were CNS (60%): SE was the prevalent species (36%), followed by SW (14%), SHO (9.4%) and SHAE (9.4%). The rate (%) of R strains was: for SW oxacillin (OX) 71, vancomycin (VA) 0, teicolanin (TE) 0, ciprofloxacin (CIP) 0, levofloxacin (LE) 25, trimethoprim/sulfametoxazole (T/S) 0, cloramphenicol (C) 9, rifampin (RI) 27, quinupristin/dalfopristin (Q/D) 12, linezolid (LI) 6; for SHO OX 57, VA 0, TE 0, CIP 0, LE 25, S/T 0, C 75, RI 0, Q/D 14, LI 12; for SHAE OX 100, VA 0, TE 9, CIP 0, LE 25, S/T 14, C 43, RI 57, Q/D 12, LI 0.
Conclusions: Our data shows the emergence of SW, SHO and SHAE as antibiotic resistant pathogens of BSIs in NICU and PICU and stress the importance in the species identification of CoNS recovered from BCs in these units to better understand their epidemiology.

P847 Interpreting PFGE patterns and antibiotypes for strain typing of methicillin-resistant *Staphylococcus aureus, S. haemolyticus* and *S. hominis*

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Objectives: The purpose of this study was to correlate antibiotypes to PFGE patterns of 69 methicillin-resistant *S. aureus* (MRSA), 21 methicillin-resistant *S. haemolyticus* and 11 *S. hominis* isolates recovered from inpatients with different pathologies and interned in different departments at the University Hospital of Patras, in Greece during a two year period (1999–2000).
Materials and methods: Biotypes of coagulase-negative staphylococci (CNS) were performed by the API Staph system. Minimal Inhibitory Concentration (MIC) to oxacillin was determined by the agar dilution method according to NCCLS guidelines. The antibiotic susceptibility testing was performed by the disk diffusion method against the antimicrobials: ampicillin, amoxicillin/clavulanic acid, cefaclor, ceftriaxone, ceftazidime, imipenem, amikacin, netilmicin, erythromycin, ciprofloxacin, fusidic acid, SXT and vancomycin. All isolates were analyzed by pulsed-field gel electrophoresis (PFGE) of small macro-fragments, transferred onto nylon membranes and hybridized with the *mecA* specific DNA probe.
Results: Five biotypes were revealed among the *S. haemolyticus* and three among the *S. hominis* clinical isolates. All MRSA showed high MIC to oxacillin (>1024 mg/L), while 24 out of 32 *S. haemolyticus*, *S. hominis* isolates

expressed lower MIC levels, ranging between 32 and 512 mg/L. All oxacillin-resistant isolates were *mecA*-positive and expressed resistance to all β-lactams, while no isolate was found to be resistant to amikacin, netilmicin and vancomycin. Four PFGE types were characterized among the MRSA (A:14, B:29, C:10 and E:16 strains), where *S. haemolyticus* and *S. hominis* were classified into two distinct PFGE types each (t':14, u':seven strains, p':10 and q':one strain, respectively). Strains of types A and C were resistant only to β-bactams while strains of types B and E were multiresistant, expressing resistance to more than three antibiotics including quinolones and fusidic acid. All *S. haemolyticus* of PFGE type t', expressed the same MIC (>128 mg/L). There was not found any correlation between the antibiotype and PFGE type of CNS isolates.

Conclusions: The MRSA clinical isolates with certain antibiotypes were correlated to common PFGE patterns, while this was not observed among the CNS strains. Four distinct PFGE patterns of methicillin-resistant *S. haemolyticus* and *S. hominis* clinical isolates are associated with nosocomial infections in our hospital during a 2-year period.

P848 Sternal wound infections due to persistence of a single clone of coagulase-negative staphylococci

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Objectives: to describe three cases of coagulase-negative staphylococci (CoNS) sternal wound infections after open heart surgery caused by the same multiresistant pathogen over a period of 10 weeks. To evaluate possible ways of persistence and spread of this CoNS clone.

Methods: Three cases of sternal wound infection after cardiac surgery caused by a multiresistant CoNS (sensitive only to vancomycin and teicoplanin) were identified by antibiogram over a period of 10 weeks. DNA-fingerprinting of the three isolates (pulsed field gel electrophoresis) showed the isolates of the patients to belong to a single clone. Chart review of the cases was performed in order to identify the pathway of persistence and spread of this CoNS clone. It included the identification of the members of the scrubbed and non-scrubbed surgical team and other hospital workers with contact with those patients during the postoperative period (such as the ECG team), identification of theatre rooms and hospital rooms. Nose and hand cultures were obtained from all identified hospital workers. The antibiotic resistance pattern of the isolates from these cultures were then matched with the previously obtained clone from the three patients.

Results: No theater room and no hospital room were common to all three patients. No surgeon, anesthesiologist or theatre nurse was involved in more than one operation in the three patients, which virtually excludes a direct contamination of the three patients during surgery from the same medical workers. A total of 34 swabs were obtained from the nose and hands of the identified medical workers. None of the isolates from those cultures showed the same multiresistant pattern of the clone. It was not possible to identify the ward nurses who took care of the patients during the postoperative hospital stay.

Conclusions: The finding of the same clone of CoNS causing sternal wound infection in three different patients over a period of 10 weeks shows that a single clone can persist over a long time period. Such persistence could be a major source of infection. Our results argue against a direct contamination during surgery from a single medical worker. We hypothesize that infection of the three patients may have occurred during the postoperative period from an unidentified colonized healthcare worker. Our study demonstrates that CoNS may also be involved in outbreaks of postoperative surgical site infections.

P849 Altered phase variation (switching) of polysaccharide adhesion production in a biofilm-forming cerebrospinal fluid isolate of *Staphylococcus epidermidis*

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Objectives: The ica operon of *Staphylococcus epidermidis* encodes the enzymes required for polysaccharide adhesion (PIA) biosynthesis and is required for biofilm formation. Expression of these genes is subject to phase-variable regulation (on–off switching), particularly in the on-to-off direction, although the molecular basis and clinical significance of this phenotype are poorly understood. We have characterized a biofilm-forming *S. epidermidis* strain, CSF41498, isolated from cerebrospinal fluid in which the on-to-off switching frequency is up to 18-fold slower than the reference strain ATCC 35984 (RP62A).

Methods: Reverse transcription PCR (RT-PCR) was used to examine the transcriptional regulation of the ica locus. Semi-quantitative assays of stained, adhered bacterial cells were conducted in 96-well tissue culture plates to assess biofilm-forming capacity. Subinhibitory tetracycline (0.06 μg/mL) or 4% NaCl/0.5% glucose were added to growth media to enhance biofilm formation. On-to-off switching rates of PIA production were measured by plating serial dilutions of phase-on (black) colonies onto Congo red agar and determining the proportion of phase-off (red) colonies.

Results: DNA sequence analysis did not identify any genetic differences between the ica loci from CSF41498 and RP62A. Using RT-PCR similar *icaA* transcription levels were detected in phase-on variants of CSF41498 and RP62A. Expression of the ica operon in phase-on variants of both strains was also found to be temporal, characterized by essentially constitutive transcription during exponential growth followed by a sharp decline in late stationary phase. Phase-on variants of CSF41498 and RP62A displayed a similar capacity for biofilm formation. Growth of both strains under biofilm/ica-inducing environmental conditions did not influence PIA switching rates. Expression of the *icaA* gene was dramatically reduced in biofilm-negative phase variants of both strains, particularly RP62A variants.

Conclusions: These data suggest that on-to-off PIA switching is contingent upon the activity of a *trans*-acting factor(s) and functions independently of ica operon regulation to control biofilm formation.

P850 Coagulase-negative staphylococci with reduced susceptibility to glycopeptides in a Greek cancer hospital

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Objectives: Staphylococci with reduced susceptibility to glycopeptides, although of uncertain clinical relevance, challenge the ability of routine microbiology laboratories to recognize them. In our study, we tried to detect subpopulations with increased resistance in clinical isolates of coagulase-negative staphylococci (CNS) with borderline susceptibility to glycopeptides.

Methods: Twenty methicillin-resistant CNS, 12 *S. haemolyticus* (shl) and eight *S. epidermidis* (sep) with vancomycin (V) MIC = 4 mg/L and/or teicoplanin (T) MIC ≥ 8 mg/L (broth microdilution: WIDER) have been isolated in the current year, mainly from blood cultures of patients with hematological malignancies. In order to detect resistant subpopulations of these strains, we used an agar screen method (Mueller–Hinton Agar [MHA] with 5 mg/L vancomycin) and the E-test 'macro' method (a heavy inoculum of two MacFarland on Brain Heart Infusion Agar [BHIA] and 48 h incubation). According to the manufacturer, strains with V ≥ 8 mg/L and T ≥ 8 mg/L or T ≥ 12 mg/L alone are considered glycopeptide-resistant and glycopeptide-heteroresistant, respectively. Fifteen glycopeptide-susceptible CNS isolates and *S. aureus* UA 166 (EARSS) were used as negative controls while vancomycin-resistant *E. faecalis* UA 605 (EARSS) was used as positive control.

Results: Of the 20 isolates, when re-tested by microdilution, five sep and one shl had lower MICs (V < 4 mg/L and T < 8 mg/L) and appeared susceptible. By the E-test 13/20 isolates (seven sep and six shl) had V <6 mg/L and T ≥ 12 mg/L ('heteroresistant'). 4/20 isolates (three shl and one sep) had V ≤ 4 mg/L and T ≤ 4 mg/L ('susceptible'). three shl had V = 8 mg/L and T = 32 mg/L ('resistant'). These were consecutive isolates from one patient with V = 4 mg/L and T = 16 mg/L when initially tested by microdilution and V = 8 mg/L and T = 16 mg/L when re-tested. Only the above mentioned three shl strains grew on agar screen plates.

Conclusion: Reduced susceptibility to glycopeptides among staphylococci is usually heterogeneous, often unreproducible and difficult to confirm. In this study, we have been able to isolate subpopulations with increased MICs for both glycopeptides from three strains. Other strains could also contain subpopulations at frequencies so low that were difficult to detect without more specialized methods like population analysis. Laboratories should be on alert for strains indicative of decreased susceptibility to glycopeptides.

P851 Why *Staphylococcus cohnii* prevail in the intensive care unit environment

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Objectives: *Staphylococcus cohnii* ssp. *cohnii* strains are the main Gram-positive flora in a environment of the pediatric hospital ICU. The species is seldom isolated from patients so that it seems strange that all strains are methicillin-resistant and resistant to many other antibiotics. Which features promote the numerous attendance of *S. cohnii* in hospital environment and their ability to gain resistance genes?
Methods: The survival period of strains in dry conditions on metal surface in albumin presence as well as the resistance to osmotic stress caused by NaCl and sucrose high concentrations were estimated. Using the plate diffusion method we assayed their ability to excrete bacteriocins active on 10 different species of Gram-positive and Gram-negative bacteria. Adherence of native (directly from culture) and sonificated cells (devoid of extracellular film) to plastic, glass and metal surfaces were controlled. We estimated hydrophobicity (SAT test) and extracellular slime as well as the relation with the ability of clusters forming and rough type of growth.
Results: *Staphylococcus cohnii* survived in dry conditions on metal surface for many months. More than a half of the investigated strains were able to grow in the presence of 2 M of sucrose and 60% of strains in the medium with 2.8 M NaCl. All the 50 investigated strains expressed bacteriolytic activity to *Micrococcus lysodeicticus* and 23 strains produced bacteriocins active to other bacteria: *Enterococcus faecalis*, *Enterococcus faecium* and *Bacillus subtilis*. As many as 66% of investigated strains produced slime, 80% were hydrophobic and 67% spontaneously formed clusters in liquid media. More than a half of the strains strongly adhered to surfaces – especially to PCV floor finish. The feature was shared by the cells covered with slime, those formed clusters and naked cells (sonificated). During the growth clusters of *S. cohnii* ssp. *cohnii* captured in themselves cells of other bacteria.
Conclusions: Unusually active adhesion to surfaces of plastic, metal and glass and high endurance are the reasons for the long-life survival of *S. cohnii* ssp. *cohnii* in the hospital environment. Activity of their bacteriocins may support their prevalence in the environment. The ability of the cells clusters to capture other bacteria and their co-growth may be the reason for genetic exchange and lead to phenotypic diversity and resistance to antibiotics.

P852 Biological properties of *Staphylococcus cohnii* strains found persisting in an intensive care unit

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Objectives: To study biological features of *Staphylococcus cohnii* ssp. *cohnii* and *S. cohnii* ssp. *urealyticus* whose numerous strains were isolated from hospital environment, from medical staff and patients of ICU in the pediatric hospital.
Methods: We performed biotyping studies of 238 *S. cohnii* ssp. *cohnii* and 15 *S. cohnii* ssp. *urealyticus* strains based on sugars decomposition on Purple Agar. We also studied the ability of these stains to express enzymes and toxins recognized as important agents of staphylococcal pathogenicity: lipase and phospholipase, thermostable DNAase, proteases (gelatinase and caseinase), elastase, hyaluronidase, fibrinolysin, enterotoxins A, B, C and D and hemolysins as well as lactate dehydrogenase and esterases. The number and size of plasmids were established by gel agarose analysis of extrachromosomal DNA of 60 strains.
Results: Most of *S. cohnii* ssp. *cohnii* strains belonged to three out of the six recognized biotypes. Almost all *S. cohnii* ssp. *urealyticus* strains were restricted to one of them. Lipases and phospholipases were produced in 40% of the 238 investigated *S. cohnii* ssp. *cohnii* but only in 15% of *S. cohnii* ssp. *urealyticus*. Almost all the investigated strains excreted thermostabile DNAase and gelatinase. All *S. cohnii* ssp. *urealyticus* produced caseinase but this was the feature of only 4% of *S. cohnii* ssp. *cohnii*. Differences between the two subspecies on intracellular level of expression of lactate dehydrogenase and esterases were also detected. None of the 116 investigated strains produced elastase, hyaluronidase or fibrinolysin. We did not find strains able to excrete enterotoxins. However, all the strains hemolyzed rabbit, sheep and human erythrocytes and caused synergistic hemolysis. Our preliminary study showed that *S. cohnii* produces highly thermostabile, low-weight delta-like hemolysin. All the investigated strains contained plasmids. Most of them were small with a size of about 1–10 kbp. The biggest ones were of 25 kbp but these were only about 7% of all plasmids. Eighty-four per cent *S. cohnii* ssp. *cohnii* but only 69% *S. cohnii* ssp. *urealyticus* strains contained 4–10 plasmids in their cells.
Conclusions: The investigated *S. cohnii* strains were phenotypically diverse. Both subspecies differ significantly. There is a series of biological traits conditioning good metabolic prospects and providing the staphylococcal species with important position in the hospital environment.

P853 The predominance of multiple resistant *Staphylococcus epidermidis* in bloodstream infections of neonates in neonatal intensive care units

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Objectives: Strains of *Staphylococcus epidermidis* have increasingly been important nosocomial pathogens, particularly in critically ill neonates. The aims of this study have been to quantify the impact of *S. epidermidis* in NICU-aquired infections occurred since January 2000.
Methods: *S. epidermidis* isolates were taxonomically identified with traditional methods. Confirmations were made in the API or ATB system. Antibiotic sensitivities were determined with the disc diffusion test, 6 μg oxacillin agar plates, and automated system of VITEK GPS 101. Oxacillin resistance was confirmed by PCR techniques. A strain was judged to be a probable cause of the infection if it was a single microorganism in the blood culture(s) of a neonate with clinical signs or symptom of infection.
Results: Of 2909 blood cultures from neonates cared at the three NICUs of the university hospitals 474 (16.29%) gave positive results and 199 isolates (42% of the total positive hemocultures) proved to be *S. epidermidis* strains. Most of the strains were resistant to β-lactamase-susceptible penicillins (99%), oxacillin (88%), macrolides (75%), fluoroquinolones (39%), gentamicin (80%) and netilmicin (62%) but sensitive to vancomycin. The strains fell into 18 distinct resistance phenotypes based on antibiotic resistance patterns, however, strains (28%) with simultaneously resistant to all β-lactams, aminoglycosides, macrolides and fluoroquinolons and those (33%) resistant to all β-lactams, aminoglycosides and macrolides were predominant. A limited number of strain showed an intermediate resistance to teicoplanin with the disc method, however, using MIC determination a reduced glycopeptide susceptibility seems to be more frequent than is currently estimated. Such a comparisons and the clonal relationship of resistant strains are in progress.
Conclusions: *S. epidermidis* strains are the most frequent Gram-positive causative agents of NICU-acquired infections because of their known high affinity to plastic devices and extraordinary ability to adapt and tolerate the environmental antibiotic pressures. We hypothesize that multiple resistant strains of *S. epidermidis* may be the reservoir of resistance genes for transfer into clones of *Staphylococcus aureus* and other *Staphylococcus* species.

P854 High prevalence of nasal carriage of methicillin-resistant coagulase-negative staphylococci in the community and in hospitalized patients

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Objectives: To evaluate the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative (MRCNS) carriage among ambulatory patients and patients interned in the hospital.
Methods:
1 *Sampling.* Nasal swabs were taken from (i) ambulatory patients attending general and ENT services; (ii) patients at four main internment services; (iii) hospital staff.
2 *Screening of methicillin-resistant staphylococci.* Staphylococci were selected in mannitol-salt media with oxacillin disks (1 μg). Individual colonies were further tested for coagulase production or clumping factor. Staphylococcal identification was performed by ITS-PCR, *mecA* detection by PCR and oxacillin resistance was confirmed by disk diffusion.

Results: A total of 3890 staphylococcal isolates were recovered from 2806 individuals attending the two ambulatory services. Carriage rates (%) were 81 for MSCNS, 49 for MRCNS, 17 for MSSA and 0.05 for MRSA. From 353 patients of the four internment services, 555 samples were obtained: 89 patients were screened at admission only (<48 h) and 264 were also followed during internment. Carriage among patients at admission and during internment was, respectively, 85 and 60% for MSCNS, 72 and 85% for MRCNS.

MSSA carriage was identical in both groups (9%) and MRSA was detected in 2% of the inpatients. MRCNS carriage among the 99 healthcare workers sampled was also high (80%) but only two MRSA carriers were isolated. Contact with hospital and age were identified as risk factors for colonization by MRCNS. A representative sample of 39 strains from 34 patients from orthopedics was analyzed for the presence of *mecA* and species identification. Out of 39 *mecA* + MRCNS isolates, 21 were *S. haemolyticus* and 16 *S. epidermidis*; we found that carriage of *S. haemolyticus* increased with the length of the internment period whereas the opposite was observed for *S. epidermidis*.
Conclusions: Infection by MRSA or MRCNS was very low (0.9%) in contrast with the high frequencies reported for Portuguese hospitals. This difference is probably due to the characteristics of this health institution. A low frequency of MRSA carriage was also found among both the community and inpatients whereas a high rate of MRCNS carriage was detected amongst all study populations. This evidence together with the capacity of prolonged carrier status may be challenging since it constitutes a reservoir of drug-resistant bacteria inside and outside the hospital.

P855 Molecular characterization of methicillin-resistant *Staphylococcus epidermidis* (MRSE) clones: evidence of geographic dissemination

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Objectives: The aim of this study was to: (i) identify the MRSE clones circulating in one central hospital in Iceland and five hospitals in Greater Copenhagen, Denmark and (ii) compare the MRSE clones from Iceland and Denmark with representative MRSE strains isolated in hospitals from other countries in Europe, South America and Africa, in order to track the geographic spread of MRSE clones.
Methods: The MRSE isolates were tested for mannitol fermentation and coagulase production and identified by Internal Transcribed Spacer PCR (ITS-PCR). Antimicrobial susceptibility was determined by disk diffusion method (NCCLS). The *mecA* gene was detected by hybridizing the SmaI digests with a DNA probe internal to the *mecA*. The molecular typing of isolates from Denmark ($n = 136$) and Iceland ($n = 94$) collected in 1997 and 1998 was performed by pulsed-field gel electrophoresis (PFGE) and visually compared with the patterns obtained from a sample of MRSE from Greece ($n = 34$), Uruguay ($n = 10$), Mexico ($n = 74$) and Cape Verde ($n = 48$). Cluster analysis was performed using Dice similarity metric and single linkage amalgamation scheme. The representativity of the dendogram was verified by determining the cophenetic coefficient. A test of differences between proportions ($P < 0.5$) was performed to analyze possible relations between the different clones and the clinical data.
Results: The 230 MRSE strains from Denmark and Iceland were classified in 40 different PFGE patterns. Twelve PFGE types were common to both countries and five (A, B, C, G and K) accounted for the majority of strains. Three PFGE types were also shared by isolates recovered in Denmark (type A and C), Iceland (A, GG and C), Mexico (GG), Uruguay (GG), Greece (A, GG and C) and Cape Verde (GG). The cluster analysis reflected the correctness of our visual PFGE pattern analysis considering a similarity distance of 78% and the cophenetic value of 0.84 validated the dendogram representation. The PFGE type K was found to be associated with an infection origin ($P < 0.05$).
Conclusions: PFGE met our aims for molecular characterization of the MRSE strains, identifying the major clones as well as the diversity among them. Notably, some multiresistant MRSE clones appear to be highly geographically disseminated. This suggests that in the future it may be necessary to establish infection control procedures directed at MRSE in order to prevent infection with multiresistant endemic/epidemic hospital flora.

P856 Detection of oxacillin-resistance in coagulase-negative staphylococci

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Objectives: To study the effect of lowering the breakpoint for oxacillin for resistance from 4 to 0.5 mg/L to improve sensitivity for the detection of oxacillin-resistant coagulase-negative staphylococci (NCCLS, 1999).
Methods: We tested 142 consecutive clinical isolates from primary sterile sites and urine using a custom-made microbroth-dilution assay (Merlin Micronaut) and Vitek 2 AST-P515 cards (bioMérieux) to determine minimum inhibitory concentrations (MIC). *mecA*-PCR served as gold standard.
Results: The prevalence of oxacillin-resistance was 53.5% as determined by *mecA*-PCR. Merlin Micronaut detected 98.6% (91.9–99.9; confidence interval 95%), Vitek 2 97.4% (90.0–99.5) of *mecA*-positive isolates. At MICs ≥ 4 mg/L 100% of isolates were *mecA*-positive and at MICs ≤ 0.25 mg/L 98.3% of isolates were *mecA*-negative. The rate of false-resistance was 10.6% (4.7–21.2) for Merlin Micronaut and 12.1% (5.7–23.0) for Vitek 2. *S. saprophyticus* and *S. lugdunensis* were over-represented in the false-resistant group.
Conclusions: The new breakpoint for oxacillin of 0.5 mg/L yields high sensitivity. Vitek 2 and Merlin Micronaut are equivalent. The relatively high rate of false-resistance may be addressed by testing all isolates with MICs from 0.5 to 4 mg/L with specific secondary assays.

P857 Rapid entrapment of vancomycin in dense cultures of slime-producing *Staphylococcus epidermidis*

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Methods: Slime-producing *Staphylococcus epidermidis* ATCC 35984 at different inocula (10^5, 10^6, 10^7, 10^8 and 10^9 cfu/mL) were exposed to vancomycin (0.5–16 mg/L). The bacterial numbers during the 24 h drug exposure were determined with bioluminescence assay of bacterial ATP. Control experiments included heat-treated bacteria. An agar well bioassay was used for the determination of vancomycin in the broth during drug exposure. Samples were centrifuged and filtered before assay.
Results: At low inocula (10^5 cfu/mL) the bacteria were inhibited at 2 mg/L. At a dense inoculum (10^8 cfu/mL) a fourfold higher concentration (8 mg/L) were needed for growth inhibition. A dense inoculum (10^8 cfu/mL) reduced the vancomycin concentrations after 30-min incubation approximately 50% in cultures exposed to 1–8 mg/L. After 3 h incubation the concentrations in cultures containing an initial concentration of 1–4 mg/L were below the detection limit of the bioassay (0.5 mg/L). Control experiments with non-growing (10^9 cfu/mL) and dead bacteria (heat-treated) showed similar results.
Conclusion: The decrease of vancomycin concentrations in cultures containing large numbers of dead, nongrowing and growing cells is probably due to binding of vancomycin to cells and cell debris. This entrapment may be an explanation for treatment failure of staphylococcal infections with high densities of bacteria (e.g. abscesses, foreign body infections and endocarditis).

P858 Effects of antibiotics on slime-producing *Staphylococcus epidermidis* adhered to pacemakers

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Objectives: Bacteria adhered to foreign bodies are often more resistant to antibiotics than bacteria of the same strain growing planktonically. Some strains also encapsulate themselves in slime consisting of extracellular biopolymers. The aim of this study was to investigate the biofilm formation on pacemakers and the antibacterial effect by rifampicin alone and combined with ofloxacin on a slime-producing strain of *Staphylococcus epidermidis*.
Methods: *S. epidermidis* ATCC 35984 was adhered to Microny® pacemakers (St Jude Medical) in supplemented Mueller Hinton Broth (MHB) for 24, 48, 72, 96 and 120 h, respectively. Adherence and slime production were studied in an environmental scanning electron microscope (ESEM). The adhered bacteria were washed and exposed to rifampicin (8 mg/L) alone or in combination with ofloxacin (16 mg/L) for 20 h at 37 °C. The bacterial numbers on the pacemakers were determined by bioluminescence assay of intracellular bacterial ATP after extraction of ATP in boiling Tris/EDTA-buffer.
Results: After 24-h incubation of the pacemakers in broth containing a start inoculum of 1×10^4 cfu/mL ESEM showed adhered bacteria. The intracellular ATP level on these pacemakers was 2.0×10^{-9} M ATP corresponding to 2.0×10^9 bacteria. After further 24 h incubation of the pacemakers in fresh broth ESEM showed adhered slime-producing bacteria. The intracellular

ATP level on these pacemakers was 2.6×10^{-8} M ATP corresponding to 2.6×10^{10} bacteria. The minimal inhibitory concentrations (MICs) of ofloxacin and rifampicin were 0.125 and 0.008 mg/L, respectively. During treatment of the pacemakers with rifampicin alone development of rifampicin resistance occurred with a MIC increase to >256 mg/L. Development of rifampicin resistance was prevented when rifampicin was combined with ofloxacin. The reduction of intracellular ATP on pacemakers with bacteria adhered for 24 h was 1.4 $\log_{10}$ (meaning 101.4) after exposure to rifampicin in combination with ofloxacin. After the same treatment of the 48 h slime-producing bacteria corresponding figure was 0.87 $\log_{10}$.

Conclusions: This study shows that ofloxacin can prevent development of rifampicin resistance. Furthermore the bactericidal effect was more pronounced on adhered bacteria prior slime production. This indicates the importance to assess the age of the biofilm and whether slime production occurs or not in models studying antibacterial effects on bacteria adhered to foreign bodies.

P859 Synergy of glycopeptides and β-lactams against teicoplanin-resistant *Staphylococcus haemolyticus*

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Objectives: Synergy between glycopeptides (GPs) and β-lactams has been reported in staphylococci. The purpose of this study was to compare the effectiveness of the association of GPs and β-lactams against *S. haemolyticus* strains susceptible or resistant to teicoplanin (TE) and methicillin.

Methods: Four isogenic pairs of TE-susceptible/resistant strains of *S. haemolyticus*, two (1S/1R and 2S/2R) *mecA*-negative and two (3S/3R and 4S/4R) *mecA*-positive, were used. Resistant derivatives were obtained from susceptible clinical parents by exposure to GPs. MICs were determined by broth microdilution. Drug combinations were tested by disk diffusion and checkerboard assays. Autolytic activity was determined by a turbidimetric assay.

Results: Compared with the TE-susceptible parents, all resistant derivatives exhibited an 8–16-fold increase in TE MICs and no variation in vancomycin (VA) and oxacillin (OX) MICs; penicillin (PE) MICs dropped by >64 and 8-fold in the two *mecA*-negative derivatives (1R and 2R, respectively), but remained unchanged in the two *mecA*-positive derivatives. Ampicillin (AM) MICs remained unchanged in all derivatives with the exception of 1R (32-fold decrease). Combinations of TE or VA with OX, PE, or AM were synergistic against all parents and derivatives; however, a paradoxical effect, associated with reduced autolysis, was observed with some β-lactam concentrations. Synergy was most evident against the *mecA*-positive TE-resistant derivatives. The latter strains, in agar plates containing TE, showed a double zone around PE and AM disks, and an incomplete zone around OX disk. By population analysis, heterogeneous or homogeneous resistance to TE associated with homogeneous susceptibility or heterogeneous resistance to VA was observed.

Conclusion: Synergy between GPs and β-lactams was particularly evident against *mecA*-positive TE-resistant derivatives of *S. haemolyticus*. The double zone shown by these strains around PE and AM disks and the incomplete zone around OX disk appeared to correlate with OX resistance rather than with a heterogeneous phenotype of GP susceptibility. A reduced autolytic activity in the presence of particular concentrations of β-lactams could be responsible for the paradoxical effect and the double zone around PE and AM disks.

P860 The relationship between antibiotic susceptibility and pathogenicity of staphylococci isolated from clinical samples

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Introduction: In this study, we aimed to investigate the relationship between antibiotic susceptibility and pathogenicity of staphylococci isolated from clinical samples. Pathogenicity criteria including slime production, crystal violet reaction were investigated.

Methods: In this study, total 200 staphylococci strains were isolated from various clinical samples for control groups 64 staphylococci strains and 56 staphylococci strains were obtained from nasal culture of hospital personals and nasal swabs from persons who had no relation with the hospital. Congo–Red Agar method was used to investigate slime production. Bacterial strains were studied for crystal violet reaction on a nutrient agar medium containing one part in 100 000 crystal violet. Antibiotic susceptibility testing was performed using agar dilution method as described by the National Comittee for Clinical Laboratory standards.

Results: Out of 200 staphylococci strains isolated 117 (58.5%) were identified as coagulase-negative *Staphylococcus* (CNS) and 83 (41.5%) *Staphylococcus aureus* (68 MRCNS, 49 MSCNS, 48 MRSA, 35 MSSA). Slime production was detected in 14.3% of MRCNS and 29% of MSCNS. 76 of *S. aureus* strains gave positive crystal violet reaction which is composed of 93.8% MRSA, 88.6% MSSA were determinated. No statistically significant difference between crystal violet reaction (CVR), slime production and/or methicillin resistance ($P^{®}$ 0.05). All of the strains were susceptible to vancomycin whereas 0.5% of pathogenic strains were resistance to teicoplanin. The antibiotic resistance ratio of slime-producing CNS was summarized like this: isepamicin 0.9%, cefepim 0.9%, ampicillin–sulbactam 0%, tetracyclin 5.1%, erythromycin 7.7%, rifampicin 6.8%. *S. aureus* strains with positive crystal violet reaction have shown antibiotic resistance like this: isepamicin 2.6%, cefepim 9.2%, ampicillin–sulbactam 10.5%, tetracyclin 13.2%, erythromycin 13.2%, rifampicin 13.2%.

Conclusions: Our results showed that positive crystal violet reaction was reliable marker for pathogenecity of *S. aureus* and slime production was an important marker for pathogenecity of CNS strains However, there has been no relationship observed between antibiotic resistance and/or crystal violet positive *S. aureus*, slime-producing CNS strains.

P861 Identification of *Staphylococcus* spp. in blood cultures by a rapid DNA probe test

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Objectives: To evaluate sensitivity and specificity of 'Accuprobe *Staphylococcus aureus* probe' (Gene Probe Incorporated, USA) for direct, rapid identification of staphylococci (*S. aureus* vs. coagulase-negative strains) from blood cultures bottles *Staphylococcus* positive at the microscopic smear.

Methods: A total of 70 blood cultures broths were tested by traditional techniques (direct coagulase test, culture onto Blood Agar plates, followed by identification with API Staph, bioMérieux and Crystal GP, BD) and by 'Accuprobe *Staphylococcus aureus* probe'. Accuprobe uses a chemiluminescent DNA probe that detects specific rRNA sequences of the target microrganism. Two centrifugation steps were done before the Gene Probe procedures. The results of the test are based on the following cut-off values: *S. aureus* > 1500 AccuLDR, coagulase-negative staphylococci < 1200 AccuLDR. Samples included in the 'repeat range' (1200–1499 AccuLDR) were re-tested, utilizing a preliminary additional wash with saline and ultrapure water.

Results: Ten/70 samples were positive for *S. aureus* (range: 3911–11012 AccuLDR) and 60 for coagulase-negative staphylococci (range: 87–1166 AccuLDR) using Accuprobe *Staphylococcus aureus* probe. Cultures confirmed the Accuprobe identification. Three samples were in the 'repeat range' (AccuLDR: 1201, 1210 and 1362, respectively), but after the additional wash test results confirmed the coagulase-negative staphylococci growth.

Conclusions: *Staphylococcus* sp. is the most frequent microrganism from blood: *Staphylococcus aureus* is 7.8% and coagulase-negative staphylococci are the 43.4% of our blood cultures isolates. It is important the rapid identification of the species level of *Staphylococcus*, considering the clinical role of *S. aureus* and the doubtful significance of coagulase-negative strains. Our data confirm that Accuprobe *Staphylococcus aureus* probe is characterized by sensitivity and specificity values of 100.0%. This test is time-saving (1 h in a routine activity) versus traditional techniques (coagulase test: several hours to overnight), if done directly from broth. This method is an excellent method for preliminary testings permitting a more rapid clinical management.

P862 A comparison of phenotype and genotype methods for the identification of coagulase-negative staphylococci

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Objectives: As coagulase-negative staphylococci (CNS) have become increasingly recognized as clinically significant pathogens, the need for them to be

identified to species level occurs with greater frequency than before. Phenotypic methods have largely been used in the past, but problems such as variable accuracy with test substrates and subjectivity exist. Genotypic methods have now been developed to speciate CNS. It is the aim of this study to compare and evaluate three phenotypic methods, with two genotypic methods, for their ability to identify CNS.
Methods: A collection of 99 isolates comprising 48 clinical isolates and 51 reference isolates, were identified using three phenotypic methods (API Staph, ID 32 Staph and an in-house method) and two genotypic methods: the 16S-23S Spacer Length PCR (SL-PCR) of de Baere et al. (Abstract 1573, ICAAC 1999) and the eight CNS species-specific primers of Gribaldo et al. (1997, J. Med. Micro. 45: 45–53). The species-specific primer PCR method (SS-PCR) was regarded as the 'gold standard' and all other methods were evaluated against it.
Results: The SL-PCR identifications corresponded to those obtained with the SS-PCR method. One isolate could not be identified by SS-PCR but produced a unique pattern by SL-PCR. Intra-species pattern variations were observed with SL-PCR, however, these were easily distinguishable from interspecies patterns. The phenotypic methods identified the CNS with varying degrees of accuracy: API Staph (84%); ID 32 Staph (81%); In-House Method (58%).
Conclusions: Genotypic methods were shown to be superior to the phenotypic methods employed in this study for the identification of CNS to species level. Problems remain with the ability of each of the phenotypic methods to identify CNS. The SL-PCR was demonstrated as an excellent alternative to SS-PCR. It is more cost effective than SS-PCR requiring only a single PCR amplification, compared to the seven required for SS-PCR. In addition, SL-PCR can identify species for which there are no species-specific primers available.

P863 Teicoplanin in outpatient/home parenteral antibiotic therapy (OHPAT): the Dundee experience

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Objectives: To describe the experience of using teicoplanin by a well-developed United Kingdom OHPAT service.
Methods: Basic demographic and clinical data about all patients who had received teicoplanin as part of OHPAT were acquired from the Dundee OHPAT database (1998–2001). Microbiological results and teicoplanin levels were ascertained from the computerized laboratory results service.
Results: One-hundred and five (105) patient-episodes were identified in 98 patients (63% male). The most common infections were bone or joint (including prosthetic) (53%), skin or soft tissue (34%) and endocarditis (10%). Fifty-three patients (50%) had positive microbiological specimens, of which 41 (39%) were from bone or joint tissue/pus or blood culture. The most commonly cultured organisms were methicillin-resistant *Staphylococcus epidermidis* (32%), MRSA (24%) and MSSA (16%). At predischarge outpatient review, 58% of patients were deemed to have been cured and 41% to have improved. One patient died, but this was unrelated to the underlying infection or therapy. Five (5%) patients are known to have subsequently relapsed requiring further intervention. Adverse events requiring cessation of therapy occurred in only two (2%) patients. Five (5%) patients, however, required a PICC line change due to catheter blockage.
Conclusions: Teicoplanin appears to be a safe and efficacious antimicrobial when used as OHPAT.

Resistance in Gram-positive bacteria

P864 High-level glycopeptide-resistant *Enterococcus faecalis*: a second isolate from Turkey

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Objective: We aimed to present a high-level glycopeptide-resistant *Enterococcus faecalis* strain which is the second isolate from Turkey.
Methods: Patient from whom the bacteria was isolated, was transferred to our hospital with the diagnosis of acute abdomen + pelvic peritonitis and sepsis. After the pelvic abscess operation, patient was given many antibiotics including teicoplanin for 2 months. Strain isolated from two blood cultures of this patient was identified as *Enterococcus* by classical methods. API 20 STREP and VITEK (BioMerieux, France) were used for the analysis of species. By these systems, strain was identified as *E. faecalis*. Susceptibility of antibiotics were evaluated by disk-diffusion, agar dilution and E-test (AB Biodisk, Solna, Sweden) methods. Strain was tested for all antibiotics used for enterococci suggested by NCCLS.
Results: Isolated strain was found sensitive to ampicillin and nitrofurantoin. Minimal inhibitory concentration (MIC) level was 256 mg/mL both for vancomycin and teicoplanin. *VanA* gene responsible for high-level glycopeptide resistance was shown by PCR. High level resistance to aminoglicoside was also detected. β-Lactamase activity was shown negative with nitrocefin.
Conclusion: High-level resistant *E. faecalis* to glycopeptide was firstly isolated in our hospital. It should be noticed that *E. faecalis* can cause a problem both in therapy and in prevention of its spreading. Therefore, investigation of glycopeptide resistance of isolated enterococci is especially suggested in routine bacteriologic tests.

P865 Characterization of a novel gene *aac(6′)* from *Enterococcus hirae*

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The objective was to characterize a new *aac(6′)* activity in *Enterococcus hirae* strains. In this study, 10 *E. hirae* strains were included. They were identified by biochemical and molecular methods and were probed to be unrelated by PFGE-*Sma*I analysis. All strains were susceptible to penicillin (MIC: 0.5–4 μg/mL) and none of them showed high-level aminoglycoside resistance. Synergistic studies were performed by time–kill curves using different aminoglycosides and penicillin combinations. All strains showed synergy when streptomycin or gentamicin were combined with penicillin, but not when penicillin was combined with tobramycin or kanamycin. A typical *aac(6′)* activity, that modified tobramycin, kanamycin and netilmycin but not 6′-netilmycin, was demonstrated by the radioenzymatic assay method. Negative results were obtained when presence of *aac(6′)-Ii* gene was tested by PCR or by dot blot hybridization with an *aac(6′)-Ii* internal probe. Degenerated primers were designed based in the comparison of four different *aac(6′)* genes, that yielded a 240-bp PCR product with all these strains. Uniform DNA sequences were obtained from amplicons of three of the *E. hirae* strains that were analyzed. The deduced amino acid sequence from PCR products showed an identity of 61% with *aac(6′)-Ii* from *E. faecium*.
Conclusion: An *aac(6′)-Ii* activity was detected in *E. hirae* strains. This enzyme does not confer high-level tobramycin or kanamycin resistance, but inhibit the synergistic effect between these aminoglycosides and penicillin. A novel *aac(6′)* gene, that showed an identity of 61% with *aac(6′)-Ii* from *E. faecium*, has been characterized.

P866 Molecular characterization of *van* genes related to vancomycin-resistant enterococci isolated from hospital infections in Brazil

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Enterococcus spp. is part of the intestinal normal flora, together with close to 100 other species of aerobic and anaerobic bacteria. Initially, the enterococci were considered as being low-virulent microorganisms, but the rapid emergence and dissemination of vancomycin-resistant enterococci strains (VRE) has completely changed the clinical relevance of such pathogens. At present, VRE is an important pathogen related to hospital infections in many countries, presenting limited or no therapeutic options for treating serious infections. The present study was initiated to investigate the prevalence of *van* genes among clinical VRE strains related with a possible outbreak (under investigation) that occurred at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP). The samples were isolated from 48 different patients. Five strains were identified as *Enterococcus*

faecium and 43 as *E. faecalis*. The oligonucleotide primers directed to *vanA*, *vanB* and *vanC* genes in enterococci and the polymerase chain reaction (PCR) technique applied were the same as previously described by CDCs Antimicrobial Resistance Working Team (Clark et al., 1998; Tenover et al., 1998). The results showed that all strains tested presented the *vanA* gene and produced the expected 1030 bp amplicon with *vanA* primers. No strains with *vanA* gene showed positive PCR results when tested with *vanB* or *vanC* primers. As all strains in our study presented the *vanA* gene, there is a potential high risk for spreading into different hospitals. Consequently, restricted infection control measures to prevent spread of VRE have been implanted since then. The HCFMUSP is a large complex tertiary hospital with 1500 beds that attends seriously ill patients with high risk for acquiring hospital infections. This fact increases the risk of the dissemination of VRE. Besides that, as enterococci are enteric bacteria and the intestinal lumen offers an environment for bacterial conjugation, the dissemination of *vanA* gene could occur among other enterococci species and also to other bacteria genus bringing an important issue to be monitored and solved by the hospital infection control team and the microbiology laboratory.

P867 Characterization of teicoplanin-susceptible and teicoplanin-resistant *Staphylococcus aureus* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and peptidoglycan muropeptide analysis

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Background and purpose: Teicoplanin (Tec) is a glycopeptide antibiotic that inhibits the polymerization of peptidoglycan (PG) glycan chains, and hence the crosslinking of its muropeptides. Glycopeptide-resistant (r) *Staphylococcus aureus* isolates emerge under therapy with Tec. Unexpectedly, Tec-r subpopulations also emerged in the tissue-cage fluid of rats infected with *S. aureus*, never exposed to this drug (J. Antimicrob. Chemotherapy 2001; 47:163–70). The present study used two different techniques to analyze perturbations of the cell wall present in such Tec-r subpopulations. One examined the ionizable components on the surface of intact bacterial cells by MALDI-TOF MS. This provides information on global modifications of cell surface components. The other analyzed the PG muropeptide pattern after digestion of their glycan chains, a method generating structural information on the PG scaffold skeleton.
Methods: The Tec-sensitive parent (isolate MRGR3) and its stable Tec-r variant recovered from tissue-cage fluids (isolate 14–4) were studied. MALDI-TOF MS was performed directly on colonies picked off agar plates, embedded in matrix and the ionisable cell surface components directly analyzed. For muropeptide analysis walls were purified, stripped off their teichoic acids, their glycan chains digested with mutanolysin and separated by HPLC. Eluted muropeptides were detected by UV absorbance.
Results and conclusions: MALDI-TOF MS revealed fingerprints compatible with reference *S. aureus*. However, each isolate contained unique and very reproducible peaks that enabled them to be differentiated. For example, an intense 825 Da peak was present in the Tec-r variant, but was practically absent from the Tec-sensitive parent. These results indicate that Tec-sensitive and Tec-r variants differed in some surface components that affected their ionization. On the other hand, both strains revealed indistinguishable muropeptide patterns that were compatible with published *S. aureus* PG. Thus, although surface alterations were present in the Tec-r variant, they were not related to an abnormal PG skeleton. The combination of these two techniques is an elegant new strategy to help determine the localization of wall alterations in different bacterial variants. The ionizable nature of the altered molecules and the fact that they are not associated with an altered PG scaffold should help target further investigations on other surface components such as teichoic acids or proteins.

P868 Emergence of a teicoplanin-resistant small colony variant of *Staphylococcus epidermidis* during vancomycin therapy

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Basel, CH

Small colony variants (SCVs) of *Staphylococcus aureus* have been reported to cause persistent and relapsing infections. Little is known about SCVs of coagulase-negative staphylococci.

Objective: To characterize a teicoplanin-resistant SCV of *S. epidermidis* that developed in an immunocompromised patient during vancomycin therapy for blood stream infection (BSI).
Methods: Bacterial isolates were identified based on their biochemical profile with the API Staph (bioMerieux). Susceptibility testing was performed according to NCCLS guidelines by broth microdilution. Resistance to methicillin was confirmed by detection of penicillin binding protein 2′ (MRSA-Screen, Denka Seiken Co.). MICs of teicoplanin and vancomycin were determined by E-test methodology (AB Biodisk). Molecular typing was performed by pulsed-field gel electrophoresis (PFGE).
Results: On day 1 and day 2, *S. epidermidis* was isolated from blood cultures of a patient with acute myeloic leukemia undergoing chemotherapy. The isolates were resistant to methicillin and susceptible to both vancomycin and teicoplanin. On day 3, the patient was treated for catheter-related BSI with vancomycin including a vancomycin-lock. Blood cultures taken 1 h before vancomycin-lock grew again teicoplanin-susceptible *S. epidermidis*. From blood cultures taken 11 h after vancomycin-lock, *S. epidermidis* with the same susceptibility profile was isolated. In addition, a methicillin-resistant SCV of *S. epidermidis* was detected which was susceptible to vancomycin (MIC, 3 mg/L) but resistant to teicoplanin (MIC, 24 mg/L). The intravenous catheter was replaced on day 5. Despite therapy with vancomycin plus other antibiotics, teicoplanin-resistant SCVs were repeatedly isolated until day 15 and teicoplanin-susceptible large colony forms were isolated until day 21. The patient died on day 23. Typing with PFGE showed that all isolates belonged to the same clone.
Conclusions: A teicoplanin-resistant SCV of *S. epidermidis* developed rapidly during vancomycin therapy and persistent *S. epidermidis* BSI. Small colony variants of coagulase-negative staphylococci can cause serious and persistent infection. Due to their slow growth and their tendency to revert to the large colony form they can easily be missed in culture.

P869 Competition between antibiotic sensitive and resistant coagulase-negative staphylococci (CoNS) on skin

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Objectives: To study how CoNS, with resistance to fusidic acid, ciprofloxacin and streptomycin compete with its isogenic sensitive strain on human skin.
Methods: A CoNS was isolated from a healthy person, and a rifampicin-resistant (rifR) clone was selected. The rifR marker was used to distinguish between the inoculated strains and the resident CoNS strains. The rifR strain was used to select for mutants resistant to fusidic acid, ciprofloxacin and streptomycin. The rifR strain was mixed 1:1 with each double-mutant strain (fusR, cipR and smR). The mixture was applied on the forearms of 18 volunteers. Samples were taken on day 1, 3 and 10, and cultured on blood agar plates containing 50 mg/L rifampicin. Colonies were counted and replicated to selective plates containing fusidic acid, ciprofloxacin or streptomycin.
Results: The ciprofloxacin- and streptomycin-resistant strains had a similar biological fitness as its isogenic sensitive strain. During 10 days the ratio varied for cipR/cipS between 1 and 0.65 and smR/smS 1.3–0.76. The fusidic acid resistance caused a clear fitness loss. The ratio fusR/fusS day 1, 3 and 10 was 0.60, 0.13 and 0.23, respectively. The fusR-strain had the same generation time as fusS in broth, but in the natural environment the fusR mutation became detrimental.
Conclusions: Resistance to ciprofloxacin and streptomycin in CoNS did not confer a biological cost, and the bacteria had the same ability to survive on the skin as the sensitive isogenic strains. Resistance to fusidic acid was a disadvantage for the bacteria and the resistant bacteria could not compete with the sensitive strain in the natural environment.

P870 PFGE-determined clonal linkages of fusidic acid resistant (FusR) versus sensitive (FusS) *Staphylococcus aureus* (SA)

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Objectives: To test skin and soft tissue isolates of SA for sensitivity to fusidic acid.
Methods: SAs ($n=255$) which caused skin and soft tissue infections in outpatients were tested. E-test was used for MIC determination, nitrocefin test for β-lactamase expression, and PFGE (*SmaI*) for testing of genotypes.

Results: FusR (MIC 1 mg/mL) was shown by 83/255 (32.5%) of the SAs. Of the FusR SAs, 80/83 (93%) produced β-lactamase versus 114/172 (66%) of the FusS SAs. Among 24 FusS strains tested by PFGE, 16 different patterns were recorded without dominance of any given pattern. Among 48 FusR SAs tested, 11 different patterns were recorded and 36/48 (75%) of these strains produced identical or nearly identical patterns.
Conclusion: FusR was more common among the SAs than we had expected. This was largely due to spread in the population of SAs which exhibited similar PFGE patterns, probably a particular clonal lineage of the FusR SAs.

P871 In vitro activity of daptomycin against staphylococci isolated in Europe during 2000–2001

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Objectives: *Staphylococcus aureus* (SA) and coagulase-negative staphylococci (CNS) resistant to oxacillin (OX) are prevalent in many countries. Daptomycin (DAP), a novel cyclic lipopeptide currently in Phase III clinical development, has been reported previously to possess in vitro bactericidal activity against OX-resistant SA (ORSA), multidrug-resistant (MDR; resistant to ≥3 antimicrobial classes) SA and CNS. The current study surveyed DAP activity in Europe and provided a baseline to monitor the activity of DAP against both susceptible and resistant (R) organisms.
Methods: During 2000–2001, 1222 SA and 1040 CNS were collected from 40 laboratories in 15 European countries. All isolates were centrally tested by NCCLS broth microdilution against OX, vancomycin, teicoplanin, erythromycin (ERY), clindamycin (CLI), gentamicin (GEN), ciprofloxacin (CIP), quinupristin–dalfopristin (Q–D), linezolid (LZD), cotrimoxazole (COT), and DAP.
Results: Overall percent resistant (R) rates for SA and CNS were ERY (34.1, 49.9), OX (27.3, 53.3), CIP (30.6, 31.6), CLI (19.0, 22.0), and GEN (14.6, 31.4). At the national level, OX resistance ranged from 4.0% in the Netherlands to 36.9% in Portugal. MIC_{90} (mg/L) for the new directed-spectrum agents against all SA and CNS were DAP (0.5, 0.5), Q–D (0.5, 0.25), and LZD (2, 2). Against ORSA, DAP and Q–D were the most active agents, based on MIC_{90} (0.5 mg/L) compared with LZD (2 mg/L). Among the SA tested, 301 (24.6%) were MDR with resistance to OX, CIP, and ERY being the most common phenotype (44.2% of MDR isolates). DAP MIC_{90} (mg/L) against SA were 0.25 for non-MDR isolates and 0.5 for MDR isolates. Against OX-R CNS, Q–D and DAP were the most active agents, based on MIC_{90} (0.25 and 0.5 mg/L, respectively) compared with LZD (2 mg/L).
Conclusion: ORSA and OX-R CNS are prevalent in many countries in Europe. DAP is a novel antimicrobial that is active against both susceptible and resistant isolates and shows promise for the therapy of infections caused by these organisms.

P872 In vitro activity of daptomycin against streptococci isolated in 15 countries in Europe during 2000–2001

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Objective: The emergence of *Streptococcus pneumoniae* (SP) multidrug resistance (MDR; resistance to ≥3 antimicrobial classes) and *S. agalactiae* (GBS) resistance to erythromycin (ERY) and clindamycin (CLI) is a concern in many countries. Daptomycin (DAP) is a new cyclic lipopeptide agent reported to be active against clinically important Gram-positive pathogens, including SP and GBS, resistant to currently available agents. This study was designed to assess the current activity of DAP against streptococci in Europe and provide a baseline by which to monitor future studies.
Methods: During 2000–2001, 865 SP and 367 GBS were collected from 40 hospitals in 15 European countries. Isolates were tested by NCCLS broth microdilution against DAP, PEN, cefuroxime (CEF), other β-lactames, vancomycin, ERY, CLI, levofloxacin (LEV), linezolid (LZD), quinupristin–dalfopristin (Q–D) and cotrimoxazole (COT).
Results: Overall resistance rates (%) among SP were ERY (24.4), COT (18.9), CLI (18.0), CEF (18.2), PEN (9.4) and LEV (0.6). At the national level, PEN resistance in SP ranged from 0% in the Netherlands to 20.7% in Portugal. Based on MIC_{90}, DAP was the most active agent tested against SP (0.25 mg/L). The prevalence of MDR in SP was 3.6% (31 isolates) with resistance to ERY, PEN and COT being the most frequently encountered MDR phenotype (29 isolates; 93.5% of MDR isolates). PEN-resistant SP were cross-resistant to many other agents including other β-lactames, ERY, CLI and COT. The activities of DAP, QD and LZD were not affected by PEN resistance with MIC_{90} (mg/L) of 0.25, 0.5 and 1, respectively, against both PEN-susceptible and -resistant isolates. DAP was also equally active against both MDR and non-MDR SP. Among GBS, resistance rates (%) were ERY (10.4), CLI (6.5) and LEV (0.3). The MIC_{90} (mg/L) for DAP, Q–D and LZD against GBS were 0.25, 0.25 and 1, respectively.
Conclusions: Antibiotic-resistant streptococci continue to occur in many European countries, and it is prudent to develop new therapeutic agents to combat these organisms. DAP, one such novel agent, demonstrated potent in vitro activity against both SP and GBS including isolates that were resistant to currently available agents. DAP shows promise for the therapy of infections caused by organisms resistant to other agents.

P873 Antimicrobial susceptibility of 60 *Streptoccocus oralis* isolates

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Objectives: To investigate the antimicrobial susceptibility of 60 *Streptococcus oralis* isolates to seven antimicrobial agents as several studies reported an increased resistance of these bacteria to some commonly used antibiotics. The strains were isolated in pus sample collected from Romanian patients with different oral and maxillofacial (OMF) infections. Correlating the microscopy with the culture results, Streptoccocus oralis was the only microorganism found to be involved in infection in four cases.
Methods: The E-test (AB Bio-Disk, Sweden) was used according to the manufacturer recommendations to test susceptibility of the isolates to the following antibiotics: benzylpenicilin (PG), ampicillin (AM), cefotaxime (CT), erythromycin (EM), clindamycin (CM), chloramphenicol (CL) and tetracycline(TC).
Results: The MICs (μg/mL) ranges were as follows: PG 0.016–0.75 (83.3% susceptible, 16.7 intermediate susceptible), AM 0.016–0.5 (95% susceptible, 5% intermediate susceptible), CT 0.016–0.38 (100% susceptible), EM 0.016–3 (91.7% susceptible, 5 intermediate susceptible, 3.3% resistant), CM 0.016–0.047 (100% susceptible), CL 0.5–4 (100% susceptible) and TC 0.047–64 (48.3% susceptible, 11.7% intermediate susceptible, 40% resistant).
Conclusions: (1) The resistance found to some antimicrobial agents recommends periodical susceptibility testing of *S. oralis* and other oral streptococci isolates of clinical significance; (2) CM or CL might be alternatives to the treatment of OMF infections involving oral streptococci with reduced sensitivity to β-lactam antibiotics.

P874 Effects of *Helichrysum italicum* extracts on growth and in vitro adherence of cariogenic streptococci

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Objective: Phytochemicals have recently been shown to be a good alternative to synthetic chemical substances for caries prevention, one of the most ubiquitous disease in the modern society. The purpose of the present study was to examine the susceptibility of cariogenic streptococci to *Helichrysum italicum*, a plant rich in flavonoids and terpenes with antinflammatory, antiallergic and antierythematosus activity, for future usage as antimicrobial agent in oral hygiene formulations.
Methods: We tested the activity of various extracts of *H. italicum* (infusion, aqueous macerate, diethyl ether and ethanolic extract) against *Streptococcus mutans* ATCC 35668, *S. salivarius* ATCC 13419, *S. sanguis* ATCC 10556 and the effect on adherence to smooth glass surface of *S. mutans*. Antimicrobial activity was determined by the disc diffusion test, MIC and MBC. To study the influence on adherence, the organisms were grown for 18 h at 37 °C in BHI broth containing 2% sucrose and various concentrations of the extract, in test tubes inclined at 30° angle. The bacteria adhering to the glass surface were removed by sodium hydroxide 0.5 M, centrifuged and suspended in saline. The adherent and nonadherent bacteria were quantified spectrophotometrically at 540 nm.
Results: The diethyl ether and ethanolic extract inhibited all streptococci tested, showing the largest inhibitory zone (25–30 mm in diameters)

and the best MIC (31.25–62.5 μg/mL) and MBC values (250–500 μg/mL). The infusion and aqueous macerate demonstrated a lower activity. Cell adherence was almost completely inhibited by both the diethyl ether and ethanolic extract at concentration equal to 0.5 and 0.25 MIC.

Conclusion: In conclusion, being the adherence of *S. mutans* to teeth the essential step for caries formation, the data presented in this study suggest that *H. italicum* could be proposed for preventive treatment of dental caries. Research is in progress to evaluate the biological effects of this plant under conditions similar to those existing 'in vivo'.

Community-acquired RTI

P875 Community-acquired pneumonia: analysis of 977 cases in a clinical setting

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Objective: Analyze the clinical characteristics of all the patients with community-acquired pneumonia (CAP) attended in a tertiary hospital during a 3-year period (1998–2000).
Methods: All the patients diagnosed of CAP in the Emergency Department were included. The diagnosis was made on basis to clinical and radiological criteria. The data recorded were: (a) sex, (b) age, (c) seasonal distribution, (d) Fine defined risk-class, (e) time until arrival to the hospital, (f) presence of underlying diseases, (g) admissions, (h) hospital stay, and (i) outcome.
Results: Nine hundred and seventy-seven consecutive patients (525 male, 472 female) with a mean age of 64 ± 19 years were included. Mean age of male with CAP was higher than mean age of female (66 ± 18 vs. 62 ± 20). Seasonal distribution was not homogeneous: the incidence of CAP in summer was lower than in winter. Fine defined risk-class was as follows: I, 254; II, 198; III, 204; IV, 251; V, 60. Mean time until arrival to the hospital was 3.8 ± 3 days. Three hundred and forty-seven patients (35%) had one or more underlying diseases. Five hundred and fifty-seven patients (57%) were admitted; 18 in a ICU. Mean hospital stay was 7.8 ± 4.9 days; it became progressively longer as Fine risk-class increased. Thirty-seven patients (4%) died; 18 in Fine risk-class IV and 19 in Fine risk-class V.
Conclusions: Fine defined risk-class is useful to identify patients with a high risk of death. Mean hospital stay is related with Fine risk-class.

P876 Validation of the pneumonia port prediction rule in HIV-infected patients with community-acquired pneumonia (CAP)

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Objectives: The use of the pneumonia PORT prediction rule has been recommended to evaluate the risk and to determine the site of care in non-HIV-infected patients. The objective of this study has been to validate the use of this rule in patients with HIV infection and CAP.
Methods: A prospective, observational, hospital-based study of consecutive cases of CAP was carried out in 12 hospitals in Andalucia (southern Spain), between July 2000 and September 2001. Patients were stratified in risk classes according to the PORT score, $P < 0.05$ was considered significant.
Results: A total of 408 CAP were included, 394 of which could be stratified in risk groups. In 248, the etiology was bacterial and in 139 nonbacterial. The overall mortality rate was 9.5% (9.5% for bacterial CAP and 9.1% for nonbacterial CAP). Following the PORT prediction rule, the mortality for each risk class for the total of CAP was: group I 4/227 (1.7%), group II 8/79 (10.1%), group III 3/38 (7.8%), group IV 15/42 (35.7%) and group V 5/8 (62.5%), $P < 0.0001$. In the group of bacterial CAP, the mortality of the different groups was: group I 3/147 (2%), group II 3/42 (7.1%), group III 2/24 (8.3%), group IV 7/27 (25.9%) and group V 5/8 (62.5%), $P < 0.0001$.
Conclusion: The pneumonia PORT prediction rule is useful to identify a high-risk population in patients with CAP and HIV infection. However, this prognostic system, if not modified, might not be used to determine the site of care the patients, given the high mortality of the 'low risk' groups.

P877 Etiology of respiratory tract infection in a primary care setting

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Vitoria, Bilbao, E

Objective: The aim of this study is to evaluate the rate of specific pathogens and clinical syndromes associated with upper respiratory tract infection in adults attending Primary Care.
Methods: Design: prospective descriptive study, without intervention.
Setting: Primary Care Setting in the south of Vitoria (Basque Country).
Participants: Subjects aged 25 years and older of a consult of Primary Care with clinical of upper respiratory tract infection (cough, coryza, sore throat or hoarseness with or without fever) in the later 6 days and gave informed consent to participate in the study. The following patients were excluded (women who were pregnant, HIV-positive, patients with coagulopaties and therapy with Sintrom, mental disease, sinusitis, otitis and tonsillitis).
Measurements: Demographic, interval between onset of illness and medical consultation, chronic medical conditions and symptoms were collected when ill. A throat and nasal swabs were collected for viral culture (cell culture and shell vial). Cell lines were observed for cytopathic effect for 10 days. Acute and convalescent sera (15 days after onset of illness) were also collected and examined for viral and *Coxiella burnetii* by complement fixation test, *Chlamydia* spp. and *Mycoplasma* by EIA, and *Legionella* by IFI.
Results: From October 18, 1999 through May 31, 2001, 290 patients participated in the study, with 330 episodes. A total of 146 (44.2%) males and 184 (55.8%) females. The mean age was 49.08 years (range 25–88 years). The interval between onset of illness and medical consultation was 3.16 days. The 26.4% of individuals had chronic medical conditions (cardiac 2.12%, pulmonary 12.72%, diabetes 5.15%, liver disease 3.33%, renal disease 1.21%, anemia 0.9%, immunodeficient 1.81%). The 0.9% of subjects received pneumococcal vaccine and 23% received influenza vaccine. Of these, active smokers 24.5%, had young children 26.1%. The symptom more frequent was cough (77.27%). An etiological diagnosis was established in 118 (35.75%) episodes with 126 isolations (38.18%). Sixty-seven influenza A, 6 influenza B, 15 parainfluenza 1, 4 parainfluenza 2, 5 parainfluenza 3, 9 respiratory syncytial virus, 2 adenovirus, 5 enterovirus, 1 *Chlamydia* spp., 4 *Mycoplasma pneumoniae*, 7 Q fever, 1 *Legionella*. The parainfluenza was more of fall and influenza and RSV more of winter.
Conclusions: Infections were most common in fall and winter months. The pathogen most common was influenza A. The RSV was associated to chronic pathology.

P878 Pneumonia in nursing home residents

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Golnik, SLO

Objectives: RTI are major cause of morbidity and mortality in the elderly. In analysis, we wanted to establish clinical, rentgenologic and microbiological characteristics of pneumonia in nursing home residents.
Methods: Nursing home residents hospitalized in our hospital in year 2000 due to CAP were included into analysis.
Results: Among 367 patients hospitalized in our hospital in year 2000 there were 30 nursing home residents (17 women). Their average age was 82.5 years. A total of 60% of patients had at least two accompanying chronic diseases, most frequently cardiovascular and neurologic diseases. A total of 83% of patients presented with severe clinical picture. In all, 93% of patients had dispnea, 67% cough, 67% elevated body temperature, 47% were confused. The 93% were xypoxemic, had electrolyte disturbances, 67% had elevated urea. Isolation of etiologic agent were rarely successful. We isolated *S. pneumoniae*, *H. influenzae*, anaerobes, Gram-negatives. 63% of patients were treated

with parenteral hydration, 90% with oxygen inhalation, all of them received antibiotic, in 70% amoxycillin with clavulanic acid (AMC) was used. Nine patients died (30%).
Conclusions: Patients were characterized with advanced age, numerous comorbidities, unspecific and severe clinical picture. Mortality is high. Treatment includes effective support measures (hydration, oxygenation) and choice of proper empirical antibiotic-AMC, high generation cephalosporines or new fluoroquinolones.

P879 Community-acquired pneumonia in a medical department: characteristics and clinical management

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Objective: To study the demographic, clinical and laboratory characteristics, as well as the management of patients admitted for a community-acquired pneumonia (CAP) in the medical department of a tertiary hospital.
Methods: A retrospective study of the data of all patients consecutively admitted for a CAP in the period from January 1998 to June 2001.
Results: Admissions for CAP accounted for 6.8% of all admissions during this period. The mean age of the patients was 64.7 ± 17 years. Fifty-two percent were males. Eight percent were residents of a nursing home. Comorbid conditions were present in 71%, the most prevalent of them being chronic pulmonary disease (18%), immunosuppression (17%), cerebrovascular disease (16.5%), diabetes mellitus (8%) and alcohol abuse (6%). The most frequent clinical findings on presentation were: cough 70%, fever 82%, dyspnea 40%, tachypnea 38%, chest pain 24%, positive findings on auscultation 84%. Leucocytosis was present in 62% and hypoxia in 59%. Criteria of sepsis were present in 17% of the patients on admission. Blood cultures were taken from 68%, sputum examination from 29% and serologic studies in 11%. A pathogen was identified in 26%. The most frequent were: *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Mycoplasma pneumoniae* and *S. aureus*. Combination therapy was used as treatment in 51% of the patients. The mean length of hospitalization was 10.2 ± 5 days. Mortality was 7%. Admission to ICU was necessary for 3.6%.
Conclusions: In the majority of the patients with CAP, the laboratory studies did not reveal the pathogen, so as to determine the antimicrobial prescription. Nevertheless, the success rate of the first empirical antibiotic regimen was satisfactory (86%). Half of the patients were treated initially with combination therapy.

P880 Epidemiology of recurrent bacterial pneumonia in AIDS patients in Europe, 1993–1999

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Objectives: To assess the association between recurrent bacterial pneumonia (RBP) as an AIDS-defining illness in the European population and the following data: age, sex, country of residence, risk factors for HIV infection and year of AIDS diagnosis, in order to better characterize the epidemiological characteristics of this clinical condition.
Methods: Data on all adolescent and adult cases of AIDS diagnosed in Europe between 1993 and 1999 were analyzed using the June 2000 update of the European Non Aggregate AIDS Dataset (ENAADS) prepared by the European Centre for the Epidemiological Monitoring of AIDS, Paris, France. ENAADS contains standardized information for each individual AIDS case recorded in the 36 European countries which have a national surveillance system. Odds ratios (OR) and 95% confidence intervals (CI) were computed to assess the association between RBP and age, sex, country of residence, risk factors for HIV infection and year of AIDS diagnosis, using a multiple logistic regression analysis.
Results: A total of 130 208 AIDS cases were diagnosed in Europe between 1993 and 1999. PBR was the AIDS-defining condition in 4765 cases (3.7%). A sharp variation in the frequency of RBP was noted among different geographical areas. RBP was more frequent in eastern (11.8%) than in northern Europe (2.4%). In comparison with heterosexuals, the highest risk for of RBP was seen among intravenous drug users (IVDU) (OR = 2.9) and female sex (OR = 1.4); men who have sex with men (MSM) who were not IVDU had a 40% reduction in the RBP risk. Finally, an upward persisting trend was registered in the frequency of RBP over time, which was apparently not modified by the introduction of HAART.
Conclusions: The frequency of RBP as an AIDS-defining condition is strongly associated with geographic factors and individual risk factors. The persisting upward trend since 1993 deserves a better monitoring of this condition.

P881 Incidence of community-acquired pneumonia in a 10-month period: serological tests

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Madrid, E

The aim of this study was to determinate the incidence of pathogens involved in community-acquired pneumonia in a 10-month period in a 500-bed teaching hospital and to know the severity of the illness caused by them.
Material and methods: During a 10-month period (January–October 2001) a total of 960 serum samples from 730 adult patients were analyzed. A 2-mL aliquots were frozen until the test was performed. The diagnosis of *Mycoplasma pneumoniae* (Mp), Influenzae A and B virus (IV), parainfluenzae virus (PV), adenovirus (AV), syncitial respiratory virus (SRV), *Coxiella burnetti* (Cb), *Chlamydia pneumoniae* (Cp) were carried out by complement fixation in microtiter panels (dilutions ranging from 1/8 to 1/2048). The diagnosis of *Legionella pneumophila* (Lp) was performed by indirect immunofluorescence with a initial screening of 1/64 and further dilutions when positive. A significant result was considered when titers were ≥1/16 for Cb, ≥1/32 for Cp, ≥1/64 for Mp and AV, and ≥1/128 for IV, PV, SRV and Lp or when a four-fold rise in titer was observed.
Results: A total of 442 (46%) samples from 308 patients (42%) were positive for one or more pathogens. From the patients with positive samples, 188 (62%) showed a severe disease and they required admission in the hospital. A total of 246 patient (80%) showed a positive result for a unique pathogen and 62 (20%) for more than one. The distribution of patients with significant results for a unique pathogen was as follow:

- One hundred and twenty-nine were positive for Lp (52.4%), 90 of them required admission (69.7%).
- Thirty-six were positive for IV (14.6%), 21 of them required admission (58.3%).
- Twenty-one were positive for Mp (11.7%), eight of them required admission (27.5%).
- Twenty-eight were positive for PV (11.3%), 21 of them required admission (75%).
- Eight were positive for Cb (3.2%), seven of them required admission (87%).
- Seven were positive for SRV (2.8%), four of them required admission (57%).
- Six were positive for AV (2.4%), three of them required admission (50%).
- Three were positive for Cp (1.2%), 1 of them required admission (33%).

From the 62 patients with a significant result for more than one pathogen, 14 presented a four-fold rise in the titer of at least one of them (three patients had a four-fold rise in the titer of two pathogen). Eleven out of the 14 required admission.
Conclusion: Lp was the pathogen most frequently detected in our hospital followed by IV. Eighty-seven percent of patients with Cb required admission in the hospital. The second more severe disease was produced by Lp. The third pathogen detected was Mp, despite of being an adult population, and 27% of patients infected with Mp required admission.

P882 Role of respiratory syncytial virus in the upper respiratory tract infections in adults

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Objective: The aim of this study was to evaluate the rate of specific pathogens and clinical syndromes associated with upper respiratory tract infection in adults attending Primary Care.

Methods: Design: Prospective descriptive study, without intervention.
Setting: Primary Care Setting in the south of Vitoria (Basque country).
Participants: Subjects aged 25 years and older of a consult of Primary Care with clinical of upper respiratory tract infection (cough, coryza, sore throat or hoarseness with or without fever) in the later 6 days and gave informed consent to participate in the study. The following patients were excluded (women who were pregnant, HIV-positive, patients with coagulopaties and therapy with Sintrom, mental disease, sinusitis, otitis and tonsillitis).
Measurements: Demographic, interval between onset of illness and medical consultation, chronic medical conditions and symptoms were collected when ill. A throat and nasal swabs were collected for viral culture (cell culture and shell vial). Cell lines were observed for cytopathic effect for 10 days. Acute and convalescent sera (15 days after onset of illness) were also collected and examined for viral and *Coxiella burnetii* by complement fixation test, *Chlamydia* spp., and *Mycoplasma* by EIA, and *Legionella* by IFI.
Results: From October 18, 1999 through May 31, 2001, 290 patients participated in the study, with 330 episodes. An etiological diagnosis was established in 118 (35.75%) episodes with 126 isolations (38.18%), 9 were respiratory syncytial virus (RSV). RSV were more frequent of winter. Two (22.22%) males and seven (77.77%) females. The mean age was 59.2 years (range 44–74 years). The interval between onset of illness and medical consultation was 3.22 days. The 44.4% of individuals had chronic medical conditions as systemic sclerosis, CREST and pernicious anemia. 66.6% received influenza vaccine. Of these, active smokers 22.2%, had young children 22.2%. Symptom more frequent were cough and sore throat (100%). Nobody had fever >37.8 °C. Nobody was admitted to hospital and nobody died. RSV was yielded in four patients by culture, in other four patients by serological methods and in one patient by both methods.
Conclusions: RSV, in adult living in the community is an important etiological agent of the upper respiratory tract infections and infects mainly to patients with chronic medical conditions or in contact with children.

P883 Does addition of a macrolide to β-lactam empirical treatment improve mortality rates in patients with community-acquired pneumonia?

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Objectives: To analyze whether treatment of patients with community-acquired pneumonia (CAP) with a macrolide in addition to a β-lactam improves mortality rates compared to those of patients treated only with a β-lactam antibiotic.
Methods: A cohort of 1518 patients with CAP was studied according to a standard protocol. CAP definition was established according to ATS criteria. Severity was assessed within the first day of admission using PORT score. At least one sputum sample, two blood cultures and two serum samples for serology (4–8 weeks apart) were obtained. Pleural puncture, transthoracic needle puncture, tracheobrochial aspiration and protected specimen brush or BAL sampling were performed according to clinical indication. Urine was collected for detection of soluble pneumococcal antigen by antibody assay as well as *L. pneumophila* antigen by EIA. 1391 patients with CAP of unknown etiology, atypical, *L. pneumophila*, viral or pneumococcal pneumonia were included in the analysis. Patients treated with only β-lactams (BL) or β-lactams together with a macrolide (BL-MC) were assessed to analyze a possible relation with mortality rates.
Results: An etiologic diagnosis was achieved in 498 patients out of 1391 (257 infections due to *S. pneumoniae*). Treatment consisted of BL in 270 patients and in BL-MC in 918. Mortality rates were 13.3 and 6.9%, respectively ($P=0.001$). Distribution of patients in PORT groups was not significantly different ($P=0.17$) among BL and BL-MC treatments (BL treatment: 19.4% in group I–II, 15% in group III and 65.6% in group IV–V; BL-MC treatment: 15.6% in group I–II, 19.5% in group III and 64.8% in group IV–V). After controlling for PORT score, the odds of fatal outcome was two times higher in patients treated with only BL than in those treated with BL-MC ($P=0.0008$). Same analyses in patients without etiologic diagnosis and in patients with CAP due to *S. pneumoniae* showed higher mortality rates in patients with β-lactam monotherapy.
Conclusion: Patients with CAP treated with β-lactam have a higher mortality rate than patients treated with β-lactam together with macrolide, independently of severity of infection.

P884 Patients with community-acquired pneumonia: the frequency of atypical agents

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Objective: Some series of reports of patients with community-acquired pneumonia demonstrate atypical pneumonia agents. A single IgG elevation is commonly used as a mean of diagnosis. In this study, we aimed to evaluate a rapid and simultaneous method in diagnosis of the main etiological agents of atypical pneumonia in adult patients with community-acquired pneumonia.
Methods: This study was conducted in Chest Diseases, Infectious Diseases, and Clinical Microbiology Department of Dokuz Eylul University Hospital in Izmir. Our study included 53 adult patients with acute one or more symptoms or signs suggestive of pneumonia. Twenty blood donors were used as a control group. The atypical pneumonia agents were determined by indirect immunofluorescent assay (Pneumo-slide:Vircell SL, Spain) in a single serum sample for each patient. Antibodies detected in samples were against *Legionella pneumophila* serogroup 1, *Mycoplasma pneumoniae*, *Coxiella burnetii*, *Chlamydia pneumoniae*, Adenovirus, Respiratory Syncytial Virus, Influenza A, Influenza B, Parainfluenza serotypes 1, 2 and 3.
Results: In the study population total IgG and IgM antibodies for several etiological agents were detected in 48 (90%) and 40 (75%) patients, respectively. Etiological agents included *L. pneumophila* 23, 28%; *M. pneumoniae* 7.5, 6%; *C. burnetii* 26, 7.5%; *C. pneumoniae* 34, 4%; Adenovirus 28%; Respiratory Syncytial Virus 68, 2%; Influenza A 28, 25%; Influenza B 34, 7.5%; Parainfluenza 70, 2%, respectively. However, in control group total IgG antibodies were determined in 17 (85%) donors while IgM antibodies were positive for 8 (40%) of them.
Conclusions: A single IgG elevation may not be as specific as a fourfold rise in antibody levels, thereby falsely increasing the incidence of infections. What causes also an increase in the incidence of infections is the ages of our patients. However, indirect immunofluorescent assay is found to be a rapid microbiological method for simultaneous diagnosis of the atypical pneumonia agents.

P885 Contribution of an antigen urinary assay (Binax) to the early diagnosis of pneumococcal pneumonia

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Objective: To evaluate the yield of a rapid immunochromatographic assay (Now™, Binax, USA) to detect *Streptococcus pneumoniae* antigen in urine samples and its contribution to the early diagnosis of pneumococcal pneumonia.
Methods: Urine samples from 116 nonimmunosuppressed adult patients with CAP were tested once for *S. pneumoniae* urinary antigen (unconcentrated urine). Sensibility and specificity were calculated for all cases with etiological diagnosis obtained by standard microbiological methods (definitive diagnosis: positive sterile samples and serology; and presumptive diagnosis: positive sputum culture with a compatible Gram stain). To evaluate the assay value as a rapid diagnostic tool, it was compared with sputum Gram stain results.
Results: When using only standard microbiological methods, 23 (20%) out of 116 patients were diagnosed of pneumococcal pneumonia (8 definitive diagnosis and 15 presumptive diagnosis). Other pathogens were identified in 14 patients: *Legionella pneumophila* (7), *Mycoplasma pneumoniae* (4), *Escherichia coli* (1), *Coxiella burnetii* (1), *Haemophilus influenzae* (1). No causative agent was identified in 79 patients. Overall, urinary antigen assay was positive in 32 (28%) of 116 cases (16/23 patients with pneumococcal pneumonia, 0/14 patients with pneumonia caused by other agents, and 16/79 without an etiological diagnosis by standard methods). Sensibility and specificity of the assay were 69.6 and 100%, respectively. Assuming a 100% specificity of the assay, there was an increase in the number of pneumococcal pneumonia diagnoses from 23 to 39 cases. Sputum Gram stain results in these 39 cases were as follows: no sample collected (9 patients), bad quality specimen (10), Gram-negative coccobacilli (1) and Gram-positive diplococci (19). Compared to sputum Gram stain, the antigen urinary assay increased the rapid diagnosis of pneumococcal pneumonia from 19 to 32 cases [48.7% when considering only the Gram stain vs. 82.1% when using also the urinary test; 95% CI of the difference 13.6–66%; $P<0.016$].

Conclusions: Binax is a sensitive and highly specific assay for the diagnosis of pneumococcal pneumonia. This test permitted an early recognition of 33% more cases of pneumococcal pneumonia than sputum Gram stain did. It can be useful in the initial approach to patients with CAP in whom a demonstrative Gram stain is not available.

P886 Detection of atypical pathogens among patients hospitalized with a community-acquired respiratory infection

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Objective: To determine the proportion of atypical respiratory pathogens using PCR and serology among patients hospitalized with a community-acquired respiratory infection.
Methods: From September 1997 to May 1999, 159 patients (57% male, median age 55, range 1–88 years with 12% <13 years) admitted to three regional hospitals for a community acquired respiratory infection, were enrolled in the study. A throat swab, sputum and/or broncho alveolar lavage (BAL) was collected and processed for PCR for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* and *L.* non-*pneumophila* species. Two serum samples were collected and processed for *M. pneumoniae* (Mp ELISA, Serion/Virion(R) and Mp microparticle agglutination test, Fujirebio(R)) and *C. pneumoniae* (in-house ELISA). On request of the physician standard microbiological procedures were performed on sera and sputum or BAL.
Results: Infection with *M. pneumoniae* was detected in 18 patients (11%) (PCR positive $n=7$), with *C. pneumoniae* in six patients (4%) (PCR positive $n=3$) and with *L. pneumophila* in four patients (2.5%) (PCR positive $n=4$). In two patients, *C. pneumoniae* was detected in combination with *M. pneumoniae* and *L. pneumophila*, respectively. In total 28 patients (18%) were diagnosed with an atypical pathogen. Among them were 11 children <13 years (39%), all diagnosed with *M. pneumoniae* infection. Furthermore, *L.* non-*pneumophila* species by PCR was found in 13 patients (8%). Routine microbiological investigations revealed etiologic agents other than the three atypical pathogens in 59 patients (37%), the most frequently diagnosed pathogens being *Streptococcus pneumoniae* ($n=15$), *Haemophilus influenzae* ($n=9$), *Branhamella catharralis* ($n=5$) and *Staphylococcus aureus* ($n=5$).
Conclusion: Among patients hospitalized with a community acquired respiratory infection 18% were diagnosed with *M. pneumoniae*, *C. pneumoniae* or *L. pneumophila*, using PCR and serology. *M. pneumoniae* was diagnosed most frequently, 61% of the patients being children aged 1–13 years. The role of *L.* non-*pneumophila* species in community respiratory infection needs to be determined.

P887 *Chlamydophilla (Chlamydia) pneumoniae* as a causative agent of respiratory tract infections in children and adolescents

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Chlamydophilla (Chlamydia) pneumoniae is an important human pathogen having a significant tendency to chronic course and acute exacerbations causing far-reaching late consequences with a high worldwide population exposure. It causes first of all inflammatory diseases of respiratory tract. Its attendance in cardiovascular diseases, degenerative diseases of central nervous system, systemic immunopathologic manifestations and others have been proven. Examination of *C. pneumoniae* infection is not a routine procedure in microbiology and its importance in clinical practice has been underestimated. In the Slovak Republic no study on the prevalence of *C. pneumoniae* has been published yet. The authors have concentrated on the survey of the participation of *C. pneumoniae* in infections of respiratory tract in children and adolescents in Piešťany region. In the period from January 2000 till May 2001, the authors have examined 293 patients ranging from 0 to 18 years of age suffering from respiratory system infection, where a dry, irritative cough lasting 5 or more days was dominating and/or the clinical picture has not improved after application of β-lactam antibiotics. IgM and IgG antibodies against *C. pneumoniae* have been examined via MIF method (Euroimmun, Laboratorium fur experimentelle immunologie, GmbH). Simultaneously a routine bacteriological examination has been executed, determination of antibodies against *Mycoplasma pneumoniae* via ELISA method (Genenzyme Virotech GmbH) and in a part of the group also a cultivation of *M. pneumoniae*. In 38.8% of examined patients primary active infection of *C. pneumoniae* has been proven (0–5 years 24%, 6–10 years 33.4%, 11–15 years 32.3, 16–18 years 39.2%), but in 13.6% of patients, the rest of microbiological examinations have been negative.

P888 The role of *Chlamydia pneumoniae* infection in asthma exacerbations

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Objectives: *C. pneumoniae* is a recently described human pathogen implicated in upper and lower respiratory tract infections. *C. pneumoniae* has also been reported as a possible cause of asthma. It is suggested that acute infection with this organism can initiate and exacerbate asthma in some patients. In this study, we aimed to evaluate the role of *C. pneumoniae* infection in exacerbations of asthma in adults.
Methods: Fifty-three adult patients with acute exacerbations of asthma (37 female, 16 male and mean age of 41.6 years) and 25 control subjects matched for age, sex, and smoking status were studied. Serum samples were tested for *C. pneumoniae* IgM and IgG antibodies by using enzyme immunoassay (EIA) methods.
Results: *C. pneumoniae* IgM antibodies were found positive in three patients who had severe asthma symptoms while the others were negative. The prevalence of *C. pneumoniae* IgG was significantly higher in asthma cases than in controls (73.6% vs. 48%, $P<0.05$). Mean titer of IgG was greater in asthma cases than in controls (11.3 vs. 9.3).
Conclusions: It is becoming clear that acute *C. pneumoniae* respiratory tract infection may trigger wheezing and asthma exacerbations and our study also shows this role of *Chlamydia pneumoniae* infection in asthma exacerbations.

P889 Evaluation of sELISA medac for the detection of *Chlamydia pneumoniae*-specific IgG, IgA and IgM in comparison to microimmunofluorescence (MIF)

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Objective: The evaluation of three new *Chlamydia pneumoniae* (C.pn.) EIAs, *Chlamydia pneumoniae*-IgG/A/M-sELISA medac (medac, Hamburg, Germany), regarding precision, sensitivity, specificity, and suitability for automation.
Methods: The assays are indirect ELISAs using highly specific LPS-reduced native C.pn. antigen for the detection of specific immunoglobulins. We investigated intra- and inter-assay variation in manual and automated procedures. Specificity and sensitivity were determined using 376 MIF (MRL Diagnostics, Cypress, CA, and in-house MIF) defined sera. Regarding specificity 44 (IgG), 87 (IgA), and 121 (IgM) MIF-negative sera were investigated. In addition, 11 (IgG), 14 (IgA), and 12 (IgM) MIF *Chlamydia trachomatis* (C.tr.) as well as 2 MIF *C. psittaci* (C.ps.) positive sera (IgM) were tested to investigate cross-reactivity. For sensitivity determination 119 (IgG), 77 (IgA), and 38 (IgM) MIF-positive sera were used. Automation suitability was investigated by using two different automatic ELISA systems.
Results: Coefficients of variation from sera within the relevant OD range (positive or borderline) were low (<11%). The specificities determined were 95% (IgG), 93% (IgA), and 97% (IgM). Cross-reactivity with C.tr. was 9% (IgG) and 7% (IgA). Regarding IgM cross-reactivity with C.ps. but not with C.tr. was found. Sensitivities were 99% (IgG), 95% (IgA) and 97% (IgM). Borderline results were excluded from the calculations. Good agreement was found between manually and automatically performed test runs.
Conclusion: *C. pneumoniae*-IgG/A/M-sELISAs medac are easy to perform, provide reliable results within less than 3 h, and are suitable for automation. Cross-reactivity within the genus *Chlamydia* still occurs but the assays provide good precision and excellent concordance with the MIF. Further studies are needed to establish firm clinical interpretation guidelines for the test results.

P891 Comparative study of bacterial community-acquired pneumonia (CAP) caused by *S. pneumoniae* (CAP-SP), *L. pneumophila* (CAP-LP) and *C. pneumoniae* (CAP-CP)

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Objectives: From May 1994 to February 1996, 392 episodes of hospital assisted CAP in 389 patients were studied prospectively. A definitive etiological diagnosis was made in 173 cases (44.1%), with *S. pneumoniae* (group 1), *L. pneumophila* (group 2) and *C. pneumoniae* (group 3) being the most prevalent microorganisms. Epidemiological data, clinical manifestations and evolution were compared among the three groups.
Methods: Risk factors, clinical manifestations, laboratory and microbiological data and complications were prospectively collected. Only microorganisms with a definitive etiological diagnosis were included: *S. pneumoniae* ($n=68$), *L. pneumophila* ($n=48$) and *C. pneumoniae* ($n=41$).
Results: Enolism and tabaquism were significantly more frequent in group 2 ($P=0.016$ and $P=0.07$, respectively), chronic obstructive pulmonary disease (COPD) in group 3 ($P=0.03$) and HIV infection and neoplasm in group 1 ($P=0.001$ and $P=0.017$, respectively). Non underlying disease was of note in group 2 ($P=0.001$). Most patients in the three groups were males with a mean age of 59.5 (group 1), 55 (group 2) and 65 (group 3). Cough, expectoration and thoracic pain were more frequent in groups 1 and 3 ($P=0.003$, $P=0.003$ and $P=0.07$, respectively). Headache, diarrhea and confusion were more frequent in group 2 ($P=0.01$, $P=0.003$ and $P=0.08$, respectively). Delay in the initiation of an efficacious treatment was longer in groups 2 and 3 ($P=0.004$). Previous antibiotic treatment ($P=0.004$) specially with β-lactams ($P=0.004$) was more frequent in groups 2 and 3. 'Shift to the left' was more frequent in group 1 ($P=0.05$), hyponatremia and increase in CK were significantly more frequent in group 2 ($P=0.001$ and $P=0.03$, respectively) and increase in AST was of note in groups 2 and 3 ($P=0.003$). Complications ($P=0.01$) and specially septic shock ($P=0.003$) were more frequent in groups 1 and 2. Lastly mortality was 14.7% in group 1, 8.3% in group 2 and 4.9% in group 3 ($P=$ ns)
Conclusions: CAP-SP mainly affects patients with underlying diseases and have a high morbidity and mortality. Efficacious antibiotic treatment is started sooner in CAP-SP than in CAP-LP and CAP-CP. CAP-LP mainly affects smokers and patients without underliying diseases. Extrarespiratory manifestations and laboratory abnormalities are specially frequent in CAP-LP. CAP-CP mainly affects COPD patients and its morbidity and mortality are low.

P892 Therapy of acute exacerbation of chronic bronchitis with moxifloxacin in pneumological practice

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Objective: The aim of this post marketing surveillance (PMS) Study was to evaluate the efficacy and safety of moxifloxacin (MXF) in acute exacerbation of chronic bronchitis (AECB) therapy, the time until improvement and recovery and patient acceptance of MXF.
Methods: PMS-study conducted in Germany with pulmonologists. Physicians documented their "real world" experience in treating AECB patients with MXF (400 mg qd) by use of structured case report forms. Demographic characteristics of the patients and the course of the symptoms, fever, coughing, expectoration, dyspnoea, chest pain and auscultation were assessed. At the end of the treatment period, global assessments of MXF therapy with regard to efficacy, tolerability and patient acceptance were made by physicians. Due to the intensive efforts to monitor the documentation and to validate the patient data a high quality and consistency could be achieved in the evaluation.
Results: The data from 2338 AECB patients documented by 399 investigators could be included in the statistical analysis. For most of the patients, a 5-day (62.3%) or 6–7-day (24%) course of MXF therapy was chosen. Temporary comedications for AECB in addition to basic therapy of COPD were antitussives/expectorants in 176 cases, corticosteroids in 103 cases and other antibiotics in 25 cases. Bronchodilators/antiasthmatics were by far the most documented continuous concomitant medications (1228 cases, 52.5%). 91.6% of the patients experienced an improvement of their AECB in a range of 1–5 days after start of MXF. Cure from the acute exacerbation was seen in a mean of 6.5 ± 2.7 days after start of MXF therapy. Especially high cure rates were observed for the symptoms, fever (96.2%) and chest pain (86.0%). The global assessments of efficacy and tolerability of MXF by physicians is shown in the following table.

Physician's global assessments (percent of patients; $N=2338$)					
Efficacy	Cure	Improvement	Failure	Not assessable	Missing
	59.2	36.9	1.9	1.4	0.5
Tolerability	Very good	Good	Sufficient	Insufficient	Missing/Not ass.
	63.9	31.5	2.7	0.9	1.0

A total of 2178 patients (93.1%) rated their MXF therapy as very satisfying or satisfying. The overall incidence rate for adverse events was 1.5%. Two patients (0.1%) experienced serious adverse events (psychosis and anaphylaxis). The psychosis was assessed by the investigator as "not related to MXF" and anaphylaxis as "probably related" but with full recovery.
Conclusions: MXF 400 mg qd is a safe and effective therapy for AECB patients treated by pulmonologists and provides rapid improvement of symptoms in a population where the majority of patients was without concomitant corticosteroids.

P893 Monotherapy versus combination of antibiotics and the prognosis of community-acquired pneumonia

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Introduction: Recent publications suggest that patients treated with a combination of antibiotics in the initial treatment of CAP is associated with a lower mortality rate than those treated with monotherapy.
Objectives: To determine the impact of monotherapy versus combination of antibiotics in the prognosis of CAP.
Methods: All patients 18 y diagnosed as having CAP seen in the Emergency Department (ED) during the calendar year 1999 were included. A predesigned protocol was applied to all patients admitted to hospital including demographic data, comorbilities, PSI according to Fine et al. antibiotics and outcome. Antibiotic treatment was instituted following the recommendations of the Infections Disease group. Mortality was defined as global (30 days or hospital discharge), during the first 72 h or related to the infection.
Results: Four hundred and forty-five (out of a 447) patients seen at ED with CAP were evaluable. Three hundred and sixty-seven (82.5%) received monotherapy (aminopenicillins, 185; third gen ceph, 110; macrolides, 39; fluoroquinolones, 9) and 78 (17.5%) were treated with dual therapy (β-lactam plus macrolide, 74; other, 4). Twenty-six patients died, 13 within the first 72 h. One was in category III, 9 in category IV and 17 in category V. Of those on monotherapy, 14/367 (3.8%) died, and had a mean PSI of 96 (range 8–218) versus 12/78 (15.4%) in those treated with a combination of agents, and with a mean PSI of 107 (range 17–205).

Considered separately, 163 patients belonged to category 1 V: 137 were treated with monotherapy and 130 (94.9%) survived, whereas 26 patients were treated with the combination and 24 (92.3%) survived ($P=0.6$). Ninety-one patients belonged to Category V: 67 were treated with monotherapy and 60 (89.6%) survived whereas 24 were treated with the combination and 15 (62.5%) survived ($P=0.005$).
Conclusions: Mortality in CAP is primary determined by severity of disease and the presence of comorbidity. Monotherapy does not appear to be associated with higher mortality rates.

P894 The efficacy and safety of telithromycin (TEL) administrated once daily for 5 days at a dose of 600 mg in patients with respiratory tract infection (RTI)

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Objective: The clinical and bacteriological efficacy and safety of telithromycin (TEL), a novel oral ketolide antibiotic, administered once daily for 5 days at a dose of 600 mg to the patients with respiratory tract infection (RTI) was evaluated.

Methods: Adult patients ($n = 120$), aged 16 to <80 years, with RTI (acute upper respiratory infection, community-acquired pneumonia and acute bacterial exacerbation of chronic pulmonary disease) presumably caused by typical or atypical/intracellular (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella pneumophila*) bacteria, received TEL 600 mg once daily for 5 days. Clinical and bacteriological efficacy were assessed at the end of treatment (4–7 days after the initiation of treatment or at the discontinuation) and at test of cure (14–28 days after the initiation of treatment).
Results: The clinical efficacy rate at the end of treatment and at test of cure was 92.7% (89/96) [difference: 95% CI (87.0, 98.4)] and 85.6% (83/97) [difference: 95% CI (78.1, 93.1)], respectively. The bacteriological efficacy by subject, including microbial substitution, was 86.0% (37/43) [difference: 95% CI (74.5, 97.6)]. The eradication rate of *S. pneumoniae*, the most frequently identified pathogen in this study, was 92.3% [difference: 95% CI (74.0, 100.0)]. The MIC_{90} of TEL to *S. pneumoniae* was 0.12 μg/mL that was eight doubling dilutions lower than that of macrolide antibiotics (erythromycin (EM) and clarithromycin (CAM)). The MIC_{90} of TEL to *H. influenzae* was 2 μg/mL that was four doubling dilutions lower than that of macrolide antibiotics (EM and CAM). TEL was generally well tolerated. The incidence rate of adverse events, for which the causality of drug could not be ruled out, was 34.5% (41/119) and were considered either mild or moderate.The clinical efficacy of this study was similar to that of another study in which the clinical efficacy of TEL 600 mg once daily for 7 days was compared to levofloxacin 100 mg three times daily in patients with RTI.
Conclusion: TEL 600 mg once daily for 5 days is an effective, well-tolerated empiric treatment for RTIs in adults.

P895 Comparative study of the efficacy and safety of oral telithromycin (TEL) 600 mg once daily versus oral levofloxacin (LVFX) 100 mg three times daily in adult subjects with community-acquired pneumonia (CAP)

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Objective: This double-blind, randomized, two-arm parallel-group comparison study evaluated the clinical efficacy and safety of telithromycin (TEL), a novel oral ketolide antibiotic, versus levofloxacin (LVFX) in patients with community-acquired pneumonia (CAP).
Methods and results: Adults and adolescents ($n = 250$), aged 16 to <80 years, with CAP presumably caused by typical or atypical/intracellular (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella pneumophila*) bacteria, were randomized and treated with TEL 600 mg once daily or LVFX 100 mg three times per for 7 days. Clinical outcome was assessed 14–28 days after initiation of treatment. There were no significant differences in the baseline demographics between the TEL and LVFX groups. Clinical efficacy rates at the end of treatment were equivalent between the two groups (per protocol population): TEL 93.6% (102/109) and LVFX 87.8% (86/98) and 87.8% (86/9). Difference (95% CI): 5.8 [−3.1, 14.7]. The point estimation of successful clinical cure rate of TEL group at clinical outcome assessment was 10.1% higher than that of LVFX group, which were 91.5% (97/106) and 81.4% (79/97), respectively. The most common bacterial strains were *S. pneumoniae* including penicillin or erythromycin-resistant strains and *H. influenzae*. The clinical cure rates of in the TEL and the LVFX groups were 93.3% (14/15) and 89.5% (17/19), respectively, for *S. pneumoniae*. For penicillin and erythromycin-resistant *S. pneumoniae*, the clinical cure rates in the TEL group were 100% (5/5) and 91.7% (11/12), respectively. Five penicillin-resistant *S. pneumoniae* isolates were also resistant to erythromycin. For *H. influnezae*, the clinical cure rates of in the TEL and the LVFX groups were 93.3% (28/30) and 86.7% (13/15), respectively. There were no significant differences in the incidence rates of adverse events, for which drug causality could not be ruled out [(TEL: 33.6% (42/125) and LVFX: 33.9% (39/115)]. No possibly treatment-related serious event was reported.
Conclusions: TEL 600 mg administered once daily for 7 days is equivalent in efficacy to LVFX in CAP in adults. TEL is as well tolerated as LVFX.

P896 The efficacy and safety of telithromycin (TEL) administered once daily at 600 or 800 mg in patients with pneumonia

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Objective: This double-blind, randomized, parallel-group comparison study evaluated the clinical and bacteriological effectiveness and safety of telithromycin (TEL), a novel oral ketolide antibiotic, in patients with community-acquired pneumonia (CAP).
Methods and results: Patients ($n = 104$) with CAP presumably caused by typical or atypical/intracellular (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella pneumophila*) bacteria were treated with TEL 600 or 800 mg once daily for 7 days. Clinical and bacteriological efficacy was assessed at 6–9 days after the initiation of treatment, and relapse/reinfection was assessed at 14–28 days after the initiation of treatment. There was no significant difference in the baseline demographics between the TEL 600- and 800-mg groups except body weight. Clinical efficacy at the end of treatment was 92.9% (39/42) in the 600-mg group versus 95.8% (46/48) in the 800-mg group. There was no statistical significance between the two groups [90% CI: (−7.3, 13.3)]. Non-relapse/nonreinfection rates (percentage of patients without relapse/reinfection) were 85.0 and 85.1% in the 600- and the 800-mg group, respectively, at 14–28 days after treatment [90% CI (−17.5, 0.5)]. Bacteriological efficacy by subject was 92.9 and 95.7% in the 600- and the 800-mg group, respectively [90% CI: (− 16.2, 21.8)]. The MIC_{90} of *S. pneumoniae*, including penicillin-resistant *S. pneumoniae* (PRSP) or erythromycin-resistant *S. pneumoniae* (ERSP), to TEL was 0.12 μg/mL that was eight times or more doubling dilutions lower than to other antibiotics (erythromycin (EM), clarithromycin (CAM), cefdinir (CFDN) and penicillin G (PCG)). The MIC_{90} of *H. influenzae* to TEL was 4 μg/mL that was four times higher than to macrolide antibiotics (EM and CAM). Clinical efficacy rate, bacteriological efficacy rate in 10 subjects with *S. pneumoniae* resistant to penicillin G and/or erythromycin were excellent (100%). TEL was well tolerated and all adverse events for which drug causality could not be ruled out were mild or moderate intensity (34.8% in the 600-mg group and 50.0% of the 800-mg group). There were no significant differences between two treatment groups.
Conclusions: TEL 600 mg once daily is as effective as TEL 800 mg once daily for 7 days in the treatment of CAP, including CAP caused by PRSP and ERSP.

P897 Quantitative determination of respiratory pathogens from throat cultures: does it indicate sinusitis?

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Objectives: Sinusitis is one of the most common disorders of childhood. Due to the limited knowledge about the causative agents of sinusitis and difficulties in diagnosis of microbial etiology, treatment is almost always empirical. In this study, we tried to determine the bacterial pathogens present in children with sinusitis and correlation of the cultures obtained from throat and maxillary sinuses.
Methods: This prospective study was performed between November 1999 and August 2001 on 90 children having adenoidectomy for their chronic adenoiditis. On the day of operation, throat cultures were obtained preoperatively and sinus aspirates at operation. Throat swab suspensions were serially diluted and were inoculated onto Columbia agar (bioMèrieux) and Polyvitex Chocolate agar (bioMèrieux) using quantitative method. Maxillary sinus aspirates were also inoculated onto Columbia agar, Polyvitex Chocolate agar, Schaedler agar and incubated in 5% CO_2 at 35 °C in an anaerobic jar. The organisms were isolated and identified by the standard methods.
Results: A total of 72 isolates from 47 (52%) of the patients were obtained. Multiple growth was detected in 20 (42.5%) patients, consisting of 19 patients with chronic and subacute sinusitis and one acute sinusitis. Of these patients, three had anaerobes (*Prevotella* spp., *Porphyromonas* spp., *Peptostreptococcus* spp.) accompanying respiratory pathogens. *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *S. pyogenes* were the most common isolates of sinusitis, respectively. Relationship between pathogens from maxillary sinus aspirates and quantity of throat isolates are shown in table.

Table: Relationship of quantity of throat isolates and maxillary sinus aspirates

Pathogen	Throat colonization	
	$>10^2$ Sinus/throat (%)	$>10^5$ Sinus/throat (%)
H. influenzae	18/14 (41)	13/30 (43)
M. catarrhalis	11/15 (66)	6/9 (66)
S. pneumoniae	8/17 (47)	5/13 (38)
S. aureus	5/9 (55)	1/3 (33)
S. pyogenes	3/11 (27)	1/5 (20)

Conclusion: There are still controversies regarding relationship between throat colonization and etiology of sinusitis. Data of this study clarifies that there is not a cut off value of pathogens found in throat cultures predicting etiology of sinusitis.

P898 Therapy of acute exacerbation of chronic bronchitis with moxifloxacin in patients with prior macrolide therapy

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Objective: The aim of this post marketing surveillance (PMS) Study was to evaluate the efficacy and tolerability of moxifloxacin (MXF) therapy in acute exacerbations of chronic bronchitis (AECB), time until improvement and recovery, and patient satisfaction in comparison to previous macrolide therapies.
Methods: PMS study conducted in Germany with physicians in general practice. Investigators documented their experience with MXF therapy for AECB patients with a prior macrolide treatment by use of structured case report forms. Course of the AECB symptoms was assessed. At the end of the treatment period, global assessments of MXF therapy were made by physicians concerning efficacy, tolerability and patient acceptance. In addition, they judged efficacy and time until onset of action in comparison to prior macrolide therapies. Patients rated their MXF therapy in comparison to their prior AECB therapy with macrolides.
Results: Data from 7223 patients could be included in the statistical analysis. Thirty-nine percent of patients received prior treatment with roxithromycin, 21% with clarithromycin, 17% with acithromycin and 13% with erythromycin. For 10% of the patients other macrolides, combination therapies or no specification was documented. For most of the patients MXF therapy was administered $\leq$5 days (67.2%) or 6–7 days (20.7%) with nearly all patients receiving 400 mg qd (only five patients received a different, higher dose). 75.5% of patients had an improvement of the acute infection at day 3, and 95.9% by day 5. Cure from the acute AECB was seen on the average after 6.2 ± 2.4 days of MXF therapy. The global assessment of physicians with regard to the efficacy of MXF therapy was "cure" or "improvement" for 98.2% of the patients. The tolerability of MXF therapy was rated as "very good" or "good" for 97.8% patients. 96.3% of the patients were very satisfied or satisfied with their MXF therapy. The assessments in comparison to the prior macrolide therapy are shown in the following table.

Physician's assessments (% of patients; $N=7223$) comparing MXF to prior macrolide

Efficacy	Better	Same	Worse	Missing
	77.5	17.5	1.5	3.6
Start of action	Earlier	Same	Later	Missing
	75.8	18.3	2.3	3.6

Patient's assessments (% of patients; $N=7223$) comparing MXF to prior macrolide

General assessment	Better	Same	Worse	Missing
	76.1	19.1	1.3	3.6

The overall incidence rate for adverse events was 0.7%. There were 5 (0.07%) serious adverse drug reactions documented (anaphylactic reaction, chills and fever, hypotension, nervousness and tremor). All events were resolved.
Conclusions: Therapy with MXF leads to rapid improvements in AECB and is well tolerated. More than 75% of physicians and patients rated MXF better than their prior macrolide therapy.

P899 Otitis media due to *Vibrio alginolyticus* after Tympanoplasty

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Tènerife, E

Objective: *Vibrio alginolyticus* have been recognized in a few cases as a potentially pathogenic bacteria in otitis externa, usually after prolonged contact with sea water. We report an isolate of this microorganism in an 22-year-old man, 6 months after a tympanoplasty and colesteatoma in his right ear. The patient didn't refer a prolonged contact with sea water and presented a suppurative chronic otitis media in the same ear than surgery.
Methods: Ear drainage was processed in the Microbiology Service, getting the following results.
Results: In Gram stain we observed polymorfonuclear leukocyte and mixed flora mainly with Gram-negative rods. *V alginolyticus* was isolated in pure culture in all media plated (Columbia, McConkey, Chocolate and TCBS agar). Identification was performed by Vitek II automated system (bioMèrieux) with an tipicity index 1 and by API 20NE system (bioMèrieux) with none error test. By NCCLS disk-diffusion test the organism was susceptible to all β-lactam antibiotics as well as to macrolides, aminoglycosides and quinolones. Patient was treated with tobramicyn during 10 days, with succeeded in the remission of the symptoms.
Conclusion: *V algynoliticus* infection, besides to be related to prolonged contact with sea water, can also be favored by traumatic processes.

Legionella

P900 Epidemiological evaluation about *Legionella* incidence in Liguria in a 4-month period

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Savona, I

Legionellosis is an infectious disease of severe gravity and height mortality. The predisposition factors are, besides old age, alcohol addiction and smoking, air inhalation, especially that from air conditioning systems, and ingestion of contaminated water. There is also a comorbidity with a great number of immunodepressing illnesses like BPCO, neoplastic diseases, renal insufficiency, diabetes, heart diseases, organ transplants and corticosteroid treatments. The incidence of Legionellosis in Community Acquired Pneumoniae (CAP) depends on the age; the diagnosed cases in patients under 65 years of age are about 2%, but they can even reach 6% in over 65 ones. This percentage varies from 4 to 10% in patients with Hospital Acquired Pneumoniae (HAP). In our research, we have tested 426 urine samples of patients with pneumonia diagnosis in 25 Ligurian hospitals and in the University of Genoa (Department of General Medicine, Infectious or Pneumological Diseases). The samples' collection has been made between December 1, 1999 and March 31, 2000. The samples have been frozen at -20 °C in special containers and tested in our laboratory every 2 weeks. The kit used is 'Binax Legionella Urinary Antigen EIA,' an immunoenzymatic kit, able to reveal the serum group 1 *Legionella pneumophila* antigen in human urine.
Results:
- Total samples tested: 426
- Positive samples: 5
- Positive samples: 421
- Percentage and positive samples: 1.07

Conclusions: The *Legionella* pneumonia incidence in our province is about 1%, less than the data found in literature.

P901 Distribution of bacteria causing Legionnaires' disease at potable water sites in Istanbul, Turkey

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Objectives: *Legionella pneumophila* (especially *L. pneumophila* Serogroup 1) known as causing Legionnaires disease lives in surface waters such as river, lake, stream in nature. These bacteria pass man-made water systems via these natural waters. It is known that these bacteria cause different infections and even death in man. For this reason, in this article, it was aimed to study, in respect of *Legionella*, potable water systems in buildings (especially hotels) in the vicinity of Istanbul.
Methods: Seven hundred and one potable water samples taken from 162 buildings between 1996 and 2001 were concentrated by filtration, and inoculated onto buffered charcoal–yeast extract agar (BCYE) added glycine, vancomycin, polymyxin, cycloheximide and incubated at 37 °C for 14 days. Colonies consistent with *Legionella* morphology were subcultured to tryptone soy agar (TSA). Definitive identification was performed by latex agglutination (OXOID).
Results: The water systems of 63 (38.8%) of the buildings surveyed were found to be positive for *Legionella*. It was found that 31 (23.7%) of the 131 strains determined as *Legionella pneumophila* were *L. pneumophila* Serogroup 1, 101 strain (%76.4) were *L. pneumophila* Serogroup 2–14. The lowest and highest viable counts of *L. pneumophila* Serogroup 1 were determined as 72 cfu/L and 20 000 cfu/L, respectively.
Conclusions: According to the results, it is suggested that the large number of buildings (hotels) in the vicinity of Istanbul have low risk category as to Legionnaires disease.

P902 Sampling and evaluation of *Legionella* pneumophila in cooling tower water systems

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Objectives: *Legionella pneumophila*, the causative agent of Legionnaires' disease, is known to be colonized and grown frequently in cooling tower water. Disease is acquired by inhaling aerosol contaminated by *Legionella*. Determination of the count of *L. pneumophila* in cooling tower water may be useful for risk assessment. In our survey, 103 water samples from 50 cooling towers were examined over a 5-year period to indicate the seasonal distribution and the ecology of *L. pneumophila* regarding temperature and pH values.
Methods: Water samples were collected in sterile plastic bottles; temperature and pH were measured at the time of sampling. Water samples were concentrated by filtration and membrane filters were resuspended in sterile distilled water. Acid and heat treatment were used as a decontamination method. Treated samples were inoculated onto α-ketoglutarate-supplemented buffered charcoal–yeast extract (BCYE) agar (containing glycine, vancomycin, polymyxin, cycloheximide) and incubated at 37 °C for 10 days. Colonies consistent with *Legionella* morphology were subcultured to blood agar and BCYE agar plates. Definitive identification was performed by latex agglutination and β-lactamase activity.
Results: *L. pneumophila* was detected in 26% of the water samples and the highest viable count was determined as >100 000 cfu/100 mL. *L. pneumophila* Serogroup 1 was found in 44% of the isolated strains, which is primarily responsible for the majority of Legionnaires' disease. The temperature range for multiplication of *Legionella* strains isolated from the cooling towers varied from 4 to 30 °C and the pH range from 6.56 to 9.40.
Conclusions: Cooling towers have been linked to many outbreaks of Legionnaires' disease. The large majority of examined towers had levels of Legionellae in the low-risk category. However, the detection of low numbers of *L. pneumophila* after a single sample cannot be used reliably to predict a low risk. Appropriate maintenance and monitoring by routine sampling are the effective control measures.

P903 In vitro activities of various antibiotics against *Legionella pneumophila*

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Objectives: When *Legionella pneumophila* known as the factor of Legionnaires disease is located in different setting such rivers, lakes and stream, it does not cause a healthy risk, but it colonizes in man-made water system found suitable conditions and causes infections contaminating human beings this way. *L. pneumophila* infections which cannot be defined because of inefficiency of diagnosis very often can only be therapied by early diagnose and an effective antibiotic practice. In this study, in vitro activities of erythromycin, azithromycin, ciprofloxacin, ofloxacin, levofloxacin, doxycyclin and rifampisin against environmental *L. pneumophila* were compared.
Methods: In this study, 8 (10%) of 80 water samples which belong to 20 buildings were determined as *L. pneumophila*. The determinations of serogroup strains were done by Oxoid diagnostic test kit. The minimum inhibitory and bactericidal concentrations (MIC and MBC) of these antibiotics were determined by microbroth dilution technique against eight *L. pneumophila*.
Results: MICs determined by microbroth dilution methods were in the range 0.25–0.0078 μg/mL of erythromycin, 0.125–0.0078 μg/mL of azithromycin, 0.031–0.0078 μg/mL of ciprofloxacin, 0.031–0.015 μg/mL of ofloxacin, 0.0156–0.0078 μg/mL of levofloxacin, 0.5–2 μg/mL of doxycyclin, less than or equal to 0.001 μg/mL of rifampicin. MBCs were in the range 1–32 μg/mL of erythromycin, 0.25–8 μg/mL of azithromycin, 0.0625–2 μg/mL of ciprofloxacin, levofloxacin, 0.0625–1 μg/mL of ofloxacin, 4–32 μg/mL of doxycyclin, more than or equal to 0.015 μg/mL of rifampicin.
Conclusions: In this study, none of the strains examined showed resistance to antibiotics. Rifampicin had significantly the highest activity against *L. pneumophila*. MICs and MBCs of ciprofloxacin, ofloxacin and levofloxacin found against the strains of *L. pneumophila* showed similar concentrations. This situation shows that these antibiotics can be selected in the therapy of *L. pneumophila* infections required bactericidal activity.

Surveillance of antibiotic resistance

P904 European Surveillance of Antibiotic Resistance (ESAR) in Gram-positive alert organisms

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Background: Since 1998, European Surveillance of Antibiotic Resistance has been funded by the European Commission to provide data through alert organisms.
Methods: European tertiary referral centers are in Slovakia, Poland, Germany with a comparator in Scotland. Routine laboratory data are sent to the European Society for Biomodulation and Chemotherapy (ESBIC) data center and analyzed. Comparative data for 1999 and 2000, on more than 100 000 Gram-positive denominator isolates are presented.
Results: Macrolide resistance is stable in β-hemolytic streptococci (βHS) (1.3–1.5%), but rose in pneumococci (1.5–6.2). Low-level penicillin resistance in pneumococci increased (3.1–10.8%). A total of 33% βHS and 50% pneumococcal alert organisms were hospitalized (12 and 22%, respectively, in ICU). Most βHS were from URTI or SSTI with one disseminated infection. Over 50% of pneumococci were from URTI, 15% from LRTI and 3% from blood cultures. Glycopeptide resistance in enterococci was low but β-lactamase production and high-level gentamicin resistance doubled (1.4 and 6.1%, respectively). Greater than 90% of alert enterococci were hospitalized (50% in ICU). Greater than 15% were from UTI, 10% from LRTI and 3% from blood cultures. Methicillin resistance remains <5% with one glycopeptide intermediate strain. Over 80% of MRSA were hospitalized (30% in ICU). LRTI and SSTI were the most common sites of isolates; 3% were from blood cultures.
Conclusions: Although alert organism resistance is increasing, rates remain much lower than published resistance studies. Selection of organisms for resistance studies may be the explanation for this. Use of routine susceptibility

data from diagnostic laboratories may give a lower estimate of resistant problems. There were marked differences in the number of alert organisms between the different centers.

P905 Quality assessment of antibiotic susceptibility testing by 608 laboratories of the European Antimicrobial Resistance Surveillance System (EARSS)

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On behalf of the EARSS participants

Objectives: EARSS organized in collaboration with UK-NEQAS (National External Quality Assurance Scheme), an external quality assessment (QA) of antibiotics-susceptibility testing to test the comparability of data across European countries and guidelines.
Methods: *E. faecalis* UA605 (vanB, aac6′-aph2′, erm) and UA1529 (susceptible), *E. faecium* UA1527 (Ap®, erm), *S. aureus* UA166 (susceptible), *E. coli* UA1530 (susceptible), *E. coli* UA1526 (CTXM β-lactamase, aminoglycoside®) and *E. coli* UA1528 (TEM8 β-lactamase, aac(6′)-Ib) were distributed. The laboratories had to report the guidelines followed and the clinical categorization of the strains. The classification was considered as 'concordant' if similar to that of three reference laboratories based on 'interpretative reading' of the data.
Results: Of the 608 laboratories from 24 countries, 552 (91%) replied: 73% used the NCCLS guideline, alone (60%) or in combination (13%). The overall concordance for the three enterococci and amoxillin/ampicillin, gentamicin and glycopeptides was high (≥91%), except for vancomycin (85%) and amoxicillin (77%) for strains UA605 and UA1527, respectively. Concordance for vancomycin was higher with MIC-yielding (90%) than with non-MIC-yielding methods (75%). The overall concordance for the three *E. coli* strains for amoxicillin/ampicillin, gentamicin, tobramycin, ciprofloxacin and ofloxacin was also high (≥92%). Detection of ESBL production by *E. coli* UA1526 and UA1528 was achieved by 85 and 90% of the laboratories, respectively. For these strains, the overall concordance for cefotaxime, ceftriaxone and ceftazidime varied from 71 to 100%.
Conclusions: The QA data indicated that the resistance rates monitored by EARSS allowed comparison between the countries. The low concordance for amoxicillin could be accounted for by differences in breakpoints among guidelines stressing the need for European breakpoints. Not all laboratories that detected ESBL production altered their data to resistance to cephalosporins.

P906 Frequency and resistance rates of *Pseudomonas aeruginosa* in Europe: update from SENTRY surveillance program 2000

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On behalf of the Euro SENTRY program

Objectives: The aim of this study was to describe antibiotic resistance rates of *Pseudomonas aeruginosa* from hospitalized patients monitored in SENTRY Europe surveillance program during 2000.
Methods: During the year 2000, a total of 766 *P. aeruginosa* strains from 18 hospitals were analyzed. These centers were distributed among 12 European countries: six Mediterranean countries (Spain, France, Italy, Greece, Turkey, Israel) and six other countries (UK, Belgium, Germany, Switzerland, Poland, Sweden). Antimicrobial susceptibility for 28 antimicrobial agents was tested by broth microdilution method as described by NCCLS.
Results: Of these isolates, 48% were obtained from hospitalized patients with pneumonia, 30% were from bloodstream infections (BSI), 13% from skin and soft tissue infections and 9% from urinary tract infections. More than 60% isolates were from nosocomial infections, 16% were of undefined place of acquisition and 36% were from patients admitted to intensive care units (ICU). *P. aeruginosa* was among the five most frequently reported pathogens causing BSI. A large variation in resistance rates was seen between the centers. The mean proportion of isolates nonsusceptible to piperacillin and ticarcillin was 23 and 36%, respectively; piperacillin-tazobactam 18%; ticarcillin-clav 35%; ceftazidime 28% (31% in ICU patients vs. 25% in non-ICU patients; $P < 0.001$); cefepime 29% (35% in ICU vs. 23% in non-ICU patients; $P = 0.07$); imipenem 25% (32% in ICU vs. 20% in non-ICU patients; $P < 0.01$); meropenem 23% (31% in ICU vs. 17% in non-ICU patients; $P < 0.01$). Rates of nonsusceptibility to aminoglycosides was 27% for tobramycin, 16% for amikacin and 31% for gentamicin. The MIC_{50} and MIC_{90} results for isepamicin were 4 and >32 μg/mL, respectively. The proportion of ciprofloxacin-nonsusceptible isolates was 32%.
Conclusions: *P. aeruginosa* was a frequent pathogen causing respiratory, skin and bloodstream infection among the hospitalized patients in this European survey. Rates of resistance of *P. aeruginosa* in 2000 were >15% for all antipseudomonal agents and were higher than in the previous surveys in 1997–1998.

P907 Antimicrobial susceptibility patterns of β-hemolytic and viridans group *Streptococci*: report from the SENTRY antimicrobial surveillance program (1997–2000)

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Objective: To determine susceptibility patterns of 15 antimicrobial agents tested against β-hemolytic (BetahS) and viridans group (VgS) streptococci in four regions of the SENTRY program: Asia-Pacific (AP), Europe (EU), Latin America (LA) and North America (NA).
Methods: Between January 1997 and December 2000, SENTRY program monitors received 3400 BetahS ($n = 2248$) and VgS ($n = 1152$) isolates from four geographic regions: AP (408), EU (777), LA (332) and NA (1883). All isolates were tested by reference broth microdilution methods and interpreted using NCCLS criteria. Among the BetahS tested, 81.9% were either serogroup A (650) or B (1190). The VgS were classified as unspeciated α-strept (512; 44%), *Streptococcus mitis* (254; 22%) and other speciated *Streptococcus* spp. (386; 34%). Seven quinolones, two β-lactams, erythromycin (ER), clindamycin (CM), quinupristin/dalfopristin (Q/D), vancomycin (VA), teicoplanin (TP) and linezolid (LZ) were routinely tested.
Results: Rank order of susceptibility for BetahS was: ceftriaxone (CTX) = Q/D = VA = TP = LZ (100.0%) > gatifloxacin (GATI; 99.8%) = trovafloxacin (TROV; 99.8%) > levofloxacin (LEVO; 99.7%) > penicillin (PEN; 99.3%) > grepafloxacin (GREP; 97.4%) > CM (94.4%) > ER (85.5%). GATI, GREP and TROV all had the same MIC_{50} (0.25 mg/L) and MIC_{90} (0.5 mg/L) result against BetahS, while LEVO had MIC_{50} and MIC_{90} results that were two-fold higher. ER versus BetahS had the highest MIC_{90} value (2 mg/L) and the lowest susceptibility across all regions (range: 81.4% in NA to 97.3% in LA). Among the VgS, susceptibility rank order was: VA = TP = LZ (100.0%) > Q/D (99.1%) > GATI = LEVO = TROVA (98.0%) > GREP (96.5%) > CTX (92.8%) > CM (90.3%) > PEN (68.6%) > ER (64.5%). VgS was 62.7, 68.1 and 57.8% susceptible to ER in AP, LA and NA, respectively.
Conclusions: Among BetahS, macrolide resistances increased to 13.8% and CM resistance was at 5.3%. These rates were two-fold greater in VgS and quinolone-resistant strains were observed at 0.6–1.3%, a disturbing level as was the low 68.6% susceptibility to PEN. Like pneumococci, other streptococci have acquired resistances and require continued surveillance.

P908 Epidemiology of antibiotic resistance of bacterial pathogens from intensive care units: the SENTRY surveillance program in Europe 2000

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On behalf of the Euro SENTRY Program

Objectives: To describe the frequency and resistance rates of bacterial pathogens from patients admitted to intensive care units (ICU) from a network of European hospitals.
Methods: During the year 2000, 18 hospitals from 12 European countries referred a total of 8062 bacterial pathogens. In vitro susceptibility of 32 antimicrobial agents isolated from hospitalized patients (pts), was tested by broth microdilution method as described by the NCCLS.
Results: Among all pathogens, 30% were from pts admitted to ICU. In these patients, the most frequent pathogens were *S. aureus* (20%), *P. aeruginosa* (15%), *E. coli* (11%), *K. pneumoniae* (8%), coagulase-negative staphylococci (7%), *A. baumannii* (7%), *E. cloacae* (5%) and *E. faecalis* (4%). Isolates from ICU pts were recovered from bloodstream (49%), lower respiratory tract (39%), skin (7%) and urinary tract infection (6%). The mean (intercenter range) proportion of *P. aeruginosa* isolates nonsusceptible to ceftazidime and cefepime was 31% (0–82) and 35% (0–82), respectively, in ICU pts, versus 25 and 23%

in non-ICU pts ($P=0.07$ and $P<0.01$, respectively). Ciprofloxacin resistance rates in ICU was 33% (0–88). Rates of nonsusceptibility to imipenem and meropenem were 32 and 31%, respectively, versus 20 and 17% in non-ICU pts ($P<0.001$). In *K. pneumoniae*, the proportion of decreased susceptibility to ceftazidime (MIC 32 μg/mL) was 43% versus 29 in non-ICU ($P<0.01$); cefepime 15% versus 7% in non-ICU pts ($P<0.01$). Resistance of *A. baumannii* to meropenem was seen in 43% isolates from ICU pts versus 16 in non-ICU pts ($P<0.01$). The proportion of oxacillin resistance in *S. aureus* from ICU pts was 47% (0–100) versus 25% in non-ICU pts ($P<0.0001$). Resistance to vancomycin was similarly low in ICU versus non-ICU pts in *E. faecalis* (1% vs. 3%). High level of gentamicin resistance was expressed by 36% of *E. faecalis* isolates from ICU pts versus 32% in non-ICU pts.

Conclusions: These data confirm that the prevalence of antimicrobial resistance in several Gram-negative pathogens and *S. aureus* isolates is higher from patients admitted to ICU than to other wards in these hospitals. Large intercenter variation underline the need to adapt the therapeutic approach to local resistance data.

P909 Antimicrobial susceptibilities of hospital pathogens to ceftriaxone and other antimicrobials: outpatient/inpatient and ICU data submitted to TSN® databases (Europe and USA, 1999–2001)

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Objectives: In this study, we report current susceptibility rates of antimicrobials for key hospital pathogens as reported to physicians by clinical microbiology laboratories in Europe and the USA.

Methods: We analyzed data (January 1999–September 2001) from The Surveillance Network® (TSN) Databases in France (Fr), Italy (It), Germany (Gy) and the USA to determine susceptibilities from routine test results. Data was divided into intensive care unit (ICU), inpatient (IP) or outpatient (OP) origin, and comprised all specimen types. 2001 NCCLS breakpoints were used, except for Fr (CA-SFM) and Gy (DIN) data.

Results: More than 50% of all isolates routinely tested were *S. aureus* and *E. coli*. For ICU patients, IPs, and OPs, rates of oxacillin-resistant *S. aureus* (ORSA) were 39.4, 43.3 and 29.9% (Fr), 40.8, 62.5 and 23.4% (It), 9.2, 17.4, and 2.9% (Gy), and 45.1, 52.2 and 28.3% (USA), respectively. Oxacillin-susceptible SA (OSSA) were susceptible to ceftriaxone (CRO) (>96%) irrespective of patient location. Erythromycin and ciprofloxacin (CIP) resistance was common among OSSA irrespective of patient location. Among *E. coli* (EC), >99% of isolates in Fr, >96% in Gy, >95% in It, and >97% in the USA from ICU patients, IPs and OPs were susceptible to CRO, similar to the activity of other third-generation cephalosporins (3GCs), amikacin (AMK) and CIP. CRO susceptibility in *K. pneumoniae* (KP) in ICU, IP and OPs, respectively, was 85.2, 91.0, 91.7% (Fr), 100, 93.5, 94.4% (Gy), 77.8, 88.3, 95.2% (It), 91.7, 95.3, 97.7% (USA), similar to rates for AMK, other 3GCs, and CIP, but higher than amoxycillin/clavulanate. For *P. mirabilis* from It, lower susceptibilities were observed than in isolates from Gy, USA or Fr for CRO and other agents. Against *C. freundii* imipenem, AMK and CIP were the most active agents. Using ceftazidime nonsusceptibility as a surrogate marker, ESBL production in EC was <4% in each country, with the highest levels observed in ICUs. For KP, <10% of isolates in Fr and Gy produced ESBLs in contrast to 12.8/31.2% (It) and 7.0/11.4% (USA) of isolates from ICU patients/IPs, respectively.

Conclusions: ORSA varied by patient location, but this was not always true for Gram-negative species. Isolates from outpatients tended to be more susceptible than hospital isolates. ESBL production in EC remains low, but in KP appear to be a significant problem, particularly in ICUs in Europe and the USA.

P910 Monitoring of drug-resistant strains in intensive care units in 1998–2000

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Objectives: The increase of drug resistance in nosocomial pathogens is a worldwide problem. In this study, clinical specimens from intensive care units have been considered and resistance to specific drugs in *S. aureus*, coagulase-negative staphylococci (CNS), *E. coli* and *P. aeruginosa*, the major opportunistic pathogens in these clinical settings has been analyzed.

Methods: A total of 666 pathogens processed in the Clinical Microbiology Laboratory have been considered; specimens were collected during a 4-month period in 1998, 1999 and 2000 from the intensive care units (ICU) of the regional hospital. The collection included *S. aureus* (211 samples), CNS (164), *E. coli* (171) and *P. aeruginosa* (117).

Results: Gram-positive bacteria were tested against nine different antibiotics. The results obtained shows that teicoplanin was the more effective compound with 100% of susceptible strains, together with doxicycline (from 93.5% in 1998 to 100% in 2000) and cotrimoxazole (about 95% during the 3 years considered). The incidence of oxacillin-resistant *S. aureus* did not vary significantly in the strains analyzed (from 42.3 to 39.2%), similarly in CNS this character varied from 57.7 to 54.5%. With *P. aeruginosa*, high levels of resistance was noted, in particular for ciprofloxacin (from 26.1 to 35.1% of resistant) and piperacillin/tazobactam (21.7 to 29.8%). With *E. coli* imipenem inhibited all strains tested, while piperacillin/tazobactam (from 84 to 85.8%), ceftazidime (from 89.2 to 97.6%) and amikacin (from 98.6 to 100%) showed high rate of activity.

Conclusion: The present findings showed that antibiotic resistance in ICU was essentially stable during the period of time considered. The importance of a strategic approach in monitoring drug resistance in nosocomial infections is confirmed.

P911 The impact of hospitalization on the epidemiology of antibiotic resistance in the oropharyngeal and fecal flora of intensive care patients

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Objectives: To assess whether hospitalization leads to (1) a change in antibiotic susceptibility patterns over time, or (2) the acquisition of new and resistant strains in the fecal and oropharyngeal flora.

Methods: Oropharyngeal and rectal swabs were collected on admission in the intensive care unit (ICU), once weekly and on discharge. The swabs were inoculated on a chromogenic agar and five colonies per sample were randomly selected. MICs for 20 antibiotics were determined by Vitek 2. NCCLS breakpoints were used. A significant change in susceptibility pattern was defined as a change in category from susceptible to resistant or as a four-fold increase in MIC.

Results: A total of 75 patients were included in the analysis. The median length of stay was 10 days (range: 5–116 days) and the median number of samples collected was 2 (2–15). In 43 patients (57%), significant changes in susceptibility patterns or acquisition of new resistant strains were observed. Changes in susceptibility patterns were found in 19 out of 75 patients (25%). These changes occurred both in the fecal and oropharyngeal flora, 17/19 and 8/19, respectively. Thirty-three out of 75 patients (44%) acquired new resistant bacteria during their stay in the ICU, 18/33 of whom in their fecal flora and 17/33 in the oropharyngeal flora. Changes and acquisitions in the feces mainly concerned *E. coli*, whereas in the oropharynx *E. coli* and *P. aeruginosa* were mainly involved. Thirty percent of the overall number of changes in susceptibility or acquisition of resistance was to aminopenicillins, 23% to first- and second-generation cephalosporins, 20% to third- and fourth-generation cephalosporins, 12% to quinolones, 6% to trimethoprim/sulfamethoxazole, 6% to aminoglycosides, and 3% to carbapenems.

Conclusions: Changes in susceptibility patterns and acquisition of resistant strains occurred frequently during admission on an intensive care unit and points to a highly dynamic bacterial population in these patients.

P912 Antimicrobial resistance in Italy: preliminary results from the AR-ISS Project

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Objectives: The AR-ISS Project is a laboratory-based sentinel surveillance system on antimicrobial resistance established in 2001 by the Istituto

Superiore di Sanità (ISS), the Italian Public Health Institute. The main objective of the project is to collect and analyze antimicrobial resistance data on pathogens of particular epidemiological and clinical interest in order to describe the pattern of susceptibility and monitor changes providing the scientific evidence on which strategies for control and prevention have to be based. Preliminary results on the first 4 months of activity are presented.

Methods: Since June 2001, 69 Italian laboratories, serving a total of 77 hospitals, collect and send to ISS data on invasive isolates of *S. pneumoniae* (blood and cerebrospinal fluid), *S. aureus*, *E. faecalis/faecium*, *K. pneumoniae/oxytoca*, *E. coli* (blood only). Repeated isolates from the same infection are excluded. Data on clinical characteristics of the patients are also collected and transmitted using standard paper forms, while 10 labs transmit data electronically. Strains with a defined pattern of antibiotic resistance are collected and shipped to ISS for further characterization.

Results: From June to September 2001, 1017 isolates were reported (average of 13 isolates/laboratory, range 1–76).

1 *S. aureus* (540 isolates): all isolates were susceptible to vancomycin; 42.8% were methicillin-resistant *S. aureus* (MRSA); average age of patients was 62 years (SD = 20.6); 61% were males.
2 *S. pneumoniae* (69 isolates): 14.3% were nonsusceptible to penicillin (PRSP); 31% were resistant to erythromycin; average age of patients was 55 years (SD = 26.9); 61% were males.
3 *E. faecalis/faecium* (173 isolates): 1% of *E. faecalis* and 11.8% of *E. faecium* were resistant to vancomycin; average age of patients was 66 (SD = 19.3); 64% were males.
4 *K. pneumoniae/oxytoca* (137 isolates): 13% were ESBL-positive; average age of patients was 56 (SD = 25.5); 60% were males.

Conclusions: The proportion of MRSA higher than 40% is in line with previous observations in Italy. On the contrary, the recorded PRSP level (14.3%) is higher than that previously reported (11.4% in 1999), but the result needs confirmation. As expected, erythromycin resistance in *S. pneumoniae* is very high. The proportion of vancomycin resistance is quite different in the two species of *Enterococcus*, being higher in *E. faecium* as expected. ESBL production by *Klebsiella* spp. isolates needs to be confirmed by further tests.

P913 Analysis of alert organism and risk factor data from a European Surveillance Program

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Background: European Surveillance of Antibiotic Resistance (ESAR) is funded by the European Commission to provide alert organism data.

Methods: Slovakian, Polish and German tertiary referral centers were compared with a large Scottish center. Routine alert organism and risk factor data from 1999 and 2000 were analyzed by the ESBIC data center.

Results: Annual alert organisms increased from 4565 to 5777. Seventy-five percent were inpatients (20% internal medicine, 20% surgery) and 40% had an ICU stay. Eighteen percent of the specimens were surveillance. Forty-nine percent were definitely/probably causing infection and 33% were possibly causative. Common organisms were *Pseudomonas* (20%), *Staphylococci* (17%), *E. coli* (16%) and enterococci (10%). Isolates were from urine (34%), respiratory tract (32%), wounds (14%) and blood cultures (5%). Seventy-five percent of the cases documented an infection; UTI (29%), LRTI (13%), wounds (10%), SSTI (8%), URTI (8%) and bacteremia (3%). Prior antibiotic treatment was known in 60% of the patients; 16% received no antibiotics, 26% received one antibiotic, 12% two and 7% more than three. The commonly used antibiotics were coamoxiclav, ceftazidime, cefotaxime, ciprofloxacin, pip-tazo, metronidazole, cotrimoxazole and vancomycin. Antibiotic use varied considerably between centers. A total of 69% of the patients were known to suffer from at least one underlying disease, commonly neoplasm (14%), circulatory disease (11%), respiratory tract disease (10%), and genitourinary disease (12%).

Conclusions: Alert organisms were predominantly from inpatients (often from surgical ICUs) with underlying disease. The majority caused urinary or respiratory infection. Most patients received prior broad-spectrum antibiotics. There were marked differences in antibiotic use between countries.

P914 Analyzing the antibiotic resistance of community-acquired bacteremia in Southern Taiwan

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A total of 313 episodes of community-acquired bacteremia were collected from the emergency department of Veterans General Hospital, Kaohsiung prospectively and analyzed from July 1998 to June 1999. Microbiologically, there were 140 strains of *Escherichia coli*, 66 strains of *Klebsiella pneumoniae*, 25 strains of *Staphylococcus aureus*, which account for over 70% of the whole isolates. Most of the community-acquired bacteremia were secondary bacteremia from primary infectious foci such as urinary tract infection, biliary stone, with underlying diseases of liver cirrhosis or diabetes mellitus. The most frequently prescribed antibiotics in the emergency department, include cefazolin which is the first-generation cephalosporin, is still sensitive to *E. coli* in 84%, *K. pneumoniae* in 89%, *S. aureus* in 76%. Gentamicin also remains 72, 92, 68% sensitive to the three major pathogens, respectively. In contrast, when analyzing the trend of nosocomial bacteremia in the same period, primary bacteremia accounts for over 70% of the total isolates. The antibiotic resistance of those nosocomial pathogens against the first-line antibiotics such as cefazolin, gentamicin, oxacillin mostly exceed 70–90%. In comparison, the drug susceptibility of community pathogen is relatively constant and stable. Indicating that the community outside the hospital possesses a much huge microecological buffer system, so as to retain a greater capability to stabilize the antibiotic susceptibilities for longer years than we had expected. Cefazolin and gentamicin can be used as the first line antibiotics to treat vast majority of community-acquired infections in Southern Taiwan. Further constant monitoring on bacteremia in a nation-wide scale is needed.

P915 Are community-acquired organisms less resistant to antibiotics?

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Objectives: (1) To study the distribution of general practice organisms and (2) to determine the antimicrobial susceptibilities of these organisms.

Methods: Private doctors practicing throughout Hong Kong collected specimens such as sputum, urine, stool, etc. from patients with suspected infections. These specimens were cultured and identified in the laboratory according to standard procedures. Susceptibility to different antimicrobial agents was determined by the disk diffusion test as set out by the National Committee for Clinical Laboratory Standards.

Results: A total of 4741 specimens from 3977 patients were submitted for culture by 89 private practitioners from July 2000 to October 2001. Of these, 28% were culture-positive. Throat swabs made up the largest proportion of specimens (26%), followed by urine (23%) and sputa (15%). More than 1300 organisms were isolated, with 50% Gram-negatives and 42% Gram-positives. *Escherichia coli* was the most common organism isolated (18%), followed by *Staphylococcus aureus* (12%), β-hemolytic *Streptococcus* (BHS) group G (8%), *Candida albicans* (8%) and *Haemophilus influenzae* (6%). There were 50 isolates (4%) of *Streptococcus pneumoniae* and 47 isolates (3%) of *Neisseria gonorrhoeae*. Of the 245 isolates of *E. coli*, 61% were resistant to ampicillin, 20% to amoxicillin/clavulanic acid, 30% to cefuroxime and 14% to ciprofloxacin. There were only three methicillin-resistant *S. aureus* (2%), however, more than 30% were resistant to erythromycin or clarithromycin. All the 60 isolates of BHS group A were susceptible to penicillin, but 5% of 103 BHS group G isolates were resistant. Up to 30% of all BHS were resistant to erythromycin or clarithromycin. Nineteen percent of the 79 *H. influenzae* isolates were resistant to ampicillin, 9% to cefaclor, 26% to clarithromycin, but only 1% to amoxicillin/clavulanic acid. All the 47 isolates of *N. gonorrhoeae* were resistant to penicillin, but none to ceftriaxone, 89% were resistant to ciprofloxacin and 13% to spectinomycin. A large proportion of the 50 *S. pneumoniae* isolates (>70%) were resistant to penicillin or clarithromycin.

Conclusions: (1) *E. coli* was the most common organism isolated; (2) the level of ciprofloxacin and macrolide resistance was high; (3) the large proportion of *S. pneumoniae* resistant to penicillin was alarming; (4) community-acquired organisms were definitely not less resistant to antibiotics.

Nosocomial bloodstream infections

P916 Bloodstream infections in ICU patients: analysis of risk factors influencing outcome

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Varese, I

Objectives: Bloodstream infections (BSI) represent a serious health problem in ICU. A 2-year analysis was performed at our hospital to evaluate risk factors influencing the outcome among ICU patients that either survived or died from BSI.
Methods: During the years 1999–2000, clinical records of ICU patients with BSI were evaluated with regard to severity of underlying disease (McCabe and Jackson groups), comorbidity scores (Charlson weighted index), severity of septicemia, predisposing factors, sources of secondary BSI, and empirical treatment. Statistical analysis of patients was performed using an appropriate software (Statistica; StatSoft, Tulsa, OK).
Results: Ninety-two BSI occurred over the study period, with an incidence of 86/1000 admissions. Seventy-five patients survived (81.5%), whereas 14 died from BSI (15.2%). With respect to survived and dead patients, the following risk factors were considered: age (49.7 vs. 68.6), nonfatal (89.3% vs. 71.4%) and ultimately fatal underlying diseases (15.2% vs. 10.7%), comorbidity scores (0.9 vs. 2.7), sepsis (88% vs. 28.6%), severe sepsis (9.3% vs. 0%), septic shock (2.7% vs. 57.1%). The most frequent predisposing factors for BSI were: intravascular (93.3% vs. 100%) and bladder catheter (92.0% vs. 100%), intubation (74.7% vs. 92.9%), and previous use of antibiotics (78.7% vs. 71.4%). Overall, 54.7% of the survived and 57.1% of the dead patients had four or five predisposing conditions, whereas 29.3 and 42.9%, respectively, had six or more. Secondary BSI affected 53.3% of the survived and 78.6% of the dead patients; lower respiratory tract (67.5% vs. 42.9%), intravascular catheter (27.5% vs. 28.6%) and urinary tract (27.5% vs. 0%) were the most frequent sources of secondary BSI. Patients who either survived or died received adequate empirical treatment in 45.8 and 35.7% of the cases, respectively. Statistical analysis of independent risk factors showed that mortality was significantly related to age ($P < 0.002$), comorbidity scores ($P < 0.026$), and septic shock ($P < 0.001$).
Conclusions: Our study demonstrates that outcome is not significantly related to the number and type of predisposing factors; on the contrary, it is influenced by age, underlying diseases, and severity of septicemia. On the basis of our findings, we suggest that the treatment of BSI should be based more on the data of the epidemiological survey provided by the local microbiology laboratory than on empirical therapeutic protocols derived from the literature.

P917 Bacteria isolated from blood in intensive care unit

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Objectives: The aim of this study was to analyze the incidence of bacteria recovered from blood cultures patients from 12-bed intensive care unit in our hospital.
Methods: The study involved patients hospitalized at the ICU from January 1999 to September 2001. The samples were incubated in BacT/Alert System (Organon Teknika) and VITAL (bioMerieux). The identification was done in the VITEK system (bioMerieux).
Results: A total number of 3736 blood cultures were examined; 669 bacteria were isolated out of 605 positive blood cultures. A total of 54.3% of all were Gram-positive, 44.2% Gram-negative, and 1.8% fungi. The most frequently isolated microogranisms were: coagulase-negative staphylococci (CNS, 36.9%), *Pseudomonas* spp. (19.5%), *Staphylococcus aureus* (7.2%), *Enterococcus* spp. (7.2%), *Serratia* spp. (4.9%), *Klebsiella* spp. (3.9%), *Streptococcus* spp. (2.3%), *Enterobacter* spp. (2.1%), *Escherichia coli* (1.8%), *Proteus* spp. (1.8%), *Acinetobacter* spp. (1.5%), *Stenotrophomonas maltophilia* (1.2%), and *Candida* spp. (1.8%).
Conclusion: The frequency of positive blood culture was 16%; 51 were a polymicrobial cultures. The most common microorganisms were Gram-positive bacteria, especially CNS, which are the main etiologic agents of nosocomial bacteremia. We noted increased frequency *Pseudomonas aeruginosa* isolation from 11.1% (1999) to 18.1% (2000) and to 27.5% (I-X, 2001) and *Enterococcus* spp. from 4.8% (1999) to 8.8% (2000) and to 7.4% (I-X, 2001).

P918 Bacteremia in a general hospital, Spain, 1994–2000

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Objective: The aim of this study is to assess the features of bacteremia in the Hospital of Basurto in the last 7 years.
Methods: The Hospital of Basurto where the study was carried out is a 800-bed primary and tertiary care teaching hospital that provides care in the urban area of Bilbao. 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 bacteremia, nosocomial/community acquired, microorganism and antibiotic susceptibility, antimicrobial treatment, complications and outcome. A computer-based surveillance system (SEPSIS-DATA) was used.
Results: A total of 3874 episodes of bacteremia were studied during this period. The incidence remained stable from 1994 to 2000 at about 19 cases/1000 discharges. The higher incidence rate was registered in the nonsurgical ward. A trend towards increasing rate was observed in pediatric ward. Men (57.38%) aged over 60 years (56.91%) accounted the majority of the cases. The most frequent underlying illness was neoplasia present in 870 patients (22.45%). The urinary (28.76%), gastrointestinal (17.65%) and respiratory tract (15.69%) were the most common sources of bacteremia. Nosocomial bacteremia accounted 38.7% of the cases in 1994 and 30.39% in 2000. Etiology: in 1994: Gram-negatives 49.7%, Gram-positives 46.6%, and yeast 2.66%; in 2000: Gram-negatives 56.16%, Gram-positives 41.25%, and yeast 2.59%. *E. coli* (32.57%), *S. pneumoniae* (10.35%), *S. aureus* (8.37%), *S. epidermidis* (5.02%), *P. aeruginosa* (3.2%) and *Salmonella* spp. (2.44%) are now the most frequent agents of bacteremia. Nosocomial-acquired bacteremia: in 1994: Gram-positives 58%, Gram-negatives 36%, yeast 4.7%; In 2000: Gram-positives 42.79%, Gram-negatives 49.25%, and yeast 7.96%. Ceftriaxone and vancomycin are the most frequently prescribed antibiotics. Eighty-four percent of the correct antibiotic treatments were started the same day when the blood cultures were obtained. Crude mortality until the end of the episode was 15% in 1994 and 17.97% in 2000.
Conclusions: Incidence of bacteremia has remained stable in last 7 years. The number of cases in pediatrics is increasing. Gram-negatives are increasing in nosocomial-acquired bacteremia, *E. coli* and *P. aeruginosa* are the most frequently recovered microorganisms in nosocomial cases.

P919 Microbiological surveillance of bloodstream infections in a pediatric hospital, Italy

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Genoa, I

Objective: We conducted a study to asses the prevalence and the antibiotic-susceptibility pattern of nosocomial bloodstream isolates in an 18-month period in a large pediatric hospital.
Methods: With the help of microbiological and epidemiological management software (0/3 – Soluzioni Informatiche, Vicenza, IT) connected to laboratory instruments, we analyzed the microbiological data of blood cultures (BCs) for the period from 1 January 2000 to 30 June 2001. BCs were performed in Bact/Alert pediatric bottles (Organon Teknika); microorganisms were isolated by conventional methods, identification obtained by API system (BioMerieux); MICs of antibiotics were determined with broth microdilutions, with wider MIC panels (DADE-Soria-Melguizo) for Gram-positive organisms and Enterobacteriaceae, with sensititer MIC panels (accumed for nonfermenters, with E-test for *Candida* spp.; NCCLS-proposed breakpoints were applied. Internal (ATCC strains) and external (NEQAS) QC were carried out regularly.
Results: A total of 815 patients were monitored and total of 2949 blood cultures were performed (69% in PICU, 32.5% NICU, 16% surgery and CCV, 8% infectious diseases, 7% neurosurgery, 5.5% pediatrics. Total 169/815 pts (21%) had 388/2949 (13%) pos BCs and 444 strains were recovered: 208 (46.8%) coagulase-negative staphylococci (139 *S. epidermidis*, 32 *S. warneri*, 28 *S. hominis*, 21 *S. haemolyticus*), 59 *Candida* (10 albicans, 49 nonalbicans) (13%), 16 *P. aeruginosa* (3.6%), 16 *Enterobacter/Kebsiella* (3.6%), 15 *Enterococcus* spp.

(3.4%), 14 *S. aureus* (3.1%), 12 *Streptococcus* spp. (2.7), 10 *S. malthophilia* (2.2%). All *S. aureus* isolates were susceptible to oxacilline (OX); 82% of CoNS were OX-resistant and 4.8% resistant to teicoplanin, only one *Shaemolyticus* was resistant to vancomycin; 35% of *Candida* nonalbicans were resistant to amphotericine B and fluconazole; among *Enterobacter* and *Kebsiella*, we found 25% resistant to cefotaxime/ceftazidime and 75% resistant to cefuroxime; 20% of *P. aeruginosa* were resistant to meropenem and/or ceftazidime, no strain was resistant to piperacillin; among enterococci 38% were highly resistant to gentamicin, and no strain was resistant to vancomycin and teicoplanin.
Conclusions: Our results on bloodstream infections laboratory surveillance show a high prevalence in our institution of resistant *S. epidermidis* and the emergence of other CoNS and *Candida nonalbicans* with high resistance. This trend suggests the need to improve local infection control measures and antimicrobial therapy policies.

P920 Nosocomial bacteremia in a university hospital

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Objective: The purpose of this study was to describe the epidemiological characteristics of nosocomial bacteremia.
Methods: Prospectively, we reviewed the medical records of all patients with positive blood culture between April and September 2001 in our hospital (650-bed university hospital).
Results: There were 185 episodes of nosocomial bacteremia, which represents an incidence rate of 1.9 per 100 patients and 1.8 per 1000 patient-days. The patients most commonly affected were in Neonatal Intensive Care Unit (15.68%) and Medical-Surgical Intensive Care Unit (11.35%). Identifiable portals of entry included intravenous catheters (34), surgical site (6), genitourinary tract (4), respiratory tract (3) and intra-abdominal foci (3), but 72.97% had no recognizable source. Aerobic Gram-positive were isolated in 109 cases, aerobic Gram-negative in 73 cases, anaerobic bacteria in two cases and *Candida* in 17 cases. There were polymicrobial bacteremia in 15 cases. The five most common microorganisms were *S. epidermidis* (18.41%), *E. faecalis* (11.44%), *E. cloacae* (10.95%), *S. aureus* (8.96%; 27.78% were MRSA) and *E. coli* (7.96%). There was an outbreak of nosocomial septicemia due to *E. cloacae* in the Neonatal Intensive Care Unit in May.
Conclusions: Coagulase-negative staphylococci were the prime etiologic agents, and catheter-related bacteremia was the prime source. The patients most commonly affected were those in critical areas. So, implementation of different strategies will be necessary to reduce these infections, promoting more strict compliance with aseptic practices and a more controlled use of invasive procedures.

P921 Nosocomial bloodstream infections in an Iranian hospital, 1999–2000

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Background and objectives: Nosocomial infections, especially bloodstream infections, are serious causes of morbidity and mortality. The aim of this study was to determine agents of nosocomial bacteremia in Imam Khomeini Hospital, a 300-bed University-affiliated teaching hospital in Urmia, West Azarbayjan, Iran.
Methods: In this 2-year retrospective study, database of Microbiology Laboratory in Imam Khomeini Hospital was reviewed to identify patients who had bacteremia between 1 May 1999 and 31 March 2000. A nosocomial bloodstream infection was defined occurring in a clinically ill patient when one or more cultures of blood drawn at least 48 h after admission yielded a pathogenic organism. Isolation of potential skin contaminant (e.g. coagulase-negative staphylococci, *Propinobacterium* spp., diphtheroids and micrococci spp.) only from one specimen in asymptotic patient considered as contamination.
Results: Our analysis revealed that 587 (9%) of 6492 admitted patients in various wards of Imam Khomeini Hospital had positive blood cultures. The frequency of isolated bacteria were as follows: coagulase-negative staphylococci 111 (18.9%) strain, *Staphylococcus aureus* 107 (18.3%) strain, *Pseudomonas aeruginosa* 104 (17.7%) strain, *Enterobacter* spp. 48 (8.2%) strain, *Escherichia coli* 41 (7%), *Streptococcus pneumonia* 24 (4%) strain, *Klebsiella pneumonia* 19 (3.2%), *Citerobacter* spp. 19 (3.2%), other Gram-positive cocci 9 (1.6%) strain, and other Gram-negative rods 20 (3.4%). The highest frequency of positive cultures was found in neonatal ward (aged <45 days). At this ward, there were 323 (55.1%) patients with positive blood cultures. The mean age in this group was 8.9 days (SD ± 5.9). Levels of antimicrobial resistance in *S. aureus* were: penicillin 82.6%, oxacillin 40.7%, vancomycin 0%, gentamycin 36.5%, ciprofloxacin 0%, clindamycin 1.9%, cotrimoxale 7.6%, while resistance of coagulase-negative staphylococci against above-mentioned antibiotics were 76.8, 61.5, 0, 25, 0, 3.7 and 6.4%, respectively. Resistance of *P. aeuroginosa* to aminoglycosides included gentamycin 32%, tobramycin 21%, and amikacin 21%. There was no resistance of this organism to ciprofloxacin. Resistance of *Enterobacter* spp. to commonly used antibiotics was as following: gentamycin 71.1%, tobramycin 56.5%, ciprofloxacin 0%, cotrimoxazol 8.6%, cefazolin 30.4%, cephalothin 65.2%, while resistance of *E. coli* to the previously mentioned antibiotics were 22.5, 22.5, 0, 22.5, 12.5 and 42.5%, respectively. Finally, resistance of *S. pneumonia* to penicillin and erythromycin was 31.8 and 4%, respectively.
Conclusion: Our study reveals that *S. aureus*, coagulase-negative staphylococci and *P. aeruginosa* are most commonly isolated bacteria in nosocomial bloodstream infections in Imam Khomeini Hospital of Urmia. We have found increased rate of Gram-positive cocci in nosocomial bateremia. The reason for the high frequency of *P. aeruginosa* was due to patients in burning ward.

P922 *Haemophilus parainfluenzae*: an uncommon case of primary septicemia in adult

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Haemophilus parainfluenzae is a normal inhabitant of the upper respiratory tract that rarely causes serious infections in human beings. The most common infections described are epiglottitis, pneumonia, subacute endocarditis, meningitis, abscesses and urinary tract infections. We describe a case of primary septicemia caused by *H. parainflueznae*. A 49-year-old male was admitted to the internal medicine department with symptoms of fever, chills, malease and melena. The physical examination of the respiratory, circulatory and urogenital tract did not reveal any pathological findings. Colonoscopy was negative, while gastroscopy showed pyloric ulcer without recent hemorrhage. Moderate leukocytosis, mild anemia and elevated ESR and CRP were present. Serologic tests for various bacteria and viruses were negative. Two sets of blood culture were positive for *H. parainfluenzae* biotype I, β-lactamase negative. A second effort to find the source of infection from the upper respiratory tract was negative. Trancesophageal echocardiogram for the diagnosis of possible endocarditis was also negative. Gradual decline of fever and improvement of the clinical picture was observed after treatment with a combination of ampicillin and gentamycin i.v. In conclusion, during last decade a few cases of *H. parainfluenzae* endocarditis have been described but primary septicemia, without known infectious focus, is extremely rare.

P923 Clinical and laboratory predictive markers in septic patients

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Objectives: The aim of the present study was to estimate the prognostic value of certain early clinical and laboratory markers of outcome in severe sepsis.
Patients and methods: A total of 139 patients (68 males, 71 females, mean age 67.5 ± 13.7 years) with severe sepsis were studied. The etiology of sepsis was: pneumonia ($n = 50$), pyelonephritis ($n = 51$), intra-abdominal infection ($n = 24$), skin or joint infection ($n = 11$) and intrapelvic infection ($n = 3$). Twenty-nine patients were in septic shock. On admission, Simplified Acute Physiology II score (SAPS II) and Glasgow Coma Scale (GCS) were used to access the severity of sepsis. Blood gases, white blood cells (WBC), liver and renal function tests, serum lactate levels and the presence of disseminated intravascural coagulation (DIC) on admission were determined as well. Logistic regression analysis was used for statistical evaluation.
Results: Thirty-seven out of 139 patients (26.6%) died. Old age was a significant predictor of poor outcome ($P < 0.05$). The presence of septic shock ($P < 0.0001$) and renal dysfunction ($P < 0.05$) had a negative influence on outcome in septic patients, while no significant differences in liver chemistry, GCS and SAPS II were detected between survivors and non-survivors. Metabolic acidosis ($P < 0.005$) and DIC ($P < 0.05$) were also found

to correlate with poor outcome. On the contrary baseline lactate levels, pO_2 and WBC did not seem to predict the outcome.
Conclusion: As severe sepsis is responsible for significant mortality, determination of early prognostic markers of outcome is helpful to identify patients at high risk. Among the various baseline clinical and laboratory parameters, age, septic shock, renal failure, DIC and metabolic acidosis seem to have a significant impact on survival.

P924 Severe sepsis and septic shock in critically ill patients

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Valencia, E

Objectives: To know the prevalence of severe sepsis and septic shock among patients with bacteremia in an intensive care unit. To know their clinical and microbiology characteristics and prognosis, and to identify the risk factors for developing these forms of sepsis.
Methods: During a 43-month study period (June 1995–January 1999), we have evaluated all clinical significant ICU-bacteremias of a teaching hospital and specially those associated with severe sepsis and septic shock. These terms were defined following consensus conference of the American College of Chest Physicians, 1992. Clinical and microbiological variables were recorded. A multivariate analysis was performed to assess the risk factors for developing severe sepsis and septic shock using SPSS package (9.0).
Results: Among 166 intensive care bacteremias, 93 (56%) of them developed severe sepsis ($n = 66$) and septic shock ($n = 27$). The proportion of nosocomial acquired bacteremia was 79.4%. The mean age of the patients, 57 years for men and 36 years for women, was 63.4 ± 15.6 years. An ultimately fatal underlying disease was present in 33.4% of the patients. The most frequent focus of infection causing bacteremia were: respiratory tract (22.6%), intravascular catheter (17.2%), urinary tract (10.8%), and gastrointestinal tract (7.5%). In 28% of the cases, a source of bacteremia was not identified. One hundred and thirteen organisms were isolated, and four of them accounted for almost two-thirds of all isolates: *Acynetobacter baumanii* 19.3%, CNS 18.5%, *Escherichia coli* (14%), and *Enterococcus* spp. (12%). Global and related mortality in patients with septic shock were 74.1 and 25.9%, and in the group of severe sepsis were 54.5 and 24%, respectively. Multivariate analysis demonstrated that the communitarian origin of the bacteremia was the only risk factor for developing severe sepsis and septic shock.
Conclusions: Severe sepsis and septic shock were the forms of presentation of more than a half of ICU-bacteremias. A small number of organisms produced the majority of these cases. The communitarian origin of the bacteremia was the only predictive factor for developing these severe forms of sepsis.

P925 *Eubacterium lentum* and *Staphylococcus epidermidis* fatal septicemia in an adult

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Objectives: A case of *Eubacterium lentum* and *Staphylococcus epidermidis* fatal septicemia in an adult is presented.
Methods: Blood cultures were carried out by BACTEC system (Becton Dickinson). The identification of *E. lentum* was performed by Gram-stain, catalase test and API ID 32 A system (bioMerieux). The identification of *S. epidermidis* was carried out by standard methods and API ID 32 Staph system (bioMerieux). Susceptibility testing for *S. epidermidis* was performed by disk diffusion method. An 83-year-old woman with history of anoia who was living at nursing home was admitted to the hospital because of feeding inability. The clinical examination revealed cachexia, tachyarrythmia, swellings of the lower limbs and extensive decubitus ulcers in sacral region and hips, and less extensive in the back and lower limbs. The blood pressure was 100/60 mmHg and the temperature 37 °C. The laboratory examination revealed WBCs 9800/μL (90% neutrophils), Ht 37.2%, glucose 187 mg/dL and dehydration with urea 98 mg/dL, creatinine 1.1 mg/dL, Na 152 meq/l, K 3.7 meq/L. The ESR was 51 mm/1 h. Ampicillin/sulbactam (1.5 g × 3, i.v.) was administered to the patient. On day 3, two sets of blood cultures because of fever at 38 °C were taken. The therapy was replaced with tazobactam/piperacillin (4.5 g × 3, i.v.). *S. epidermidis* was isolated from four blood cultures, while *E. lentum* from two anaerobic cultures. *S. epidermidis* isolate was resistant to oxacillin, clindamycin, erythromycin, tetracycline, chloramphenicol and susceptible to ciprofloxacin, gentamicin, trimethoprime/sulfamethoxazole and rifampicin. Ciprofloxacin (400 mg × 2, i.v.) after the results of cultures was added. The patient on day 10 died because of septic shock.
Conclusion: The decubitus ulcers considered as a probable source of bacteremia, which is usually polymicrobial with participation of anaerobic microorganisms. *E. lentum* is an unusual cause of infections.

P926 Polymicrobial bacteremia in patients in a tertiary care hospital

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Gdansk, PL

Objectives: To establish the prevalence of polymicrobial bacteremia (pb) in patients of Public Hospital No. 1 in Gdansk.
Methods: We analyzed 7100 blood cultures, from January to November 2001 in two automated systems: BacT/Alert (Organon Tecknika) and Vital (bioMérieux). Blood cultures with more than one isolate, and with different isolates in blood samples drawn within 48 h, were recognized as polymicrobial bacteremia. We omitted coagulase-negative staphylococci with no obvious clinical importance.
Results: A total of 976 (14%) blood cultures were positive and in 83 we yielded more than one isolate. After analysis, we found 53 episodes of pb. There were 47 bacteremia with two different isolates, three with three isolates and three with four. Most of the pb were noted in hematology (32%) and ICU (22.6%). In surgery, eight were found (15%) and in the internal medicine nine (17%) – five of them from kidney transplant patients. Predominant pathogens were as follows: *S. epidermidis*: 29 isolates (25%); enterococci: 18 (15.6%); *P. aeruginosa*: 13 (11.3%); and *E. coli*: 10 (8.7%).
Conclusion: We observed polymicrobial bacteremia in most serious patients with many risk factors (immunosuppression, mechanical ventilation, vesical and central vascular catheters). They were hospitalized for a long time and often experienced multiple bacteremia, both poly- and monomicrobial. We consider that frequent blood cultures enable accurate diagnosis and optimal treatment in these patients.

P927 Outcome in critically ill patients with bacteremia involving *Klebsiella* species: results of a matched cohort study

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Objectives: To evaluate the clinical impact of *Klebsiella bacteremia* in critically ill patients admitted during a 9-year period.
Methods: A matched cohort study was performed. All ICU patients with *K. bacteremia* were defined as cases. Matching of the control patients (1 : 2 ratio) was done on the basis of the APACHE II system: an equal APACHE II score (±1 point) and admission diagnosis. As expected mortality can be derived from the severity of the disease scoring system, this matching procedure results in an equal expected mortality for cases ($n = 50$) and controls ($n = 100$).
Results: Following the matching procedure, cases and controls had nearly equal APACHE II scores (respectively, 21 ± 8.1 vs. 21 ± 7.9; $P = 0.903$) and related expected mortality (respectively, $36 \pm 26.4\%$ vs. $36 \pm 26.5\%$; $P = 0.979$). Patients with *K. bacteremia* had more acute renal failure (30% vs. 14%; $P = 0.019$) and hemodynamic instability (80% vs. 49%; $P < 0.001$). They had a longer ICU stay (38 ± 31.6 vs. 11 ± 16.7 days; $P < 0.001$) and a longer ventilator dependence (28 ± 25.6 vs. 8 ± 13.0 days; $P < 0.001$). There was no significant difference between cases and controls in age (respectively, 52 ± 15.9 vs. 56 ± 18.6; $P = 0.225$) and prevalence of acute respiratory failure (88% vs. 79%; $P = 0.176$). In-hospital mortality rates for cases and controls were nearly equal (respectively, 36% vs. 37%; $P = 0.905$). Logistic regression analysis demonstrated acute renal failure (OR: 3.7, 95% CI: 1.5–9.0; $P = 0.004$) and increasing age (OR: 1.02, 95% CI: 1.00–1.05; $P = 0.048$) to be independent predictors of mortality.
Conclusions: After accurate adjustment for severity of underlying disease and acute illness, no difference in mortality was found between ICU patients with *K. bacteremia* (36%) and their matched control subjects (37%). Bacteremia involving *Klebsiella* species does not adversely affect the outcome in critically ill patients.

P928 The frequency of isolation and resistance patterns of microorganisms from 5637 blood cultures

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Objectives: To analyze the frequency of isolation and resistance patterns of microorganisms isolated from 5637 blood cultures.
Methods: A total of 5637 blood cultures were processed by BacT/Alert system (Organon Teknika) during 1998–1999. The isolates were identified by conventional methods. Susceptibility testing was performed by Kirby–Bauer disk-diffusion method following NCCLS guidelines. For the isolates of enterococci that were resistant or intermediate to vancomycin by disk-diffusion and/or agar-screen test were determined minimal inhibitory concentration for vancomycin and teicoplanin by broth-dilution method. For the detection of extended-spectrum β-lactamase (ESBLs), we used the double-disk synergy test, based on synergy between β-lactamase inhibitor clavulanate and cefotaxime, ceftriaksone, and ceftazidime.
Results: The most frequently isolated microorganisms among a total of 1743 isolates from 1278 patients/episodes were: coagulase-negative staphylococci (CNS), 536 isolates from 405 patients (31.7%); *Staphylococcus aureus* (Sa), 289/187 (14.6%); *Enterococcus* spp., 171/128 (10.0%); *Acinetobacter* spp., 115/78 (6.1%); *Corynebacterium* spp., 69/63 (4.9%); *Klebsiella* spp., 85/59 (4.6%); *Candida* spp., 86/57 (4.5%); *Escherichia coli*, 62/48 (3.7%); *Pseudomonas aeruginosa*, 49/32 (2.5%); and *Streptococcus viridans* group 42/29 (2.3%). Gram-positive bacteria were isolated more frequently (66.4%) than Gram-negative bacteria (24.8%). Among CNS (416) and Sa (199), there were no strains resistant to vancomycin, but 76.2% of CNS and 80.5% of Sa were resistant to oxacillin. High level of aminoglycoside resistance among enterococci (69) was: 65.2% to gentamicin, 50.7% to streptomycin, and 36.2% to both of the drugs. Only one strain among enterococci was resistant to vancomycin (*Enterococcus faecium*, VanA phenotype). Among *Enterobacteriaceae* (128) and *Acinetobacter* spp. (85), no strains were found resistant to imipenem, but the rate of resistance among *Pseudomonas* spp. (48) was 39.6%. The rate of ESBLs producers among 46 *Klebsiella* spp. strains was 84.8%. All Gram-negative aerobic bacilli, except *E. coli*, expressed high frequency of resistance to ceftriaxone, aminoglycosides, and ciprofloxacin.
Conclusions: The multiresistant 'problem' bacteria dominated as the agent of bacteremia in our hospital that reflected ecosystem of the hospital.

Miscellaneous nosocomial infections

P929 Infections after open-heart surgery

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Objective: The aim of this study is to know the rates of nosocomial infection (NI) in patients operated on open-heart surgery and to compare with those of National Nosocomial Infection Surveillance (NNIS)
Patients and methods: The 222 patients operated on open-heart surgery in the hospital of Basurto between January & December 2000 were included. Preoperative protocol regarding infection control includes: shower with 4% chlrohexidine soap the night before surgery and repeated the day of surgery; and shave just before surgery. Antibiotic prophylaxis: cefuroxime 1.5 g. i.v. 8 h beginning 30 min before surgery until vascular catheters are withdrawal. The infection-control team makes infection surveillance. All the patients undergoing open-heart surgery are prospectively studied since the day they are operated until the end of the episode. A computer-based surveillance system, INOZ, designed for the incidence studies of NI is used during admission and continued 1 year after discharge. CDC definitions of nosocomial infection are used. All the nosocomial infections not only surgical-site infections (SSI) are recorded. NNIS SSI-risk score is used and results are compared with those from NNIS reports.
Results: Age and sex: 147 (66.2%) men, mean age 66.6 years. Mean preoperative stay in the hospital 6.8 days (SD 9.5). NNIS score 0, 7 patients; score 1, 156 patients; score 2, 59 patients; and score 3, 0 patients. Infection and admission: nine patients (three urinary tract, three respiratory, one skin and two endocarditis). Nosocomial infections: 55 patients acquired 68 NI, 13 of them were SSI. Surgical site infection: three incisional superficial, 5 deep incisional and 5 organ space. NNISS score 0, 0% score 1, 5.8%, score 2, 6.8%. Microorganisms: *S. aureus* three, CNS three, polimicrobial two, *S. marcescens* one, *Propionibacterium* one. Urinary tract infections (UTI): 13 patients, nine cases in patients undergoing valvular replacement. *E. coli* (4) and *E. faecalis* (4) were the most frequent etiological agents. Respiratory infections: 22 patients. Pneumonia: five cases. Bacteremia: seven cases 1 catheter related, 3 secondary to surgical site infection, one secondary to pneumonia and two primary bacteremias. Microorganisms: *S. marcescens*, *P. aeruginosa*, *S. epidermidis*, *S. aureus*, *Candida* spp., *E. cloacae* and *E. coli*.
Conclusions: Incidence of surgical infection is lower in patients with valvular replacement than in CABG. Comparing our data of SSI with those of NNIS report the results are below the rates of percentile 90.

P930 Risk factors for wound infection in oral-cavity cancer surgery with neck dissection

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Objective: Information about the wound infection (WI) in oral-cavity cancer surgery is limited. The goal of this prospective study was to determine risk factors predisposing to WI among patients with oral-cavity cancer who underwent surgical procedure with neck dissection.
Methods: Eighty-one consecutive patients (77.8% male, 22.2% female, mean age 60.43 $\pm$ 12.37 years) with oral-cavity cancer who underwent surgery with neck dissection were prospectively evaluated in the 1999–2000 period. In all cases, surgery was performed with curative purpose. Antimicrobial prophylaxis was carried out with amoxicillin–clavulanate. WI was defined as the presence of purulent drainage from the wound. For each patient, we look after the link between WI and several variables. Statistical analysis was made using χ^2- and Mann–Whitney tests.
Results: Thirty-five WI were recorded during the study period, resulting in an overall rate of 43.2%. Univariate analysis investigating the link between WI and possible risk factors revealed the following results: age ($P=0.75$), sex ($P=0.13$), chronic underlying disease ($P=0.36$), current smoker ($P=0.02$), chronic pulmonary disease ($P=0.60$), chronic alcoholism ($P=0.33$), chronic hepatic disease ($P=0.41$), diabetes mellitus ($P=0.39$), intraoperative corticotherapy ($P=0.14$), ASA score ($P=0.23$), T stage ($P=0.002$), N stage ($P=0.60$), postoperative corticotherapy ($P=0.61$), NNIS index ($P<0.0001$), lymphocyte count (0.33), duration of total preoperative stay ($P=0.11$), and duration of surgical procedure ($P<0.0001$).%. Patients with WI had a median postoperative hospital stay of 28.48 $\pm$ 13.12 days compared with 10.63 $\pm$ 5.08 days for whose without WI ($P<0.0001$).
Conclusion: The incidence of WI in oral cavity cancer surgery with neck dissection is very high. The most closely related factors to WI are advanced T stage, NNIS index and duration of surgical procedure. WI significantly increases the length of hospital stay.

P931 Surgical site infections in a cardiothoracic surgery ward: incidence and risk factors

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Objectives: To determine median sternotomy-site infection rate and safenectomy site infection rate in a Cardiothoracic Surgery Ward, we observed patients submitted to coronary artery bypass graft surgery (CABG) and patients submitted to cardiac valves interventions during a five month surveillance.
Methods: Patients admitted to Cardiothoracic Surgery Ward of Azienda Ospedaliera S. Maria della Misericordia in Udine, Italy – from 15th August 1999–15th January 2000 and submitted to surgical treatment, were observed according to these criteria: procedures were codified with specific codes (between 35.20 and 35.2 8 – for CABG – or between 36.10 and 36.19 – for cardiac valves – of ICD-9-cm classification); procedures were executed during a single access to the operating room; procedures involved at least one skin incision; wounds were closed by primam intentionem (at the end of the intervention, before leaving the operating room). Patients undergoing more than one procedure in different operating rooms were excluded. Patients under surveillance were followed during their length of stay in hospital and evaluated about surgical site infection. Surveillance continued after discharge during programmed outpatient controls were used CDC definitions of surgical site infections.
Results: Were collected 177 observation forms, whose 98 were patients undergoing CABG, 60 were patients undergoing cardiac valves interventions and 19 were patients undergoing both types of intervention in the same session. The incidence of surgical site infection was 5.6% as a whole; 8.2% for CABG (4.1% in sternotomy site and 4.1% in safenectomy site), 3.3% for cardiac valves interventions, none for both interventions associated. No deaths after wound infections were observed. Variables associated with infection by univariate analysis ($P < 0.01$) were: diabetes (OR 4.05; 95% CI between 1.2 and 16.5); obesity (OR 9.3; 95% CI between 1.6 and 25.03).
Conclusions: Rates observed are higher then NNIS System and represent a significant complication in our hospital. This fact induces us implementing a continuos surveillance system in Cardiothoracic Surgical Ward.

P932 Microbiology of nosocomial and community-acquired spontaneous bacterial peritonitis

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Clichy, F

Objectives: Ascitic fluid infection is a frequent complication in cirrhotic patients with ascites. The aim of the study was to compare the microbiological characteristics of nosocomial and community-acquired episodes of spontaneous bacterial peritonitis.
Methods: We retrospectively reviewed 95 consecutive episodes of ascitic fluid infection that occurred in 75 patients. Seventy episodes were bacteriologically documented (45 spontaneous bacterial peritonitis and 25 bacterascites). Fifty-three (55.8%) were nosocomial and 42 (44.2%) were community-acquired.
Results: The 78 isolated pathogens included 40 Gram-positive cocci (34 streptococci/enterococci, 6 *Staphylococcus aureus*), 35 Gram-negative bacilli (including 23 *Escherichia coli*), 2 Gram-positive cocci (one *Listeria monocytogenes*, one *Rothia dentocariosa*) and one yeast. There was no difference between spontaneous bacterial peritonitis and bacterascites regarding flora. Streptococci were more frequent in community-acquired episodes (53.8%) than in nosocomial episodes (33.3%). Gram-negative bacilli were significantly more frequent in nosocomial episodes than in community-acquired episodes (56.4% vs. 33.3%, $P < 0.05$). The patients receiving norfloxacin prophylaxis had more frequently Gram-positive organisms (68.7% vs. 51%) than did the patients without prophylaxis, but this difference did not reach statistical significance. Nosocomial isolates were significantly more resistant to amoxicillin–clavulanate (48.7% vs. 18.4%, $P < 0.01$) and cefotaxime (33.3% vs. 13.2%, $P < 0.05$) than community isolates, but there was no difference regarding resistance to ciprofloxacin.
Conclusions: There are significant differences in microbial epidemiology between nosocomial and community-acquired episodes of spontaneous bacterial peritonitis. The empirical treatment strategy should differ according to whether the infection is acquired in the hospital or in the community.

P933 Ceftazidime in patients with nosocomial pneumonia and septicemia: results of a German surveillance study

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Objectives: Life-threatening infections like nosocomial pneumonia and septicemia require immediate antibiotic treatment. International guidelines recommend initial therapy with an anti-pseudomonal broad-spectrum beta-lactam alone or in combination with an aminoglycoside or a fluoroquinolone. Ceftazidime is one of the drugs of choice. It has been successfully investigated in numerous clinical trials. Besides the favorable in vitro data against the most frequent pathogens including *Pseudomonas aeruginosa*, it is important to survey and document the clinical efficacy in patients under day to day routine conditions in hospital.
Methods: The present retrospective multicenter surveillance study comprised 581 patients with nosocomial pneumonia and 168 septicemia patients, recruited from February 2000 till February 2001 in German hospitals. Sixty-four percent (pneumonia) and 76% (septicemia) under intensive care were mechanically ventilated (31, 43%, respectively).
Results: Median duration of ceftazidime therapy was 9 days, median dosage 6 g. Thirty-three percent of pneumonia patients and 19% of septicemia patients received ceftazidime alone, combination therapy was used, mainly with aminoglycosides. The most frequently isolated pathogens were *Pseudomonas aeruginosa* (45%) followed by other Gram-negative pathogens (17%), *E. coli* (10%), *S. aureus* (14%), other Gram-positive pathogens (11%) and *S. maltophilia* (3%). The overall clinical-success rate at the end of therapy was 85% (47% cure, 38% improvement) in pneumonia and 76% (44% cure and 32% improvement) in septicemia. Overall tolerability was very good.
Conclusion: The study confirms the high efficacy and good tolerability of ceftazidime in hospitalized patients with nosocomial pneumonia and septicemia under day to day clinical routine conditions.

P934 *Escherichia fergusonii* strains isolated from clinical specimens

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Objective: The aim of this study is to describe two strains of *Escherichia fergusonii* isolated from the pus of an infected surgical wound of a 31-year-old woman operated at the Trauma Department of Central Military Hospital-Tirana, Albania. for '*Pseudoarthrosis cruris*'. The presented data indicates the pathogenic potential of *Escherichia fergusonii*, which is a very rarely isolated agent.
Materials and methods: Six days after surgical treatment, signs of an evident wound infection were present and from two pus specimens, taken with a 4-day interval, *E. fergusonii* was isolated. Conventional biochemical tests were performed. These strains were transported to Department of Microbiology, Gulhane Military Medical Academy for confirmation. By using conventional methods and API ID 32 GN strips confirmed as *E. fergusonii*. All the MIC determinations of these strains were performed at the same department by using E-test strips.
Results: Both strains demonstrated the morphological, cultural and biochemical characteristics of the family Enterobacteriaceae: also manifested biochemical features of the genus *Escherichia*. They are distinguished from *E. coli* by their ability to ferment adonitol and cellobiose and failure to ferment sorbitol. The three above-mentioned characteristics, combined with positive test for ornithine decarboxylase and negative tests for lactose, sucrose, raffinose and melibiose fermentation are the most important features for the identification of isolated strains as *Escherichia fergusonii*. It might be stressed that both strains showed the same biochemical profile and also the same antibiotic susceptibility pattern being susceptible to ampicillin, cefazolin, amoxicillin–clavulanic acid, ciprofloxacin, levofloxacin, amikacin, cefotaxime, netilmicin, imipenem, meropenem, ceftriaxone, nitrofurantoin, mezlocillin, gentamicin, and ceftazimide.
Conclusion: Little is known about clinical significance of some very rare isolation in patients with sepsis, urinary tract infections or diarrhea suggest its pathogenic potential. The data given in this study clearly indicate the role played by *E. fergusonii* as etiologic agent in a surgical wound infection.

P935 Monotherapy vs. combination therapy in *Pseudomonas aeruginosa* bacteremias: influence on the outcome

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Background: Infections caused by *P. aeruginosa* are very aggressive and the development of resistance to antimicrobials during the treatment is common. To overcome these problems many authors recommend the combined use of several active antibiotics.
Objective: To compare the efficacy of monotherapy (M) and combination (C) therapy in the treatment of PAB.
Methods: During a 7-year period, 211 consecutive cases of PAB were included. Microbiologic studies were performed with standard methods. Epidemiological factors, baseline disease and clinical parameters were recorded. The decision of treating and the choice of antibiotic was not randomized and taken depending on the epidemiological and clinical data. We excluded 21 patients who did not receive any active antimicrobial against *P. aeruginosa*. Minimum follow-up was 1 month after the end of antimicrobial treatment. Cure was considered when there were no relapses in the month after completing treatment or related death is (defined as occurring while the infection was active). Comparison between groups was done with de Chi-square test.
Results: Patients who received M were 132 (69%), and 58 (31%) different combinations of antibiotics. Antibiotics used as M were ceftazidime (52, 39%), ciprofloxacin (31, 23%), imipenem (24, 18%), piperacillin/tazobactam (21, 16%) aminoglicosides (4, 3%). No differences in mortality or relapse rate were found depending on the choice of antibiotics. In group C, ceftacidime plus amikacine were mainly used (32, 58%). No significant differences for sex, age, nosocomial acquisition, length of stay, origin, previous procedures, or presence of complications were found between patients receiving unique or several antimicrobials. No statistically significant differences in the cure rate (M 80, 60%; C 33, 57%), relapses (M 19, 14%; C 5, 9%) and related deaths (M 34, 26%; C 20, 34%).
Conclusions: In our study, M and C therapy have a similar effectiveness in the treatment of PAB.

P936 *Serratia marcescens* nososcomial infections: epidemic investigation using pulsed field gel electrophoresis

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Between February and December 1999, 30 strains of *Serratia marcescens* where isolated from 30 patients throughout Rangueil hospital in Toulouse, France which is a large 1000-bed teaching hospital. Bacterial isolates where identified within current phenotypic methods and antimicrobial susceptibility was tested. Because strain typing was necessary, a specific genotyping tool based on pulsed field gel electrophoresis (PFGE) was carried out. Only two isolates out of 30 had the same clonal origin. This result indicated no cross-transmission between infected patients. This typing method was used once again during another outbreak of *S. marcescens* infections occurring in the neurosurgery department during 2001 (April–December). Among 46 clinical strains isolated from 7 patients, a genetic link was demonstrated for 6 patients. The outbreak investigation identified the patient responsible for introducing the outbreak strain: he was hospitalized at first from November 2000 to February 2001, and then from April to September 2001. The antibiomicrobial susceptibility revealed a resistance evolution of his isolates which was also found for all clinical isolates through the episode (i.e. gentamicin resistance). The implementation of control measures jugulated the outbreak on December 2001. This recent episode allowed us to validate the PFGE method as a useful epidemiological tool.

Fungi and fungal infections

P937 Encapsulation of *Cryptococcus neoformans* inhibits maturation and activation of human dendritic cells

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Objective: The capacity of dendritic cells (DC) to function as antigen presenting cells is unrivalled. They play a pivotal role in initiating T-cell response to microbial antigens. In this study we evaluated the ability of encapsulated and acapsular strains of *Cryptococcus neoformans* to activate DC derived from monocytes stimulated with GM-CSF and IL-4. We observed profound differences in the response of DC to encapsulated and acapsular *C. neoformans* strains.
Methods: Human DC were obtained from monocytes cultured in the presence of GM-CSF plus IL-4. Two strains of *C. neoformans* (*C. neoformans* var. *neoformans* serotype D ATCC B3501 and its parental acapsular strain, CAP67) were used to stimulate DC.
Results: The acapsular strain was easily phagocytosed by immature DC and the process induced several molecular markers characteristic of mature DC such as MHC Class I, Class II, CD40. In contrast, there was no up-regulation of MHC Class I and Class II molecules by exposing DC to encapsulated cells. However, the addition of monoclonal antibody to capsular material (glucuronoxylomannan) to encapsulated strain promoted ingestion of *C. neoformans* by DC and facilitated APC maturation by enhancing MHC Class II, CD40 and CD86 expression through a process mediated by engagement of Fc-γ RII (CD32) and Fc-γ R III (CD16). This event led to optimal autologous T-cell activation and differentiation as documented by enhancement of lymphoproliferation and IFN-γ production.
Conclusions: Encapsulation of *C. neoformans* has an inhibitory effect on DC activation and maturation. This event could have profound implications given the preeminent role of DC in initiating the immune response against pathogens.

P938 A *Cryptococcus luteolus* strain isolated from endoscopic sinus surgery specimens of an apparently normal host

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Almost all pathogenic fungi produce air-borne conidia and via inhalation, they can enter and adhere to the mucosal surfaces of the nose and sinuses. The host immune status and the virulence of fungus are the major factors in determining the pathogenic entity. In recent years, fungal infections of the sinuses in both their invasive and non invasive forms appear to be increasingly common and normally saprophytic fungi may invade patients with underlying disease. We report a *Cryptococcus luteolus* strain isolated from endoscopic sinus surgery specimen of a patient without any underlying disease. A 45-year-old male patient, being neither diabetic nor human immunodeficiency virus positive, without underlying disease, presented with a chronic sinusitis history. There was no history of previous surgery, facial trauma, distant travel, or drug abuse. The tissue biopsy specimen obtained at functional endoscopic sinus surgery (FESS) was examined. In direct examination of microscopic slides of the specimen stained with Gram, Ehrlich–Ziehl–Neelsen, methylene blue, Giemsa techniques revealed many thinly encapsulated globose to elliptic yeast cells. Aseptically cut pieces of specimen were inoculated onto Sabouraud glucose agar, brain heart infusion agar, cooked sheep's blood agar, corn meal agar and *Guizotia abyssinica* extract agar media. The plates were incubated at 35, 30, and 25 °C. After 4 days cultures showed pink-colored yeast-like colonies grown just around the embedded pieces of tissue on all plates. The fungus was isolated as a single microorganism from all pieces and was identified as *C. luteolus* using classical mycological and biochemical methods. Owing to the patient's immunosuppressed state, inhaled fungus elements even apathogenic ones, can easily spread beyond the sinus region and may eventually lead to fulminate disease. However, in the present case, a normally

apathogenic fungi, *Cryptococcus luteolus* was isolated from FESS specimen of immunocompetent patient who has radiologically confirmed sinusitis with intractable recurrent symptoms despite the adequate treatment for bacteriological sinusitis. The fungi spectrum which have caused fungal sinusitis is broad. To our knowledge, C. *luteolus* have not previously been reported in human infection in the world.

P939 Serial isolates from a clinical case of recurrent *Cryptococcosis* exhibit differential virulence in mice

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Aim: The present work was aimed at comparing serial isolates of *Cryptococcus neoformans* (Cn) obtained from a clinical case of recurrent meningoencephalitis for virulence in mice.
Background: Recently, we provided evidence of 'microevolution' in a clinical case of recurrent Cn meningoencephalitis (Eur J Clin Microbiol Infect Dis, 20: 535–43, 2001). When comparing for genotypic and phenotypic characteristics, three serial isolates, obtained from an AIDS patient during a 3-year period, it was shown that the same microorganism persisted throughout this period, yet over time, it acquired resistance to phagocytic cells and capacity to interfere with cytokine response (impairment of IL-12 and enhancement of IL-10 production). Antifungal drug resistance remained unchanged in the three isolates, whereas extracellular enzyme production was enhanced in the relapse isolate with respect to the others.
Methods: The clinical isolates, 1525 and 1526 (blood and cerebrospinal fluid isolates from the first episode) and 1782 (cerebrospinal fluid isolate from the relapse), were inoculated intranasally (i.n.) or intracerebrally (i.c.) in adult naive mice and in iron overloaded mice (FeDextran 50 mg/kg, 10×). The outcome of the infections was evaluated as organ fungal load (cfu assay), cytokine content (ELISA) in tissues, mean survival time (MST) and percent survivors.
Results: The results of i.n. infection showed that the 1525 was the most lethal isolate, being the phenomenon associated with a rapid and irreversible colonization of the lung. Differently, both 1526 and 1782 colonized the lung transiently and to a similar extent; yet, 1782 exhibited the most marked neurotropism. This was confirmed by the i.c. infection model, where 1782 caused the most rapid brain colonization and the lowest MST with respect to the other isolates. Immune system disturbance by iron over-load exacerbated the outcome of all the infections to a similar extent. Cytokine content assays in tissues revealed that IFNg was produced in a fungal load-dependent manner, IL-12 was down-regulated by the infection especially in iron overloaded mice.
Conclusion: The 'microevolution' phenomenon, recently documented in serial isolates of Cn from an AIDS patient, allows fulfillment of phenotypes with enhanced virulence in mice, thus emphasizing the role of Cn biomolecular plasticity in pathogen persistence/reactivation and clinical relapse.
Acknowledgments: Work supported by the IX Progetto AIDS-ISS, 1998 50B.2

P940 Adhesion of *Aspergillus* spp. to biomaterials

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Introduction: The incidence of deep seated aspergillosis is increasingly growing in critical care patients, with most of the pathogenic mechanisms remaining yet unknown. Adherence to liquid hydrocarbons is considered an indirect method to assess microbial cell surface hydrophobicity (CSH), which is believed to have an important role in the adherence of pathogenic microorganisms to host cell or foreign surfaces, namely plastic.
Objective: To determine the potential ability of clinical isolates of Aspergillus to adhere and colonize the surface of medical indwelling devices, namely plastic and silicon rubber.
Materials and methods: Both resting and germinated spores (5 and 11 days old) of clinical isolates of *A. fumigatus* (three strains), *A. niger* (two strains) and *A. flavus* (two strains) were assayed (in an assay for hydrocarbon adhesion 1) for the capacity to adhere to *n*-hexadecane and xylene. The adhesion to silicon (silicon oil) was assayed according to a previously developed assay 2. All tests were run in triplicate.
Results: Non-germinated spores showed a significant adhesion to *n*-hexadecane and xylene and similarly to silicon, which did not change significantly with age. However, following germination and initial hyphal growth, both CSH and silicon adhesion increased significantly, in all tested strains.
Conclusions: *Aspergillus* shows a promoted adhesion to biomaterials of plastic nature as well as silicon materials, like vascular catheters or peritoneal shunts, particularly following hyphae formation. Such a fact may be evoked to help to explain colonization and infection of medical indwelling devices like those seen in critical care patients.

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P941 Invasive aspergillosis (IA) in the critically ill: is there a relationship between predisposing risk factors (RF) and outcome?

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Objectives: To investigate outcome in ICU patients with IA. A comparison was performed between patients with specific ($n = 17$) versus nonspecific RF ($n = 21$) for the acquisition of IA.
Methods: Retrospective cohort study (7/1997–12/2000). IA was diagnosed when proven on histology or in case of an abnormal chest radiography or CT thorax with predisposing specific RF or positive findings (microscopy or culture) on a bronchoalveolar lavage. Haematological malignancy, neutropenia and immunosuppressive therapy were considered as specific RF for the acquisition of IA. Previous lung damage (COPD, viral pneumonia, ARDS), liver failure, burns, severe bacterial infection and malnutrition were categorized as nonspecific RF in previously immunocompetent patients.
Results: All patients with IA were mechanically ventilated. Patients with specific RF had higher APACHE II scores (31 ± 7.6 vs. 22 ± 8.4; $P = 0.003$) and related expected mortality ($69 \pm 21.3\%$ vs. $44 \pm 26.8\%$; $P = 0.009$). No difference between patients with specific versus nonspecific RF were found in age (53 ± 15.3 vs. 56 ± 13.4 years; $P = 0.780$), acute renal failure (respectively, 53% vs. 42%; $P = 0.360$), hemodynamic instability (respectively, 82% vs. 86%; $P = 0.778$), length of ICU stay prior to the first positive culture (respectively, 7 ± 7.6 vs. 6 ± 6.2 days; $P = 0.506$), total length of ICU stay (respectively, 23 ± 22.4 vs. 26 ± 24.0 days; $P = 0.638$), length of ventilator dependence prior to the first positive culture (respectively, 6 ± 7.3 vs. 6 ± 6.4 days; $P = 0.975$) and total length of ventilator dependence (respectively, 19 ± 17.4 vs. 24 ± 22.6 days; $P = 0.360$). Ninety-four percent of the patients with specific RF received appropriate antifungal therapy versus 86% of patients with nonspecific RF ($P = 0.401$). For the outcome comparison, no difference was found between patients with specific and nonspecific RF in 14-days mortality (respectively, 71% vs. 57%; $P = 0.393$), 28-days mortality (respectively, 82% vs. 67%; $P = 0.275$) and in-hospital mortality (respectively, 82% vs. 71%; $P = 0.431$). In a logistic regression analysis no variables were found to be independently associated with mortality. This is probably owing to the small sample size.
Conclusion: No difference in mortality was found between patients with specific and nonspecific RF for the acquisition of IA. The absence of an association between RF for IA and death could be owing to insufficient study power. Furthermore, data are difficult to interpret because of the heterogeneous nature of nonspecific RF for IA.

P942 Albumin promotes germination and mycelium formation by *Aspergillus fumigatus*

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Introduction: *Aspergillus* spp. are increasingly common agents of fungal infections in immunocompromised patients, with *A. fumigatus* being the most frequently isolated species, particularly in case of invasive disease.
Objective: To evaluate the effect of albumin on germination of spores of *A. fumigatus* and *A. flavus*.
Material and methods: Spores (5 and 11 days old) of clinical isolates of *A. fumigatus* (4 strains) and of *A. flavus* (2 strains) were assayed for germination and hypha formation in presence of 2 and 4% albumin (human and bovine), in RPMI 1640 medium, at 37 °C.
Results: A significant, dose-related, promotion of germination rate and of hypha formation was observed, shortly after 6-h of incubation, particularly with 5 days old spores of *A. fumigatus*. *A. flavus* demonstrated a considerably slower rate of germination in comparison to *A. fumigatus*, which was further

decreased in presence of albumin. Similar results were found both in human and bovine albumin.
Conclusions: Our results may help to justify the mechanisms of infection and the interspecies variability commonly found among *Aspergillus* regarding its pathogenic potential. Albumin, by promoting mycelium formation, enhances the pathogenic potential by *A. fumigatus*, a fact that may be evoked to explain its common involvement in invasive infection.

P943 Identification and antifungal susceptibility testing of fungal strains isolated from chronic rhinosinusitis

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The fungal revolution taking place in otorhinology, inspired a study of chronic rhinosinusitis (CRS) patients (with or without polyposis), and the frequency of occurrence of fungi in their nasal mucus. This prospective study assessed, the incidence of eosinophilic fungal sinusitis (EFS) in CRS patients. A total of 96 samples were examined from patients from EFS and CRS. In 74 cases mucus was collected noninvasively, and in 22 cases during operation. The Gram-stained direct smears of all samples were also evaluated. Bacteria and fungi colonizing in the mucus were detected by culturing method. The special mould plates were incubated at 30 °C and allowed to grow for 30 days. Finally, were the total IgE levels determined. The control group consisted of the nasal secretion from 50 healthy volunteers. Typical aerobic pathogenic bacteria could be isolated from 34 patients. Fifty-seven aerobic bacteria were isolated, i.e. 1.6 bacteria/positive patient with a maximum of three different bacteria/sample. The isolated bacteria were *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *S. agalactiae* and *E. faecalis* strains. Yeasts and moulds could be detected from 79 patients (83%): *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *Aspergillus* spp., *Cladosporium* spp., *Geotrichum candidum*, *Penicillium* spp., *Scopulariopsis* spp. and *Zygomycetes*. 237 yeasts and moulds were isolated, i.e. 3.0 different fungi/positive patient, with a maximum of five different fungi/sample. In the control group no were aerobic pathogens isolated, but only apathogens. Fungi were isolated from 22 patients (44%). Antifungal susceptibility testing of the yeasts and moulds is complicated by drug, organism and medium-specific factors. The E-test (AB Biodisk) is a new agar-based MIC method that makes use of a plastic strip with a preformed exponential concentration gradient of an antifungal agent. E-test strips for fluconazol, itraconazol, ketaconazol and amphotericin B were placed on separate plates.

P944 Adhesion of *Saccharomyces cerevisiae* to biomaterials

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Introduction: An increase in fungal infections has been registered in late years, particularly in intensive care patients, in whom is common the widespread use of medical indwelling devices. Among etiological agents, the yeast *Saccharomyces cerevisiae* is being registered with increasing frequency. Adherence to liquid hydrocarbons is considered an indirect method to assess microbial cell surface hydrophobicity (CSH), which is believed to have an important role in the adherence of pathogenic microorganisms to host cell or foreign surfaces, namely plastic.
Objective: To determine the potential ability of clinical isolates of *S. cerevisiae* to adhere to components of medical indwelling devices, namely plastic and silicon rubber.
Materials and methods: Blastoconidia of eight clinical isolates of *S. cerevisiae* were assayed (in an assay for hydrocarbon adhesion 1) for the capacity to adhere to *n*-hexadecane and xylene. The adhesion to silicon (silicon oil) was assayed according to a previously developed assay 2. All tests were run in triplicate.
Results: Seven strains showed a significant adhesion to *n*-hexadecane and xylene and similarly to silicon. The single strain with reduced CSH, thus revealing a hydrophilic behavior, corresponded to a vaginal isolate (recurrent vaginosis), which however, showed a comparable greater adherence to silicon oil.
Conclusions: *S. cerevisiae* shows an overall CSH that predicts its promoted adhesion to biomaterials of plastic nature as well as silicon materials, like vascular catheters or peritoneal shunts. Such a fact may be evoked to help to explain its increasing isolation from critical care patients, frequently carriers of plastic or similar foreign bodies.

References

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P945 Species distribution and antifungal susceptibility pattern of yeasts and moulds isolated at Cerrahpasa Medical Faculty Department of Microbiology and Clinical Microbiology Deep Mycoses Laboratory between 01 April 1999–27 March 2001

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The purpose of this study was to characterize the species distribution and antifungal susceptibility patterns of yeast and mold strains isolated at a Turkish University Hospital deep mycoses laboratory. The species distribution and antifungal susceptibility patterns of the clinically significant yeast and mold isolates recovered from deep mycoses suspected patients' specimens over a 2-year period determined against amphotericin B (AMB), fluconazole (FLC), itraconazole (ITZ), ketoconazole (KTZ), miconazole (MCZ), flucytosine (5FC) and terbinafine (TRB) using the National Committee for Clinical Laboratory Standards M27-A and M38-P reference methods. A total of 146 yeast and 12 mold strains were isolated. *C. albicans* strains were 66% (97/146), and nonalbicans *Candida* species were 33.6% (49/146). The frequency of various yeast species identified was *Candida catenulata* 1 (0.7%), *C. glabrata* 6 (4.1%), *C. kefyr* 4 (2.7%), *C. krusei* 6 (4.1), *C. lipolytica* 3 (2.0%), *C. lusitaniae* 1 (0.7%), *C. parapsilosis* 8 (5.5%), *C. rugosa* 2 (1.4%), *C. tropicalis* 12 (8.2%), *Malassezia furfur* 1 (0.7%), *Trichosporon beigelii* 3 (2.0%), *Cryptococcus neoformans* 2 (1.4). The frequency of mold species identified *Aspergillus flavus* 4 (33.3%), *A. fumigatus* 2 (16.6%), *A. niger* 3 (25%), *A. versicolor* 1 (8.3%), *Cladosporium cladosporioides* 1 (8.3%), *Scopulariopsis candida* 1 (8.3%) and *Cladosporium* sp. (8.3%). Resistance to FLC (MIC higher than or equal to 64 µg/mL) as per NCCLS criteria was observed in 21 *C. albicans* (21.6%), 19 nonalbicans *Candida* strains (38.8%); resistance to ITZ (MIC higher than or equal to 1 µg/mL) in 31 *C. albicans* (32.2%), 15 nonalbicans Candida (30.6%) and resistance to 5FC (MIC higher than or equal to 32 µg/mL) in 5 *C. albicans* (5.1%), 7 nonalbicans *Candida* strains (14.3%). Multiresistant strains evaluated as resistant to one or more azole antifungal agent along with AMB were 37 *Candida* isolates. Of those, 29 was found in vitro resistant to FLC and AMB and 14 of them was *C. albicans*. Other multipresistant strains and their frequency was *C. catenulata* (1/1), *C. glabrata* (1/6), *C. krusei* (5/6), *C. lusitaniae* (1/1), *C. tropicalis* (6/12). The present study indicates that *Candida* spp. are emerging as important pathogens in immunocompromised patients and the tendency of yeasts to develop resistance to antifungal agents and the appearance of species specific susceptibility of molds, in vitro tests seem to be useful for patient management.

P946 Comparison of microdilution and E-test with reference broth macrodilution method for antifungal susceptibility determination of *Candida*, *Cryptococcus*, *Trichosporon* and *Malassezia* spp. strains

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In the present study, a comparison of microdilution and E-test with reference macrodilution method was performed to determine antifungal susceptibility of clinical *Candida*, *Cryptococcus*, *Trichosporon* and *Malassezia* strains against amphotericin B (AMB), fluconazole (FLC), itraconazole (ITZ), ketoconazole (KTZ) and flucytosine (5FC). A total of 102 clinical yeast strains and 14 ATCC reference strains were used for quality control. Macro- and microdilution methods was performed according to the recommendations of the National Committee for Clinical Laboratory Standards (Document M27-A).

The lipophilic species *Malassezia furdur* was tested according to the Nenoff's modification and E-test was performed as recommended by the manufacturer. MIC pairs were considered in agreement when the difference between the pairs was within 2 two-fold dilutions. General agreement was 95% for AMB, FLC, ITZ and 5FC, and was 96% for KTZ between the micro and macrodilution tests. The agreement between E-test and macrodilution methods was 95% for AMB and 5FC, 96% for FLC and ITZ and 56% for KTZ and high agreement was demonstrated between the methods except KTZ for the tested strains. In conclusion, microdilution and E-test methods are reliable alternatives to the reference macrodilution method for the in vitro susceptibility determination of *Candida* and non-*Candida* yeasts with conventional antifungals, however, azole resistance should better confirm by the reference tests.

P947 Resorting to the microbiology laboratory for evaluating clinical specimens submitted for fungal culture and eventual identification: the experience of a large Italian university hospital

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Objective: To carry out a 12-month prospective survey of clinical samples sent to the Microbiology laboratory of a tertiary care Hospital, for fungal culture and identification.
Methods: During the last year, 27959 specimens were processed from either inpatients (inp) or outpatients (outp). Multiple daily samples sent from one p were considered as a single submission.
Results: Most of clinical specimens regarded blood culture/intravascular catheters (9977 cases: 35.7%), but the frequency of positive assays proved very low in this field (103 cases: 1.03%), with only nine strains from i.v. lines. The genitourinary tract accounted for 7644 samples (positive in 13.2% of cases), with a significant prevalence of positive assays from the female genital tract (761) versus other samples (247 cases: $P < 0.001$). Both upper and lower airways were the origin of 5334 specimens, characterized by an overall elevated frequency of isolation (29.8%), although 36.3% of positive samples were from sputum and 12.4% from nasal/oral swabs. The gastrointestinal tract accounted for 2222 specimens, but the apparently high positivity rate (28.9%) was influenced by frequent stool colonization (1911 cases: 86%). Other samples came from skin/soft tissue (878 cases, with a 7.7% positivity rate), eye (129 samples, with a limited 1.5% fungal recovery), ear (78 cases, with a 6.4% culture rate), central nervous system (145 samples, positive in 2.8% of cases), and miscellaneous sites (1552 specimens, with a 10.3% positive culture rate). When considering some representative inp units, a greater fungal recovery was recognized in pneumology (47%), followed by infectious diseases (28.7%), internal medicine (25.5%), and overall surgical and transplant units (23.7%), while cardiology, hematology, general and specialized ICU, obstetrics/gynecology, nephrology/dialysis, and day-hospital services had a positive culture rate of 10.3–21%.
Conclusion: Tertiary care-based mycology laboratories are borne by a high request of fungal culture and identification, from a broad spectrum of inp/outp units. Whereas search from sterile sites (blood and CSF) account for the minority of positive assays (1.03–2.8% of cases), either respiratory, genitourinary, gastrointestinal tract, or skin and soft tissues, eye and ear, are interested by an elevated colonization, often caused by the concurrent use of antibiotics or underlying immunodeficiencies. Surveillance cultures should be correctly interpreted, especially when p at risk for local or systemic fungal disease are of concern.

P948 Epidemiology of dermatophytes and accuracy of direct microscopic examination: correlation with positive cultures

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Objective: Epidemiology is important in infection control and public health issues related to the different types of dermatophytoses. To our knowledge, no laboratory data concerning dermatophytoses in Lebanon were published yet. In this study, our objective was to collect epidemiological data of the specimens coming to our laboratory and to evaluate the accuracy of direct microscopic examination in detecting the presence of fungi comparing it to culture results.
Material and methods: A total number of 130 clinical specimens collected between October 1st 2000 to October 1st 2001 at the Mycology section of the Saint George Hospital laboratory, Beirut, were included in this prospective study. The information concerning epidemiological and clinical characteristics, as well as laboratory tests and results, was statistically analyzed. Identification characters of the isolates included colonial morphology and biochemical tests. Culture results were correlated to Direct Microscopic Examination (DME).
Results and discussions: Results are shown in Tables 1–3.

Table 1 Statistical characteristics of the studied population

Parameter	Description						
Nationality	Type	Lebanese	Others				
	Numbers (%)	109 (83.8)	7 (5.4)				
Age	Ranges	0–18	19–39	>40			
	Numbers (%)	24 (18.5)	48 (36.9)	58 (44.6)			
Specimen	Type	Nail (Foot)	Nail (hand)	Skin	Hand		
	Numbers (%)	60 (45.2)	31 (23.8)	32 (24.6)	7 (5.4)		
Animals	Kind	Cat	Dog	Rabbit	Bird	No	
	Numbers (%)	5 (3.8)	15 (11.5)	3 (3.1)	6 (4.6)	82 (83.1)	
Culture result	Type	N.G.	*T. violaceum*	*T. rubrum*	*T. mentagrophytes*	*E. floccosum*	*Candida* sp.
	Number (%)	100 (76.9)	4 (3.1)	12 (9.2)	8 (6.2)	1 (0.8)	5 (3.8)

The total number of isolates between 3 October 2000 and 31 October 2001 was 130.

Table 2 Correlation between direct microscopic examination (KOH) and type of specimen, previous treatment and culture's result

	No spores no hyphae	Spores and hyphae present
# of specimens	84	13
Type of specimen		
Nail (foot)	46 (55.4)	4 (30.8)
Nail (hand)	19 (22.9)	2 (15.4)
Skin	14 (16.9)	7 (53.8)
Hair	4 (4.8)	0 (0.0)
Treatment		
No	64 (77.1)	6 (69.2)
Antifugal	10 (12.0)	2 (15.4)
Corticosteroids	0 (0.0)	0 (0.0)
Chemotherapy	2 (2.4)	0 (0.0)
Others	2 (2.4)	0 (0.0)
Result of culture		
No. growth/3 weeks	82 (97.6)	1 (7.7)
T. rubrum	0 (0.0)	4 (30.8)
T. violaceum	0 (0.0)	2 (15.4)
T. mentagrophytes	0 (0.0)	6 (46.2)
E. floccosum	0 (0.0)	0 (0.0)

Table 3 Repartition of the positive cultures of filamentous fungi by clinical specimens

	Positive cultures	Nail (foot)	Nail (hand)	Skin	Hair
T. rubrum	12	3 (25.0)	3 (25.0)	6 (50.0)	0 (0.0)
T. mentagrophytes	8	4 (50.0)	0 (0.0)	4 (50.0)	0 (0.0)
T. violaceum	4	0 (0.0)	1 (25.0)	3 (75.0)	0 (0.0)
E. floccosum	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)

The majority of the specimens were foot (45.2%) and hand (23.8%) nails coming from people above 40 years of age (44.6%). Most of the patients had no animals at home and were not occupationally exposed. Our results show that 97.6% of the cultures were negative when the DME revealed absence of spores and hyphae. On the other hand, 52% of the cases with positive DME revealed positive cultures. Among all cases, *Trychophyton rubrum* followed by *Trychophyton* mentagrophytes were the most isolated fungi. The use of antifungal agents significantly affected the percentages of positive cultures. These data demonstrate the accuracy and reliability of Direct Microscopic Examination in detecting dermatophytosis.

P949 Comparison between prevalence of occult *Tinea pedis* (Athlete's foot) in professional and students soccer players

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Our purpose of this study was determination of any probably effects of duration of training and exercises and prevalence of athlete's foot. The current research has been carried out for the first time in Iran. *Tinea pedis*, a fungal infection of the interdigital toe web spaces as well as solely dermatophyte fungi cause the skin of the feet, includes *Trichophyton* spp. and *Epidermophyton floccosum*. The symptoms and signs of athlete's foot are scales, cracks, peels, redness, blisters and itching. However, it has occasionally had no any symptoms. Athlete's foot is frequently found in adult men between 20 and 40 years old. In this research, two groups include 180 elite soccer players from eight sports clubs and 272 student from 11 university teams were selected as professional and nonprofessional soccer players, respectively. Their skin samples were collected from two-forth interdigital toe web spaces and cultured on Scc agar medium and incubated for 3 weeks. The results showed that about 3.35% ($n=6$) of professional players were positive in three out of eight teams. *Trichophyton rubrum* had a most frequency (four cases). *Trichophyton mentagrophytes* and *Epidermophyton floccosum* isolated from two cases. In contrast, we find only two positive cultures at student players. Besides, some factors includes duration of training, taking a shower after exercise, foots moisture, history and symptom with positive cases detected by questionnaire. there were not any significant difference between these factors and frequency of infection ($P>0.05$). However, significant relation observed between different clubs and the frequency of infections in professional players ($P=0.035$). We could not find any correlation in factors and infection at student's teams because of low cases. This study revealed that *Tinea pedis* is endemic at some clubs which prevention and treatment operation must be done.

P950 Necrotizing fasciitis caused by *Absidia corymbifera* in a patient without local-risk factors

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Objective: We report here, the case of a patient with hyperglycemia and immunosupression secondary to steroid therapy who had no local risk factors but developed invasive necrotizing cutaneous mucormycosis caused by *Absidia corymbifera*.
Material and methods: A 78-year-old man was admitted to the emergency ward with breathing difficulties and serosanguenous nasal discharge. Six days after hospitalization a very painful, red, hot swollen area in the external side of his right thigh appeared. Several days later the nasal lesion disappeared spontaneously but the skin lesion progressed with large necrotic areas. Bacterial and fungi cultures were obtained from the black sinus discharge and from necrotic skin area. For bacterial culture we used blood, MacConkey, chocolate and Sheadler agar plates and thioglicolate medium incubated at 35 °C. For fungal cultures, we used tubes containing Sabouraud dextrose agar with and without clorammphenicol and cicloheximide that were incubated at 25 °C and 35 °C.
Results: The bacterial and fungi cultures of the black sinus discharge were negative. Histopathologic examination of the specimens of the necrotic skin area revealed fungal invasion by a nonseptate fungus. Forty-eight hours later cultures of this specimens showed a white, fluffy profusely growing fungus. No sporulation was seen in cultures even after 7 days of incubation on Sabouraud or Potato dextrose agar. Stimulation of sporulation was attempted with culture in malt extract agar and we identified the fungus as *Absidia corymbifera*. Owing to the continued progression of the necrosis successive debridement were performed and lipososmal amphotericin B was given (total dose 5100 mg). Amputation of the limb was necessary at last.
Conclusion: The significance of this case is that the origin of the *Absidia* infection in this patient is unknown as we failed to observe any recognizable entry portal for the fungi and no apparent local trauma was identified.

P951 Fungemia in patients at a university hospital: impact of concurrent infections, cancer, coronary artery disease, diabetes, and renal failure on short-term mortality

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Objective: The current study was performed to determine the association of host factors, and nosocomial interventions on the outcome of fungemia.
Methods: During 01 January 1998–31 December 1999, all consecutive episodes of fungemia were evaluated retrospectively. Mortality was death during the course of antifungal therapy, and/or <30 days of last positive blood culture during the same hospitalization. Mortality is abbreviated (M%).
Results: Seventy-five patients (37 male) developed 81 episodes of fungemia. Median age was 48 ± 29 years and WBC 10 ± 9.3 k/μL. Overall mortality was 44.3%. Five among eight neutropenic patients expired (M 62.5). Forty-five had fever (>100.5 F) (M 44.2) and mortality in 25 patients without fever was (M 48). In 47 individuals fungemia developed while on hyperalimentation (M 49). Sixty (80%) received antibiotics within 30 days of bloodstream invasion (BSI) (M 43.3). Similarly, 22 were exposure to antifungals (M 55); 15 tirazole-based agent (M 57.1), 5 amphotericin B (M 75), and in 5 two antifungals (M 75). Forty-two developed fungemia during postsurgical period (M 40), including 22 abdomino-pelvic (M 40), 6 vascular (M 33.3), and 7 following severe trauma (M 42.9). Among 81 episodes, 37% were owing to *C. albicans* (M 42.3), 30.9% *C. glabrata* (M 58.3), 17.3% *C. parapsilosis* (M 21.4), 7.4% *C. tropicalis* (M 50), 6.2% *C. krusei* (M 20) and 1.2% *C. lusitaniae* (M 100). Sixty-nine had single species fungemia (M 45.3), and six patients with dual spp. BSI (M 33.3). Forty-three had a concurrent infection (M 51.2); 21 with a single microorganism (M 50) and 22 patients with polymicrobial BSI (M 52.4). An underlying malignancy was present in 34 (M 59.4); 18 solid-organ (M 56.3), 16 hematological (M 62.5), and 9 allogeneic marrow graft recipients (M 44.4). Sixteen had coronary artery disease (M 80), 14 renal failure (M 61.5), including 6 on hemodialysis (M 40), 1 peritoneal dialysis (M 100), and 7 patients with acute azotemia (M 71.4). Thirteen had diabetes (M 58.3), 2 pancreatitis (M 50), and four patients had cirrhosis of liver (M 25). No deaths were observed in patients with AIDS, short bowel syndrome, and 11 premature infants including three being treated in the neonatal ICU.
Conclusions: *Hematogenous candidiasis* is a serious complication even in the immunocompetent individuals, especially those with coronary artery disease or acute azotemia. Universal response to treatment in premature infants in this setting was encouraging.

P952 Consequences of nephrotoxicity in hospitalized hematology patients receiving amphotericinB

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Objectives: Amphotericin B desoxycholate (amB) has been the standard treatment for most systemic mycoses although it carries significant risk of nephrotoxicity (NT). A study was conducted in a hematology ward of a district hospital located in Lyon, France to estimate the incidence and consequences of NT among hospitalized patients receiving amB.
Methods: Data were collected through a retrospective review of medical records. Consecutive patients receiving amB therapy were identified. The first day of amB constituted the beginning of the evaluation period. NT was defined a priori to data collection as: none; slight: increase in serum creatinine (SCr) ≥ 0.5 mg/dL above baseline; moderate: 50% increase in baseline SCr with a maximum of ≥2.0 mg/dL; or severe: baseline SCr tripled or reached a peak ≥3.0 mg/dL (each category mutually exclusive). Hospital costs were estimated by multiplying length of stay (LOS) by the average hospital per diem cost (expressed as 2001 Euros).
Results: From February 1997 to March 2001, 129 eligible patients were identified. Mean age at admission was 51 years; 61% were male. Reason for initiating amB, using EORTC criteria, was proven 10%, probable 36%, and possible 54% fungal infection. All patients received pre-medications and had concomitant use of other nephrotoxic medications. 56% of patients developed some degree of NT after starting amB [44% none (SCr change = +32%); 44% slight (SCR change = +88%); 7% moderate (SCr change = +134%); 5%

severe (SCr change = +235%)]. 46% of patients presented with NT that led to the discontinuation of amB. AmB treatment duration and cumulative dose did not differ among patients who did and did not develop NT (15 days, $P = 0.968$; 820 mg, $P = 0.651$). In hospital mortality was significantly higher for patients developing NT (none: 4%, slight: 17%, moderate: 29%, and severe: 50%). LOS after starting amB and adjusting for mortality was also significantly longer for patients experiencing NT ($P = 0.02$). Lastly, hospital costs after starting amB and adjusting for mortality were also significantly higher for patients experiencing NT (0.04).
Conclusion: NT occurred frequently in this cohort of patients receiving amB and may contribute to increased in-hospital mortality, LOS and hospital costs.

P953 Alveolar macrophages are destroyed by their interaction with *Aspergillus fumigatus* conidia and lung surfactant

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Introduction: *Aspergillus fumigatus* conidia are ubiquitous, airborne, might colonize the lungs and cause chronic inflammation. We have found that when alveolar macrophages (AM) are challenged with *A. fumigatus* conidia, they increase their production and extracellular release of reactive oxygen species (ROS). This increases lipid peroxidation (LPO) of lung surfactant by AM.
Objective: To determine whether products from the increase in oxidative metabolism or those of LPO kill AM.
Methods: We cultured AM with *A. fumigatus* conidia for 48 h with or without a surfactant preparation (Curosurf) and then measured the malonaldehyde levels, which reflect the degree of LPO activity. At the same time, we counted the numbers of AM that had become detached from the surface of the culture dishes during the culture. Finally, we studied DNA fragmentation of the AM with agarose gel electrophoresis.
Results: After the 48-h incubation period, about 15% of the AM population had separated from the culture dish into the medium. In the presence of surfactant, the amount of detached AM increased in a dose-dependent way and was about 50% with 1.6 mg mL^{-1} Curosurf. In parallel experiments the MDA level increased with the concentrations of surfactant. The correlation between the MDA level and cell detachment was highly significant. In similar experiments we found DNA fragmentation of the AM already after 24 h, with Curosurf against 48 h without this agent.
Conclusions: On the basis of this and our previous studies (Nessa K 1997, Gross NT 2000), we suggest that (1) *A. fumigatus* conidia may in the lung activate AM to increase their oxidative metabolism. (2) In the AM the conidia are often located in open phagolysosomes from which ROS are released extracellularly. (3) Lung surfactant is peroxidized by ROS. (4) Products of surfactant LPO together with ROS cause death of AM and presumably also of other cells in the lungs. LPO products, rather than ROS, are primarily responsible for the cell destruction. Thus AM stimulated by fungi tend to be destroyed early because of their exposure to surfactant lipids. Cystic fibrosis is a genetic disorder with pathological secretions in the airways. Sputum samples from such patients contain *A. fumigatus* in about 30% of the cases. We believe that the LPO of lung surfactant and subsequent cell death, found in this study, contribute to the lung destruction seen in these patients.

P954 Epidemiology of *Pneumocystis carinii* dihydropteroate synthase mutations associated with drug resistance

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Pneumocystis carinii is a fungus which causes severe pneumonia (PCP) in immunocompromised patients, mainly those infected with HIV. To prevent and treat this disease, sulfa drugs are widely used. Failure of sulfa prophylaxis and treatment has been shown to be associated with two mutations altering the active site of the dihydropteroate synthase (DHPS), the target of sulfa drugs. Theses mutations result in an amino acid change at position 55 or 57 of DHPS, and were observed either as a single or double mutation in the same isolate. We developed a rapid and simple method which discriminates between the different DHPS genotypes. It consists of the amplification by PCR of the DHPS gene, followed by the detection of its polymorphism using the single strand conformation polymorphism technique. The method was applied to 397 specimens collected from Swiss and French hospitals. The frequency of DHPS mutant genotypes was lower in Swiss hospitals (10%) than in the French ones (33%) (P-value < 0.0001). The use of the sulfa drug sulfadoxine (as part of Fansidar) for prophylaxis in some French hospitals was associated with the DHPS mutant genotype carrying a single amino acid change at position 57 (P-value = 0.001), whereas the use of sulfamethoxazole (as part of cotrimoxazole) was associated with the double mutation at positions 55 and 57. Analysis of cases who had two episodes of pneumonia suggested that selection of the mutant genotype occurred between the two episodes.

P955 Surveillance for antifungal resistance of clinical yeasts isolated in a general hospital during a 3-month period

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Introduction: Surveillance for antifungal resistance of clinical yeasts is necessary for help guide empiric therapy of yeast infections.
Objectives: To determine the antifungal susceptibility patterns of all available clinical yeasts isolated in a General Hospital during a 3-month period (September–November 2001).
Material and methods: Susceptibility testing was performed using a commercial microdilution method (Sensititre Yeast-ONE). Antifungal drugs tested were amphotericin B (AB), flucytosine (FC), ketoconazole (KZ), fluconazole (FZ) and itraconazole (IZ).
Results: A total of 162 yeasts from 157 specimens were tested. Specimen types were as follows: profound (71). mucocutaneous (63). and superficial (23). The MIC_{90} (μg/mL) for anphotericin, fluocitosine, ketoconazole, fluconazole and itraconazole in the species tested were as shown: 0.5, 0.5, 0.008, 1 and 0.125 for *C. albicans*; 1, 0.25, 0.25, 4 and 0.5 for *C. parapsilosis*; 0.5, 0.06, 1, 32 and 2 for *C. glabrata*; 1, 0.125, 0.03, 1 and 0.25 for *C. tropicalis* and 4, 32, 2, 128 and 1 for *C. krusei*. In overall, the MIC_{90} (μg/mL) were 1, 0.5, 0.5, 16, 1. There were no differences in MIC_{90} values for *C. albicans* when the specimen types were compared. Four *C. albicans* strains (3.6%) (each one from wound, urine, pharingeal, and esophagus specimens) had high azole MICs (KZ: 0.25, 0.25, 4 and >16 μg/mL; FZ: 32, 64, 128 and >256 μg/mL; IZ: 0.5, 1, 16 and >16 μg/mL.
Conclusions: AB showed the best activity with an overall MIC_{90} of 1 μg/mL. Globally, the azoles were active against most isolates except for *C. glabrata* and *C. krusei*. FC showed good activity against most isolates tested with the exception of *C. krusei*. In spite of antifungal therapy over last years, the clinical yeasts isolated in our hospital showed an expected pattern of susceptibility.

P956 Assessment of the identification of 271 fungal strains by the VITEK 2 (R) system

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Objectives: The bioMérieux VITEK 2 system enables the identification of clinical yeasts using the VITEK 2 ID-YST card. This system was evaluated in our lab at the Brest University Hospital (France), in comparison with the ID32C method (bioMérieux) using 271 clinical yeast strains.
Methods: A total of 271 strains representing 30 different species were studied. The strains were isolated from patients and/or hospital environment. Tests were performed from subcultures grown for 24–55 h on Sabouraud–chloramphenicol–gentamicin agar plates. Inocula were adjusted to a two McFarland standard in 0.45% aqueous NaCl. Filling, sealing and reading of cards were automatically processed by the VITEK 2 system. Fluorescence measurements every 15 min for 15 h were computerized to give the final identification. The data base allows the identification of 51 species of clinical yeast. Each strain was tested in parallel using the ID32C strip, according to the manufacturer's recommendations. The results obtained with the VITEK 2 system and the ID32C strips were classified into four categories: Concordant (identical identification with both methods, including low discrimination

solved with supplemental test), Low Discrimination not solved with supplemental test, Mis Identification (VITEK 2 system leads to an error of identification), and No Identification (VITEK 2 gives no identification answer).

Results: Of the 271 strains tested, 247 (91.1%) were correctly identified, including low discrimination solved with supplemental test. For 12 (4%) strains, no additional test was available to finalize identification. Nine (3.3%) strains were Mis identified, and 3 (1.1%) were not identified with the system.

Conclusions: The VITEK 2 system represents a significant advance for identification of clinical fungal strains. The most common strains in our laboratory were all correctly identified without additional tests. Its major advantages are:

- Simplicity of the procedure, including fully automated card filling and reading;
- Identification results obtained within only 15 h;
- The presentation of reagents in the form of sealed cards, which is hygienic and ensures user safety.

Abdominal infections – empiric treatment

P957 Risk factors for in-hospital mortality in patients undergoing surgery for intra-abdominal infections

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Objectives: Intra-abdominal infections (IAI) requiring surgery can result in substantial morbidity and mortality. This study examined the risk factors for in-hospital mortality (IHM) in patients undergoing surgery for IAI in Australia.

Methods: Data were extracted from the National Hospital Morbidity Database (Australian Institute of Health and Welfare) for all states/territories except Australian Capital Territory and Victoria. Patients hospitalized between 1995 and 1999 who had a primary discharge diagnosis suggesting IAI and at least one surgical procedure were included. The following covariates were evaluated in multivariate logistic regressions to identify risk factors for IHM: infection type, age category, gender, existence of comorbid conditions, public versus private hospital and whether patient had private insurance.

Results: A total of 15 876 admissions for IAIs requiring surgery were identified. Mean age was 38; 43% were female. Seventy-eight percent occurred in public hospitals. Twenty-six percent had private insurance. Fifty-five percent had acute appendicitis with generalized peritonitis (ICD 540.0), 21% had acute appendicitis with peritoneal abscess (540.1), 5% had perforation of intestine (569.5), 3% had abscess of intestine (569.83), and 16% had other secondary peritonitis (567.0, 567.1, 567.2, 567.0). Almost two-third had at least one comorbid condition. Average IHM rate was 2.6% (95% CI: 2.4–2.9%) compared to 1.2% (1.2–1.3%) for all conditions. The following risk factors were independently associated with a greater IHM rate: perforation of intestine (odds ratio: 17; 95% CI: 12–25; relative to acute appendicitis with peritonitis or abscess), other secondary peritonitis (7.9; 5.6–11), abscess of intestine (2.2; 1.1–4.3), age $\geq$ 65 (3.9; 3.1–5), public hospital (1.5; 1.1–2.2), neoplasms (21; 8.9–50), mental disorders (6.9; 1.9–25), diseases of blood/blood-forming organs (21; 7–66), nervous system (9.2; 1.7–49), skin and subcutaneous tissue (8.2; 1.5–44), musculoskeletal (9.8; 1.8–52), circulatory (12; 5.5–29), respiratory (13; 5.7–32), digestive (11; 5 ÀV 24) and genitourinary systems (10; 4.3–24). Patients with private insurance had a lower IHM risk (0.7; 0.5–0.9).

Conclusion: Among patients undergoing surgery for IAI, the risk of IHM was independently associated with infection type, older patient age, existence of certain comorbid conditions, public hospital admission, and lack of private insurance.

P958 Determinants of hospital length-of-stay in patients undergoing surgery for intra-abdominal infections in Australia

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Objective: Intra-abdominal infections requiring surgery can result in substantial healthcare resource use. This study examined the determinants of hospital length-of-stay (LOS) in patients undergoing surgery for IAI.

Methods: Data were extracted from the National Hospital Morbidity Database (Australian Institute of Health and Welfare) for all states/territories except Australian Capital Territory and Victoria. Patients hospitalized between 1995 and 1999 who had a primary discharge diagnosis suggesting IAI and at least one surgical procedure were included. The following covariates were evaluated in multivariate linear regressions to estimate the determinants of LOS (log transformed): type of infection, age category, gender, comorbid conditions, hospital type (public vs. private) and whether patient had private insurance. P-value ≤ 0.05 was considered significant.

Results: A total of 15 876 admissions for IAI requiring surgery were identified from 1995 to 1999. Mean age was 38; 43% were female. Seventy-eight percent of admissions occurred in public hospitals. Twenty-six percent were covered by private health insurance. Fifty-five percent had acute appendicitis with generalized peritonitis (ICD 540.0), 21% had acute appendicitis with peritoneal abscess (540.1), 5% had perforation of intestine (569.5), 3% had abscess of intestine (569.83), and 16% had other secondary peritonitis (567.0, 567.1, 567.2, 567.0). Majority of patients (60%) had at least one comorbid condition. Average LOS was 6.7 days (SD = 7.8). The following IAIs were significantly associated with longer LOS: perforation of intestine, abscess of intestine, other secondary peritonitis, and acute appendicitis with peritoneal abscess (all $P = 0.0001$, relative to acute appendicitis with peritonitis). Other determinants of longer LOS were age $\geq$ 65, neoplasms, endocrine/nutritional/metabolic diseases, mental disorders, diseases of the blood and blood-forming organs, skin and subcutaneous tissue, musculoskeletal system, circulatory system, respiratory system, digestive system, genitourinary system (all $P < 0.01$). Patients with private insurance were associated with shorter LOS ($P = 0.0001$).

Conclusion: Patients undergoing surgery for IAI in Australia stayed an average of 6.7 days per hospitalization. The LOS was independently associated with the type of infection, age $\geq$ 65 and certain comorbid conditions. Patients with private insurance were also associated with shorter LOS.

P959 Empirical treatment of patients with bloodstream infection: evaluation of efficacy and possible strategies to achieve better results

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Objectives: The study analyzed the efficacy of empirical treatment (ET) in patients with bloodstream infection (BSI). Based on bacterial identification and drug susceptibility results, we wanted to suggest possible local guidelines in order to improve ET strategies.

Methods: Clinical records of patients with BSI were studied to evaluate the effectiveness of ET. Administered drugs were grouped on the basis of the antimicrobial class. Based on patient admission, results were grouped in ICU, medical or surgical.

Results: During 1999, we found 251 BSI (125 medical, 81 surgical, and 45 ICU patients). In medical wards, the most frequent agents were *Staphylococcus aureus* (27.2%) and *Escherichia coli* (22.4%). Patients receiving ET were 85.6%; in 60.7% of the cases, it was adequate. Third-generation cephalosporins (C3G) were the most frequently used drugs (63.6%) either as monotherapy (48.6%) or associations (15%). In surgery, *Candida* spp. (23.5%) overcame *S. aureus* (19.8%). Patients receiving ET were 75.3%, being adequate in 60.7%. C3G accounted for 36.1% of the therapeutic regimens (monotherapy 26.2%, associations 9.8%). In ICU, 33.3% of BSI were caused by Enterobacteriaceae and 28.9% by coagulase-negative staphylococci. All patients were empirically treated (53.3% with associations), but only 37.8% adequately. Aminoglycosides plus C3G was the most commonly used association (15.6%). Monotherapy was mainly administered using β-lactams plus inhibitor (15.6%) or glycopeptides (11.1%). In medical BSIs, the best in vitro association was C3G plus aminoglycosides (84.1%), in surgical ones C3G plus cotrimoxazole (88.5%), and in ICU C3G plus fluoroquinolones (57.8%).

Conclusions: Based on these results, monotherapy with C3G remains an acceptable option for noncomplicated septic patients in medical and surgical wards. However, C3G plus aminoglycosides may be needed in medical patients with severe sepsis and septic shock, whereas association with cotrimoxazole may be the best choice in surgical wards. Therapeutic failures are frequent in ICU, even if C3G are used in association. Wide diffusion of resistance makes effectiveness of ET barely predictable. In fact, we did not detect any C3G association that could guarantee acceptable success. Thus, with special regard to severe septic patients, monotherapy with glycopeptides or carbapenems remains the only choice in life-threatening situations.

P960 Simple categorization of patients with positive blood bacterial and yeast cultures guides empirical antimicrobial therapy

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Objectives and methods: Gram-stain of blood cultures offers the first microbiologic data to guide therapy usually within 24 h after blood samples are drawn. We categorized 989 consecutive positive culture findings from 1 January 2000 to 30 September 2001 into four groups: patients with community-acquired infection (CAI, 437 cases), hospital-acquired infection (HAI, 328 cases), infections in hematological units (HEAI, 116 cases), or contamination (108 cases). Categorization is done routinely at our hospital immediately after Gram-stain findings; the clinician responsible for treatment of each patient is consulted by phone. Aim of the study is to create empirical treatment guidelines for septic infections based on patient category and stain result.
Results: Gram-negative rods were found in 215 (49.2%) cultures of CAI, 110 (33,5%) HAI, and 23 (19,8%) HEAI patients. *E. coli* was the most common Gram-negative rod with 133 (61.9%), 48 (43.6%), and 7 (30.4%) findings. *Klebsiella* strains were found in 21 (9.8%) CAI, 19 (17.3%) HAI, and 11 (47.8%) HEAI patients. *Enterobacter* strains were concentrated in HAI class (16 cases, 14.6%). Gram-positive cocci in clusters (staphylococci) was in 79 (18.1%) CAI, 127 (38,7%) HAI, and 51 (44.0%) HEAI cases. Proportion of *S. aureus* among the staphylococci was 77.2% in CAI, 31.5% in HAI, and 21.5% in HEAI patients. Percentages of coagulase-negative staphylococci (CNS) were 22.8, 68.5, and 78.5%, respectively. Gram-positive cocci in chain were found in 51 (11.2%), 62 (18.9%), and 26 (22.4%) in CAI, HAI, and HEAI categories, respectively. Within these cases, proportion of enterococci was 25.5% in CAI, 33.8% in HAI, and 38.5% in HEAI findings. Gram-positive diplococcus (*S. pneumoniae*) was in 74 (16.9%), 9 (2.7%), and 5 (4.3%) cases in these categories. *Candida* strains (18 cases) were seen in HAI and HEAI categories only. Contamination was caused by Gram-positive bacteria: CNS were in 74 cases (68.5%), *Propionbacterium* sp. in 15 cases (13.9%), *Bacillus* sp. in 7 cases (6.5%), and other Gram-positive cocci in 10 cases (9.3%).
Conclusions: We conclude that classification of positive blood cultures according to Gram-stain and simple clinical data generates practical information for empirical antimicrobial treatment. Combining these statistics to locally collected antibiotic resistance data and good clinical judgment should offer means for creating more accurate treatment guidelines for septic infections.

P961 The use of time-trend analysis in the design of empirical antimicrobial treatment of urinary tract infection (UTI)

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Background: Selection of empirical treatment of UTI is traditionally based on urine culture results from the local microbiology laboratory. These results may be too general to be applicable to treatment of the individual patient.
Methods: We analyzed a large database of urine cultures from the first 3 months of each year over a 10-year period (1991–2000). Included were only samples, from which one organism was grown at >105 colony forming units (cfu) per milliliter. Trend statistical tools were applied to assess the decay in activity of individual antibiotic agents over time and to calculate susceptibility rates of subsets of urine samples.
Results: During the study period, 5272 (14%) of all sent samples grew a single organism at >105 cfu/mL: 36% of positive cultures were catheter associated. The most frequently isolated organisms were *E. coli* (48%), *K. pneumoniae* (11%), *P. aeruginosa* (9%), *Enterococcus* spp. (8%), and *P. mirabilis* (6%). In the presence of a urinary catheter, compared to a clean-catch specimen, the relative risk of isolating *E. coli* was 0.7 (95% CI: 0.7–0.8), *K. pneumoniae* 1.3 (1.1–1.5), *P. aeruginosa* 1.5 (1.3–1.8) and *E. faecalis* 2.1 (1.7–2.5) ($P < 0.001$ for all). Of 1923 positive cultures obtained in the emergency department (ED), 1586 (82%) were *Enterobacteriaceae*, compared to 2216/3349 positive cultures (66%) from inpatient departments ($P < 0.001$). *E. coli*, *Pseudomonas* and *Enterococcus* were isolated in 63, 6 and 3% of ED samples, respectively, compared to 38, 11 and 11% of hospital-acquired specimens ($P < 0.001$). Susceptibility rates of *E. coli* ($n = 2508$) of 10/14 (71%) tested drugs decreased annually by $1.7 \pm 0.6\%$ from 1991 to 2000; that of *P. mirabilis* ($n = 333$) decreased to only four drugs. Susceptibility of *E. cloacae* ($n = 129$), *P. aeruginosa* ($n = 461$) and *Enterococcus* ($n = 414$) remained unchanged; that of *K. pneumoniae* did not change significantly over the decade for various subsets of isolates, except for amikacin and ciprofloxacin.
Conclusions: Trend analysis helps identify and quantify the presence of decay in antimicrobial susceptibilities. Stratified trend analysis is a readily available tool which is useful in designing and adapting guidelines for the selection of empirical antibiotic treatment for the individual patient with UTI.

Anaerobes

P962 Bacterial Endotoxins Test (BET) application to determine the biological activity of *Bacteroides* lipopolysaccharides and capsular polysaccharides

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Objectives: To determine the biological activities of LPS and CPS preparations isolated from *Bacteroides fragilis* and *B. thetaiotaomicron* strictly anaerobic rods using quantitative BET (formerly LAL) method.
Methods: Lipopolysaccharides were extracted with the method of Westphal and Jann (1965) from three *B. fragilis* strains – two nonenterotoxigenic (NTBF) NCTC 9343 and IPL E 323 and one enterotoxigenic (ETBF) ATCC 43858, as well as from three *B. thetaiotaomicron* strains of different origin – reference NCTC 10582, clinical 312/85 and fecal 9/18. LPS preparations were purified according to the procedure described by Gmeiner (1975). Capsular polysaccharides were prepared from two strains: *B. fragilis* ATCC 43858 and *B. hetaiotaomicron* 312/85 by the method of Poxton and Ip (1981). Biological activity of bacterial compounds was determined with the use of quantitative BET method with chromogenic substrate S-2423 (ENDOCHROME kit). The test was performed according to the recommendations of producer (Charles River Endosafe Ltd, USA). *E. coli* O55:B5 LPS (Sigma Chemical Co., USA) was applied for comparison of LAL activities.
Results: Lipopolysaccharides showed considerably greater activity with LAL (Limulus Amoebocyte Lysate) reagent than capsular polysaccharides. The results obtained for all *Bacteroides* lipopolysaccharides (except of LPS from ETBF strain) were comparable. The most active among determined preparations was *E. coli* O55:B5 LPS. *E. coli* LPS was generally several times more active than lipopolysaccharides isolated from strictly anaerobic rods of genus *Bacteroides*.

Conclusions: It can be stated that the quantitative, chromogenic BET test with application of LAL reagent is an useful method for determination of the biological activity of cell-surface antigens (LPS and CPS) isolated from the *Bacteroides fragilis* group (BFG) rods.

P963 Enterotoxin-producing *Bacteroides fragilis* (ETBF) strains-susceptibility to antimicrobial agents

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Twenty-two *Bacteroides fragilis* strains isolated from clinical samples in different countries (England, France, the Netherlands, Poland and USA) were used in the experiments. In all strains the presence of enterotoxin (fragilysin) gene was found by PCR with primers 404/407. Drug susceptibility of *B. fragilis* strains was determined with Etest (MICs for penicillin G, ceftriaxone, amoxicillin/clavulanic acid, imipenem, clindamycin and metronidazole). MICs were estimated in accordance to the NCCLS recommendations (1999). All tested strains were susceptible to imipenem and metronidazole. Twenty-one strains were susceptible and one was intermediate susceptible to amoxicillin/clavulanic acid. Fourteen strains were resistant to ceftriaxone and five were found highly resistant to clindamycin. All examined strains were resistant to penicillin G. Four tested strains were simultaneously resistant to penicillin G, ceftriaxone and clindamycin (three French human strains isolated from postoperative wound, peritoneal fluid and bone inflammation, and one strain isolated from pig).

P964 The increasing and decreasing antimicrobial resistance (AR) of the *Bacteroides fragilis* group (Bfg): comparison of 1989–1990 and 1998–1999 susceptibility results

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Objectives: Because of numerous reports worldwide of increasing antimicrobial resistance (IAR) among the Bfg we compared the MIC results of two studies 10 years apart (1989–1990 vs. 1998–1999) to establish any IAR or conversely decreasing antimicrobial resistance (DAR) among selected antimicrobial agents (AA).
Methods: A total of 1240 isolates were tested during 1989–1990 and 401 during 1998–1999. All test isolates of the Bfg were from clinical specimens and identified using selective media, biochemical profiles and GLC methods. Susceptibility testing was performed using a broth microdilution method as recommended by the NCCLS with a standard inoculum size and MIC determination after 48 h of anaerobic incubation at 35 °C.
Results: Comparison of the results showed both IAR and DAR to various AA by the Bfg. Methronidazole was active against all test isolates. Imipenem and piperacillin/tazobactam (P/T) showed equal activity at both time periods (>99%). Ertapenem (ETP), a new β-lactam agent, had DAR by 6% (92 and 98%) while AR to meropenem remained virtually unchanged. The MIC90 values for ETP and P/T were four- and eight-fold lower, respectively, for the 1998–1999 study. Cefoxitin resistance decreased by approximately 10% from 1989–1990 to 1998–1999. Clindamycin resistance remained stable but high (76 and 77%) at the two time periods. Conversely, ampicillin/sulbactam was less active against Bfg with IAR from 3 to 8% predominantly among non-*B. fragilis* species. Trovafloxacin activity remained comparable for the two time periods (AR-<10%). All isolates from both time periods were resistant to penicillin G based on MIC values and/or β-lactamase production.
Conclusion: Unlike IAR among aerobes which can be linked to antimicrobial selective pressure, the changes in AR among the Bfg lack such an established link. The present data emphasize changing susceptibility of the Bfg to AA and the need for continued monitoring of AR among the Bfg pathogens to encourage appropriate selection of AA for effective patient care.

P965 Increased cross-resistance to antimicrobials among the *Bacteroides fragilis* group (Bfg) from blood using clindamycin (CL) and cefoxitin (FOX) as phenotypic markers

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Objectives: Bfg bacteremia has been shown to be a risk factor for increased morbidity and mortality in patients receiving inappropriate antimicrobials based on in vitro susceptibility. This study compares cross-resistance parameters of blood isolates showing reduced susceptibility (rs) to CL and FOX.
Methods: Cumulative MIC data was collected over a 10-year period for Bfg blood isolates (maximum of 542 isolates). MIC values were determined using a broth microdilution method as recommended by the NCCLS. Rs was defined as an MIC value interpreted as intermediate or resistant by NCCLS guidelines.
Results: The overall incidence of rs to CL was 22% and rs to FOX was 16%. Isolates with rsCL showed decreased antimicrobial susceptibility (DAS) of 34–54% to ceftriaxone (CTR), cefotaxime (TAX), and cefotetan (TAN) while rsFOX showed DAS of 36–66%. For ertapenem (ETP), meropenem (ME), and imipenem (IM) DAS of 2.5–14% for rsCL isolates and DAS of 4–34% for rsFOX isolates was noted. For ticarcillin-clavulanate (TC), ampicillin-sulbactam (AS), and amoxicillin-clavulanate (AC) DAS of 13–23% for rsCL isolates and DAS of 15–19% for rsFOX was found. No resistance to piperacillin-tazobactam (PT) was found among any isolates. Isolates with double phenotypes of rsCLFOX showed DAS of >25% for CTR, TAX, TAN, ETP, ME, TC, AS, and AC.
Conclusion: Continued increases in antimicrobial resistance among Bfg to individual agents has been repeatly documented. This study has documented that rs to CL and FOX compromises the activity of other agents in the same and other classes of antimcrobial agents against Bfg isolates.

P966 Fatal gas gangrene and septicemia caused by *Clostridium septicum*: a case report

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Septicemia caused by *Clostridium septicum* is an uncommon but almost invariably fatal infection. Most of the cases have been described in patients with colonic carcinoma, hematologic malignancies and cyclic neutropenia. We describe a case of gas gangrene and septicemia in a patient without any underlying disease. A 64-year-old male was admitted to the emergency department with clinical signs of acute abdomen, in severe septic shock. Skin lesions characteristic of gas gangrene (tense, white, cyanotic skin) were observed during inspection of the perineum and scrotum. Treatment with penicillin and clindamycin initiated immediately. The laboratory tests at the time of admission showed coagulation disorders while the abdominal CT-scan revealed gas in the peritoneal cavity and retroperitoneal space. During sigmoidoscopy a penetrating injury, 4–5 cm from the anal sphincter was found. During exploratory laparoscopy signs of myonecrosis (gas gangrene) and focal necrotic lesions of the intestine were observed. After that Hartman's operation was performed. The patient died 6 h after the operation. Gram's stain smear showed the presence of large Gram-positive rods and absence of neurophils. *C. septicum* was isolated from blood, peritoneal and perianal tissue culture. Gas gangrene and septicemia, caused by *C. septicum* is not common and has been described mainly in patients suffering from malignant diseases, cyclic neutropenia, diabetes mellitus and severe cardiovascular disorders, but our case shows that it occasionally may occur in patients without any underlying disease.

P967 Rate of anaerobic and nonanaerobic bacteria in human mixed infections

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Objectives: It is now generally recognized that anaerobic bacteria may be involved in most human bacterial infections that follow any form of surgery or are related to those body sites that have a large anaerobic population. The aim of this study was the detection of anaerobic and nonanaerobic bacteria in human mixed infections.
Methods: In this study, samples of 498 patients with periodontitis, abscess and sinusitis were collected. Then the samples were cultured under anaerobic, capnophilic, microaerophilic and aerobic conditions using selective and nonselective media. Isolates were characterized to species level by conventional biochemical tests and by a commercial rapid test system.
Results: In the patients with periodontitis, detected bacteria were *Porphyromonas gingivalis* (45%), *Actinobacillus actinomycetemcomitans* (36.8%), *Eikenella corrodens* (34.4%), *Prevotella intermedia* (33.1%), *Capnocytophaga* spp. (31.9%), *Fusobacterium nucleatum* (22.5%) and *Prevotella melanogenica* (17.5%). In the head and neck abscesses, isolated bacteria were *Staphylococcus aureus* (15.8%), *Prevotella melaninogenica* (15.8%), *Esherichia coli* (14.9%), *Klebsiella pneumonia* (14.9%) and *Pseudomonas aeruginosa* (13.2%). The predominant bacteria species of intra-abdominal abscesses were identified as *Staphylococcus aureus* (77.6%), *Streptococcus* group A (46.3%) and *Bacteroides fragilis* (37.3%). Brain abscesses had *Streptococcus* nongroup A (36.4%) and *Peptostreptococcus* spp. (10%). Also, *Streptococcus* nongroup A (36.4%) and *Staphylococcus epidermidis* (15.2%) were isolated from sinusitis.
Conclusion: The studies and our findings have shown that many of the infectious processes involving anaerobes are polymicrobial, consisting of mixtures of obligate anaerobes or mixtures of obligate or microaerotolerant anaerobes and facultative organisms. Symbiotic relationships frequently exist between some of the bacteria involved in polymicrobial infections, which can act synergistically in the production of diseases. Anaerobes must therefore be sought in a wide variety of clinical specimens.

P968 Prevalence rate of monobacterial and polybacterial infections in patients with adult periodontitis

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Objectives: There is no doubt that anaerobic and capnophilic bacteria play a major role in the pathogenesis of human periodontal disease. Microbiological studies have identified more than 242 bacterial species in periodontal pockets which less than 20 species are periodontopathogen. The purpose of this study was the detection of monobacterial and polybacterial infections in the patients with adults periodontitis.
Methods: In this study, samples of 242 patients with adult periodontitis were collected with sterile paper points from the deepest periodontal pockets. The samples were cultured under anaerobic and capnophilic conditions using selective and nonselective media. Isolates were characterized to species level by conventional biochemical tests and by a commerical rapid test system.
Results: *Actinobacillus actinomycetemcomitans* (25.2%), *Porphyromonas gingivalis* (20.7%) and *Eikenella corrodens* (16.5%) were the most frequently detected microorganisms in all diagnostic groups. The other bacterial species, including *Prevotella* spp., *Fusobacterium* spp., *Peptostreptococcus* spp. and *Wolinella recta* were detected in the samples of patients. The data show prevalence rate of monobacterial and polybacterial infections in the patients with adults periodontitis were 47 (19.4%) and 70 (28.9%), respectively.
Conclusion: The data of the present investigation suggest that there is great heterogeneity in the subgingival bacteria among subjects. Cultural methods for recovery of bacteria are still standard in microbiology. However, as many bacteria in the oral cavity cannot be cultured, it is likely that these still uncharacterized bacteria might play a role in the initiation and progression of periodontal diseases.

P969 In vitro activity of moxifloxacin in patients with severe odontogenic infections

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Introduction: Penicillin resistance is reported in more than 50% of patients with severe odontogenic infections (Lewis 1995).
Objectives: Prospective longitudinal study on patient related in vitro activity of moxifloxacin in episodes with severe odontogenic infections.
Methods: In 19 patients with severe, life-threatening odontogenic abscesses microbiologic samples were drawn by extraoral incision or intraoral needle aspiration. Clinical data on prior therapy and duration of post operative treatment was collected. After cultivation and identification of aerobic and anaerobic pathogens MIC were determined using E-test stripes for penicillin, amoxicillin + clavulanic acid (amoxiclav), levofloxacin (levo), moxifloxacin (moxi), clindamycin (clinda), metronidazole (metro).
Results: From 19 episodes, of which 1 was culture negative, 28 aerobes and 14 anaerobes were isolated. The rate of resistance using DIN breakpoints is given in the table.

	Penicillin (%)	Amoxiclav (%)	Clinda (%)	Levo (%)	Moxi (%)	Metro (%)
Aerobes ($n = 28$)	11	0	7	0	0	100
Anaerobes ($n = 14$)	0	0	8	8	0	15
Patients ($n = 18$)	13	0	20	6	0	100

Prior treatment: Four patients developed the severe infection in spite of adequate antibiotic therapy, one patient, who received metronidazole had resistant bacteria, 14 patients had no prior therapy. Patients were hospitalized for a mean of 8.25 days. The mean total treatment time was 16.7 days. Most patients received amoxiclav postoperatively.
Conclusion: Although metronidazole is found in many guidelines for treatment of odontogenic infections its routine use should be discussed critically. The role of penicillin and clindamycin in life-threatening situations is questionable. In none of the patients resistance against amixiclav or moxifloxacin was found. A possible role of moxifloxacin as an alternative for patients with hypersensitivity to β-lactam antibiotics is to be tested in future prospective clinical trials.

Community-acquired UTI

P970 Acute pyelonephritis: 5-year experience in a general hospital

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Introduction: Pyelonephritis is one of the most prevalent cause of morbidity and mortality. Our goal is to analyze the clinic and epidemiologic characteristics of all the inpatient pyelonephritis treated in our hospital.
Methods: This is a retrospective study which analyzes all the pyelonephritis attended in our center from 1 January 1996 to 31 December 2000. Data were obtained from administrative and microbiology records.
Results: From a total of 59 329 admissions, 353 were diagnosed of acute pyelonephritis (0.59%). This was the main diagnosis in 85% of the patients. The mean age was 47 years (ranged from 1 to 92 years). The rate of female/male was 1.7. The mean length of stay was 5.97 days.
Concurrent problems: Diabetes was found in 61 patients (17.28%), pregnancy in 19 patients (5.38%), chronic renal failure in 19 patients (5.38%), and congenital anomalies in five patients (1.41%). Urine culture were carried out in 284 (83%) cases with the next results: 149 positives (52.46%), 110 negatives (38%), and 25 contaminated (8%). Causal microorganisms were identified in 160 cases (45%): *Escherichia coli* 107 (66.85%), *Proteus* 10 (6.32%), *Enterococcus* 9 (5.69%), *Klebsiella* 8 (5.06%), *Staphylococcus* 7 (4.43%) and *Citrobacter* 5 (3.16%). The *E. coli* antibiotic-susceptibility study demonstrated resistance to: quinolones in 30 cases (28.04%), amoxicilin-clavulanate in five cases

(4.67%), cefotaxime/ceftriaxone in two cases (1.87%). *E. coli* quinolone resistances increased from 27% in 1996 to 38.5% in 2000. Abdominal ultrasound scans were done in 230 patients (82.43%) and intravenous urography in 21 patients (6%). Obstructive uropathy was detected in 98 patients (27.76%), the main cause was kidney and ureteral lithiasis (71 patients). Therapeutic procedure was required in 42 cases (12%), mainly nephostomy (36 patients), and in six partial nephrectomy or ureter-nephrectomy. Acute renal failure was detected as complication in 28 cases (7.93%). Mortality was 1.7% (seven patients).

Conclusions: In our hospital, the inpatient pyelonephritis is an infection affecting predominantly women, in the middle of their life, with obstructive uropathy in one-third of the cases. Causal agent is detected in about half of the cases. *E. coli* causes more than 65% of these cases with a significant increment of quinolone resistance. So in our area, severe pyelonephritis must be treated with other antibiotic than quinolone, as amoxicilin-clavulanate or cephalosporins.

P971 Acute focal bacterial nephritis in children

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Objective: Acute focal bacterial nephritis (AFBN) is a focal bacterial infection localized within the parenchyma of the kidney which may develop with abscess formation. Clinical features of such evolution include fever, chills, flank pain and the hematological findings of infective disease. The aim was to review the presentation, methods of diagnosis, incidence of concomitant urological abnormalities and treatment effectiveness in children with AFBN, also known as lobar nephronia, a severe nonliquefactive infection involving one or more renal lobules.

Patients and methods: Twenty-eight children (age range of 6 days to 15 years with an average of 8 years) diagnosed with AFBN over a 5-year period in Vilnius University Children Hospital were retrospectively reviewed. Their age, gender, presenting symptoms, presence of urinary tract abnormalities, methods of diagnosis and treatment were assessed.

Results: All the patients presented a fever and persistent flank and abdominal pain on affected kidney. Ultrasound showed local enlarged renal volume and space-occupying masses with low-level echoes. CT scans demonstrated focal enlargement of affected kidneys and low-density solid space-occupying lesions. Predisposing conditions were found in 10 patients: vesico-ureteric reflux (three), urethra diverticulum (one), hydronephrosis (one), nephrosclerosis (two), and urethra stenosis (three). Evolution to renal abscess occurred in 10 patients. All 28 children received intensive intravenous and oral antibiotics.

Conclusions: Acute focal bacterial nephritis should be strongly suspected when a patient presents fever, chills and pain on the affected side and has a history of urinary tract infection. In addition, ultrasound and CT examinations suggest evidence of parenchymal space-occupying lesion, and the renal mass and its clinical symptoms disappear following anti-infection treatment.

P972 Asymptomatic microbiuria in diabetic females

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Objective: To study the prevalence of asymptomatic microbiuria (ASM) in diabetic females.

Methods: The study included 408 females with diabetes mellitus type I and II and no abnormalities of the urinary tract (mean age: 62 ± 9 years; range: 19–88 years) and 410 nondiabetic females (controls) of a mean age of 61 ± 8.6 years, all admitted in our department in a 4-year period. We defined ASM as the presence of at least 10 (five) colony-forming units per milliliter of one or two bacterial species in a culture of clean-voided midstream urine from a female with no symptoms of a urinary tract infection. The patients were distinguished in two groups according to their glycemic control indicated by their HbAa1c: in group A, HbA1c was <8.0, and in group B, HbA1c was >8.0.

Results: Group A included 150 patients (36.7%). The prevalence of asymptomatic microburia was 22% in diabetic females and 6% in nondiabetic ones ($P < 0.01$). The prevalence of asymptomatic microbiuria was 23.6% in group A and 21% in group B. No association was evident between current HbA1c levels and the presence of ASB. The pathogens isolated in diabetic and nondiabetic females were: *E. coli* (64.2% vs. 66.8%), *Proteus* sp. (8.1% vs. 7.6%), *Pseudomonas* sp. (7.6% vs. 8%), *Enterococcus* sp. (7.6% vs. 8.8%).

Conclusions: The prevalence of ASM is increased in women with diabetes mellitus. The commonest microorganism isolated is *E. coli*. The good glycemic control does not decrease the frequency of ASM.

P973 Antimicrobial therapy for lower urinary tract infections (LUTI)

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Background: Ethiological therapy of LUTI changes because of antimicrobial resistance of germs and of improved efficiency–cost relation need.

Objectives: To evaluate some LUTI therapy regimens in accordance to: known or unknown ethiology uncomplicated or complicated/iterative LUTI, respectively.

Methods: There was a 192 LUTI patients retrospective study. The suggestive symptoms for LUTI associated with positive uroculture or leukocyturia represented inclusion criteria. High fever suggested upper UTI, represented exclusion criterias. Ethiology has been identified for 96 patients. Therapy results were defined on the basis of clinical (symptoms remission in less than 72 h) and bacteriological results.

Results:

1 Clinical success rate (CSR) has been 91.6% and bacteriological eradication (ER) 86.45%. Aminopenicillins and first-generation cephalosporins have been less effective (76.19 and 66.67% with $P = 0.008$ and $P = 0.001$).
2 Uncomplicated LUTI (114 patients) CSR has been 93% and ER 87.3%, respectively. Among the used regimens, more effective than the average proved to be fosfomycin (CSR = 100%, ER 8/8 cases) and F-quinolones (CSR = 98.5% and ER = 97.5%, respectively). We noticed a quite high frequent ≤3 days therapy rate: 50.7%; the efficiency being approximately the same for short or standard (7 days) therapy; CSR having $P = 0.47$, ER = 0.72.
3 The CSR regarding the complicated LUTI has been 97.3% and ER = 84.6%. According to this, <3 days therapy proved to be a clinical failure (14.2% vs. 4.65% for uncomplicated LUTI, $P = 0.025$) and a bacteriological failure (8/21 cases vs. 20/21 cases) more frequently. The most useful antimicrobials proved to be the F-quinolones (CSR = 94.1% and ER = 97.7%).
4 The CSR for unidentified ethiology (96 cases) has been 90.6% and the most useful antimicrobials proved to be the F-quinolones (96.2%), fosfomycin (100%) and cotrimoxazole (9/11 cases).

Conclusions: The most-effective first-line therapy for uncomplicated LUTI proved to be fosfomycin in monodose and F-quinolones for 3 days (F-quinolones monodose not evaluated). A 7-day course of F-quinolones proved to be effective for the complicated or recidivant LUTI. If we cannot identify LUTI's causative agent, we may use F-quinolones or cotrimoxazole. Using aminopenicillins or first-generation cephalosporins meant an unacceptable failure rate.

P974 Urinary tract infections in adults with diabetes mellitus

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Objectives: Urinary tract infections (UTIs) are common and sometimes life-threatening infections in diabetic patients. The aim of this study was to investigate the epidemiologic, microbiologic and clinical features of UTI in adults with diabetes mellitus (DM).

Methods: Patients were identified from a retrospective study of diabetic adults hospitalized with UTI. The medical records of 101 patients with type 2 DM and UTI, 65 (64.4%) female and 36 (35.6%) male, were reviewed on the basis of a specific questionnaire.

Results: The mean age was $70.7 + 12.8$ (range 18–95) years with mean diabetes duration of $14 + 8$ years. Ninety-six out of 101 (95.1%) were community-acquired UTIs. Pyelonephritis had 97 (96.1%) patients. Thirty diabetics (29.7%) were classified as complicated UTI and the majority of underlying conditions found, were indwelling urinary devices in 16 (15.8%), calculi in 14 (13.9%), and residual urine in 8 (7.9%) patients. Common clinical

features included fever, dysuria, flank pain and vomiting noted in 100, 51, 46.9 and 35.7%, respectively. Urine analysis showed pyuria in 89%, and bacteruria in 84% of the patients. Bacteremia in 17% and increased levels of urea and creatinine in 23% were common complications. From 102 available urine cultures, 81 microorganisms were isolated. *Escherichia coli* was the most-common bacteria isolated in 49 (60.5%) cases followed by *Enterococcus faecalis* and *Proteus* in six (6.2%) and *Klebsiella pneumoniae* in three (3.7%) cases. All patients received antibiotics. Apyrexia was obtained in a mean time of 5.2 + 3.7 (1–20) days. Four patients (3.96%) died from the underlying infection.

Conclusion: Our data suggests that *E. coli* was the most common bacteria isolated in elderly diabetic patients with pyelonephritis. Complicated UTIs and bacteremia was another quite common finding among these patients. Apyrexia occurred in a mean time of 5 days, which probably means a more difficult eradication of infection.

P975 Effect of pivmecillinam in acute uncomplicated urinary tract infections due to ampicillin-sensitive and ampicillin-resistant *Escherichia coli*

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Objective: Current level of resistance to ampicillin in *Escherichia coli* is such that alternative first-line empiric treatments for acute uncomplicated urinary tract infection are required. The efficacy of pivmecillinam in urinary tract infections due to *E. coli* in relation to ampicillin susceptibility has been determined.

Methods: Data were taken from a large prospective double study. The analysis involved women with community-acquired infection who were given a 7-day course of pivmecillinam of 200 mg two or three times daily. Bacteriological efficacy (*E. coli* eradication) and clinical response (cure or significant improvement) were determined at day 8–10 (short term) and day 35–49 (long term). Short-term outcome was used when no long-term data was available. Ampicillin and mecillinam susceptibility was determined by agar disk diffusion using the SRGA methodology. Isolates reported as sensitive or intermediate were considered susceptible for the analysis.

Results: No significant differences were seen between the two dosages, so the data were pooled.

- Ampicillin- and mecillinam-susceptible *E. coli* ($n = 288$ bacteriological; $n = 299$ clinical): bacteriological success was achieved for short-term in 273 (94.8%) and for long term in 240 (83.3%). Clinical response was achieved for short term in 272 (94.1%) and for long term in 253 (87.5%).
- Ampicillin-resistant and mecillinam-susceptible *E. coli* ($n = 54$ bacteriological and clinical): bacteriological success was achieved for short-term in 54 (100%) and for long term in 44 (81.5%). Clinical response was achieved for short term in 54 (100%) and for long term in 51 (94.4%).

There were no statistically significant differences in outcomes for ampicillin-susceptible/resistant *E. coli* [1].

Conclusions: Pivmecillinam is bacteriologically and clinically effective in patients with acute uncomplicated urinary tract infection due to *E. coli*, regardless of ampicillin susceptibility. Pivmecillinam is therefore a suitable empiric first-line treatment, particularly in areas where resistance limits the value of ampicillin.

Reference

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P976 Once-daily versus thrice-daily gentamicin in the treatment of acute pyelonephritis in children

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Objectives: Urinary tract infections (UTI) are a common cause of hospital admissions in children. The aim of this study was to examine the safety and efficacy of once-daily (OD) gentamicin compared to conventional eight hourly dosing (TDS) in children with acute pyelonephritis.

Methods: This is a prospective, randomized-controlled trial of children between 1 month and 13 years of age with presumed UTI. They were randomized, with parental consent, to OD gentamicin 5 mg/kg/day or TDS gentamicin 6 mg/kg/day divided eight hourly. Oto-acoustic emission hearing tests, serum creatinine were done at baseline and at the end of gentamicin treatment. When the patient was afebrile, the gentamicin was converted to a suitable oral antibiotic to complete the course of treatment. A renal ultrasound and dimercaptosuccinic acid (DMSA) scan were done within 5 days of admission. A follow-up micturating cystourethrogram was done at 6 weeks and repeat DMSA at 3 months to look for renal scarring.

Results: A total of 220 patients with presumed UTI were recruited out of which 134 had pyelonephritis and 117 were analyzable (57 OD, 60 TDS). The median age was 7 months, males constituted 50%. The majority were due to *E. coli* (89%) of which 93% were sensitive to gentamicin and eight (6.8%) had positive blood cultures. Comparing the two groups, there was no significant difference in age, sex, duration of fever before admission, pyuria, nitrite positivity, initial total white blood count. All had negative urine cultures after 2–3 days of treatment, demonstrating microbiolgic efficacy. There was no difference between the two groups in terms of ototoxicity, nephrotoxicity, duration of gentamicin treatment, time to fever defervescence and renal scarring.

Conclusion: OD gentamicin is as efficacious as TDS gentamicin in the treatment of pyelonephritis in children with no difference in ototoxicity and nephrotoxicity. More importantly, there was no difference in the incidence of renal scarring in the long term.

P977 Readmission factors in infectious pyelonephritis

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Objective: To analyze factors providing to hospital readmission in 6 months after discharge by infectious pyelonephritis (IP).

Methods: This is a retrospective and descriptive study of IP cases treated as inpatient in our center from January 2000 to December 2000. We have considered the new admissions 6 months later. Readmitted IP (RIP) patient characteristics were analyzed and compared with the no-readmitted IP (NRIP) group. Student's *t* test was used to compare and Chi-square to rate the comparison.

Results: From 73 IP patients, 13 (17.8%) were RIP patients. The mean age in RIP group was 66.38 ± 21 versus 47 ± 24 in NRIP patients ($P = 0.046$). We found no differences when compared: gender, previous urine tract infection, previous urological procedure, urine tract abnormalities, pregnancy, diabetes, neurogenic bladder, permanent urinary catheter or clinical data (dysuria, fever, shaking chills, flank pain, nausea, vomiting and painful kidney percussion) or general laboratory data (leukocytes, platelets or creatinine). We found differences in the presence of obstruction in the urine tract (by ultrasounds or intravenous urography): there was obstruction in 26% in RIP group versus 9% in NRIP group ($P = 0.018$). Rate of positive blood cultures and urine cultures were similar in both groups and we found no differences in the isolated microorganisms. The invasive procedures (nephrostomy or surgery) were more frequent in RIP patients compared with NRIP (53% vs. 9%, $P = 0.001$). The length of stay in RIP group was 6.85 versus 6.01 in NRIP patients.

Conclusion: In IP, the urine tract obstruction and the need of invasive procedures (nephrostomy or surgery) are associated with a higher rate of readmission.

P978 Acute obstructive pyelonephritis: epidemiologic and clinical characteristics

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Introduction: Obstruction of the genitourinary tract can facilitate pyelonephritis (obstructive pyelonephritis, OP). Our goal is to analyze the clinical and epidemiologic characteristics of all the inpatients suffering from pyelonephritis treated in our hospital compared with OP cases.

Methods: Retrospective and descriptive study of OP cases treated as inpatient in our center from January 2000 to December 2000. We have considered OP, all cases with kidney litiasis and/or pyelocalicial enlargement, both detected by ultrasound scan and/or intravenous urography. Student's *t* test was used to compare and Chi-square to rate the comparison.

Results: A total of 73 inpatients were treated of infectious pyelonephritis, 39 of them (53.4%) were OP. Mean age was 51 ± 22. Compared with non-obstructive pyelonephritis (NOP), there were no differences in age, gender, previous urine tract infection or previous urological procedures. In OP group, the abnormalities of urine tract were significantly higher (82% vs. 47%, $P = 0.003$). There were no differences in incidence of diabetes, pregnancy,

HIV infection or existence of permanent urinary catheter. There were no significant differences in the clinical data (dysuria, fever, flank pain, nausea, vomiting and painful kidney percussion) or general laboratory data (leukocytes, platelets or creatinine). Rate of positive blood cultures and urine culture were similar in both groups (blood: 24.75% vs. 26.11%; urine: 48.7% vs. 50.0%). In OP group, the microorganisms isolated by incidence were *E. coli* (52%) and *Klebsiella* (13%), without differences in comparison with NOP group. Invasive procedure were used more frequently in OP patients (38% vs. 15%, $P=0.041$), mainly nephrostomy (13 patients, 33%) and kidney surgery (two patients, 5%). We found no differences in time of antibiotics course or length of stay. In OP group, two patients died, and in NOP one person. The readmission rate in 6 months was higher in OP group, but without statistical significance (26% vs. 9%, $P=0.061$).
Conclusion: The only difference found between OP and NOP patients is that OP is more frequent in patients suffering from urine-tract anomalies. In our study, there were no clinical differences defining OP and OP need of aggressive therapeutical procedure in more than 30% of the cases. So this suggests that an ultrasound scan, to rule out OP, is always necessary in patients with pyelonephritis requiring hospital admission.

P979 Rate of *Chlamydia trachomatis* and genital *Mycoplasma* in men with nongonococcal urethritis and its comparison with control group

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Objectives: Urethritis in men has been categorized historically as gonococcal or nongonococcal (NGU). The major pathogens causing NGU are *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *Trichomonas vaginalis* may be involved occasionally. In up to one-half of the cases, an etiologic organism may not be identified. The aim of this study was the detection of *C. trachomatis* and genital *Mycoplasma* in men with nongonococcal urethritis and its comparison with control group.
Methods: In this study, urine urethral swab specimens and blood samples collected from 125 men with nongonococcal urethritis and 125 men without NGU as control group were examined by culture, microimmunofluorescence and cell culture methods.
Results: *C. trachomatis* seropositive NGU was detected in 20% of the cases and 0.8% of control group. *U. urealyticum*, *Mycoplasma hominis* and *M. genitalium* were isolated from 22.4, 7.2 and 0.8% of the patients, and 7.2, 6.4 and 0% of the control group, respectively.
Conclusion: *Z*-statistical analysis test with 95% confidence interval showed that the difference rate of isolated *C. trachomatis* ($P<0.00003$) and *U. urealyticum* ($P<0.0004$) in case and control groups were significant.

P981 The role of O-antigen of *Proteus bacilli* in infection-induced urinary stones' formation

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Proteus bacilli causes urinary tract infection (UTI) primarily in the complicated urinary tract, most frequently in patients with indwelling catheters or structural abnormalities of the urinary tract. Urinary stones are a common complication of these infections. Urease is the essential virulence factor in stones' formation. Ammonia, produced by the enzymatic hydrolysis of urea, elevates urine pH causing a supersaturation and crystallization of magnesium and calcium ions as struvite (ammonium magnesium phosphate) and apatite (calcium phosphate), respectively. Additionally, extracellular polysaccharides of *Proteus* sp., which have negative charged residues may accelerate crystals' growth and calculi formation by concentrating magnesium and calcium ions during crystals formation. The goal of this study was the analysis of the in vitro crystal growth in the presence of urease-negative mutants of various *Proteus* strains or their O-antigen of known chemical structures. Polyvalent cations (magnesium and calcium) binding by O-antigen and whole cells of urease-negative mutants in artificial urine was measured by atomic absorption spectroscopy. In crystallization experiment, polysaccharide or urease-negative mutant was added to artificial urine, pH was elevated to 8.0 (to mimic urease activity) by addition of 1 M ammonium hydroxide to induce crystallization. Crystal formation was examined by phase-contrast microscopy and by particle analysis with Coulter Counter. It was found that all of the examined strains bound cations through the electrostatic interactions from artificial urine but enhancement of struvite formation was inversely proportional to their binding ability. Of all tested strains and their O-antigens, only *P. vulgaris* O12, which bound cations very weakly, enhanced the formation of crystals in vitro. Most probably, in this case O-antigen binds cations, which then are easily released to the surrounding urine. Subsequent supersaturation of the magnesium and calcium leads to the precipitation of struvite and apatite crystals. We hope that our results will be confirmed in the future using an in vivo experimental model. The knowledge concerning molecular basis of urinary calculi formation will be useful in the diagnostics and treatment of *Proteus* infections which lead to this complication.

P982 Uropathogens: a laboratory view

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Objective: To find out the frequency and antimicrobial susceptibility of urinary pathogens in hospitalized patients.
Methods: All urine isolates from urine cultures were collected from inpatients in Urology and Nephrology Center in Mansoura University. The analysis was based on the microbiology laboratory data. Urine cultures were done in laboratory according to the standard techniques. The identification of isolates and antimicrobial susceptibility tests were performed using the MicroScan Walk Away 40 dried MIC/ID panels.
Results: A total of 235 isolates from urine cultures were analyzed. There were 89.4% Gram-negative pathogens among them and 9.4% were Gram-positive pathogens. The predominant species was *Escherichia coli* which represented 29.4% of the isolates. The isolates that followed were *Klebsiella* spp. (19.6%), *Pseudomonas aeruginosa* (11.9%), *Enterobacter* spp. (8.9%), *Serratia* spp. (8.5%), *Citrobacter* spp. (5.5%) and coagulase-negative staphylococci (4.7%). These seven pathogens accounted 98.8% of the isolates. The highest rate of susceptibility among all Gram-negative pathogens was that of imipenem (93%) and amikacin (83.3%), the susceptibility rate of amoxicillin-clavulanate was (59%) and trimethoprime/sulphamethoxale was (36.9%). *E. coli* alone was susceptible to imipenem (92.8%), amikacin (76.8%), nitrofurantoin (58%), aztreonam (55%), cefotaxime (53.6%), amoxicillin/clavulanate (52.2%), and gentamicin (42%). *Klebsiella* spp. was susceptible to amikacin (92.5%), ceftazidime (60%), cefotaxime (52.5%), gentamicin (50%), and amoxicillin/clavulanate (50%). The susceptibility of *P. aeruginosa* to imipenem was 85.7% and to ceftazidime 60.7%. The susceptibility of *Enterobacter* spp. to imipenem and amikacin was 85.7 and 71.4%, respectively. The susceptibility of coagulase-negative staphylococci to penicillin was 81.4%, cefotaxime 54.5% and was lower (50%) to the rest of the tested antimicrobials. All the tested Gram-positive pathogens were susceptible to vancomycin.
Conclusion: The most common pathogen among isolates from urine cultures was *E. coli*. There was no single antimicrobial agent with acceptable rates of activity against all the isolates.

HIV II

P984 In vitro action of the HIV regulatory protein TAT in human mammary cells

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Objective: We aimed to assess the possible influence of pleiotropic factor TAT on mammary epithelial cells for a better understanding of the mechanism on the basis of the vertical transmission of HIV during breast feeding. We also evaluate the pattern of basal expression of the chemokine receptors and some interleukines in this system for analyzing possible level modification due to the specific treatment with the protein TAT.
Methods: We used cellular culture of different gland epithelial cell lines: mec-1 and mec-2 (SV40 immortalized primary mammary epithelial cells), MCF-10A (spontaneously immortalized mammary epithelial cells), and MCF-7 lines. The evaluation of the cellular proliferation was made with

an image acquisition computer system linked to a camera microscope utilized at different time after stimulation with exogenous TAT (100 ng/mL with DTT). For neutralizing the action of TAT, we used an antibody anti-TAT. The expression of the chemokine receptors and interleukine was seen at cellular mRNA level with an extraction of the nucleic acid, the spectrophotometric quality control, the retrotranscription and the final amplification of the selected regions through the specific primers.

Results: After 24 h, TAT starts stimulating the proliferation of the MCF7 line cells in the order of the 25% with respect to not stimulated cells, this effect is neutralized specifically by the co-stimulation with TAT and antibody anti-TAT (1 mg/mL). In MCF 10A the proliferating effect achieve the level of the 50% after 24 h of stimulation respect to untreated cells; at the same manner the proliferating effect is neutralized by the anti-TAT. In mec-2, there is a proliferating effect in the order of the 25% after 24h but not in mec-1 line. The mec-1, mec-2, MCF7 and MCF10A express CD4 and CXCR4, but not CCR5, CCR1, CCR2, CCR3. Mec-1 and mec-2 express IL-6 and IL-6 receptor, MCF-7 only IL-6 receptor and MCF-10A no one. No lines express IL 8, while the three isoform of vascular endothelial growth factor (vegf) are expressed from mec-1 and mec-2.

Conclusion: The human mammary epithelial cells lines respond to the stimulation with TAT proliferating 24 h after, this action is specifically blocked with anti-TAT demonstrating that the proliferating effect is mediated by TAT. The line express CD4 and CXCR4 that sinergically can mediate the, already demonstrated in those lines, virus entry.

P985 Role of cytokines and C-reactive protein (CRP) in the diagnosis and outcome of HIV-1-infected patients with pulmonary infections

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Objective: To evaluate the role of several cytokines and CRP in the diagnosis and outcome of HIV-1-infected patients with PI.

Methods: Prospective study of all consecutive HIV patients diagnosed of PI in our institution between April 1998 and May 2001. Plasma CRP and IL-1β, IL-6, IL-8, IL-10 and TNFα were performed at admission and 5 days later. Patients were included in a protocol addressed to study the etiology and outcome.

Results: A total of 249 PI were diagnosed in 220 patients (160 males). Mean (SD) age was 39 (11) years. The main etiological groups were: bacterial pneumonia (BP) in 114 episodes (59 caused by *S. pneumoniae*), *P. carinii* pneumonia (PCP) in 37 episodes and tuberculosis (TB) in 36 cases. A total of 24 patients died (10%) – PCP, 6 cases; BP, 4 and TB, 1 case. Median levels at admission of CRP and cytokines for BP, PCP and TB were: CRP (normal values <0.8 mg/dL) 10.2, 3.75 and 5 mg/dL, respectively ($P=0.0001$); IL-8 (NV < 10 pg/mL): 19, 3 and 2.9, respectively ($P=0.045$); TNFα (NV < 20 pg/mL): 46.5, 44 and 75 pg/mL, respectively ($P=0.029$). Concerning the levels of IL-1β, IL-6 and IL-10, there were no statistically significant differences among the three etiological groups. Neither there were correlation between the etiologies and the values of cytokines and CRP performed on the 5th day. There was also no correlation between the CD^{4+} cell count and the levels of CRP and cytokines. Patients with undetectable plasma RNA HIV-1 viral load (VL) had higher levels of IL-1β than patients with a positive VL (13 pg/mL vs. 4 pg/mL, $P=0.01$). Patients with VL > 200.000 copies/mL had higher levels of TNFα (75 pg/mL vs. 46 pg/mL; $P=0.006$). Compared with survivors, patients who died had at admission higher levels of IL-6 (NV < 5 pg/mL) 95 pg/mL vs. 36 pg/mL ($P=0.014$), IL-10 (NV < 10 pg/mL) 22 pg/mL vs. 6 pg/mL ($P=0.01$) and TNFα 70 pg/mL vs. 46 pg/mL ($P=0.041$). At day 5 post-admission, the IL-6 remained statistically higher in those patients who died (126.5 mg/dL vs. 24 mg/dL; $P=0.043$).

Conclusion: At admission, plasma CRP and IL-8 levels were higher in HIV-1 patients with BP and TNFα was higher in those with TB. There was no correlation between the CD^{4+} cell count and levels of CRP or cytokines. Patients with high plasma VL had high plasma levels of TNFα and patients with undetectable VL had high levels of IL-1β. Patients with PI and a worse outcome had higher plasma levels of IL-6, IL-10 or TNFα at admission and maintained high plasma CRP levels on the 5th day

P986 Longitudinal study of density of expression of CD38 antigen on $CD8^+$ cells in HIV/AIDS patients treated with HAART

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Objective: HIV infection is associated with chronic immune activation including increased proportion of $CD8^+$ cells expressing CD38 antigen. Furthermore, a high percentage of $CD8^+/CD38^+$ cells in HIV patients may correlate with the disease progression. However, less is known about the density of expression of this activation molecule. The aim of our study was to analyze the density of CD38 expression on $CD8^+$ cells in HIV-1 associated disease treated by HAART and assess whether the degree of CD38 expression on $CD8^+$ cells correlates with the $CD4^+$ T cell count, plasma HIV-1 RNA level or stage of the illness.

Methods: In a longitudinal prospective study of cohort HIV-positive patients, the patients were followed for a period of 12 months. Seronegative controls were chosen at random to obtain comparison values. T-cell subsets were examined by two-color flow cytometry (FACScalibur, Becton-Dickinson, San Jose, USA). CD38 expression on $CD8^+$ cells was measured as relative fluorescence intensity using monoclonal antibody (mAb) anti-CD38PE in combination with gating on cells stained by mAb anti-CD8PerCP to select CD8 bright cells – CD8high; fluorescence linearity was assessed using QuantiBrite tubes (all BD, Heidelberg, Germany). Clinical evaluation was performed at 6 months intervals.

Results: Although the percentage of CD8high/$CD38^+$ cells correlated ($P=0.05$; $r=0.40$; Spearman's test) with the disease progression, the density of expression of CD38 antigen did not show any correlation.

Conclusion: Our results indicate that an increase of the percentage of CD8high/$CD38^+$ cells in HIV-infected patients reflecting the progression of HIV infection is significantly influenced by subset with low density of expression of CD38 antigen (CD8high/CD38low cells). However, whether CD8hi cells with different density of expression of CD38 antigen might play a role in the disease immunopathophysiology needs to be further clarified, and CD38 immunofluorescence quantification may serve as an useful tool in such a study.

Acknowledgement: The study is supported by the Grant IGA MZ CR NI 6303-March 2000.

Results I	Healthy controls ($n=18$)	HIV/AIDS patients ($n=24$)	
		Year 2000	Year 2001
$CD4^+$ T cells (cells/mm^3)	1007 ± 95	565 ± 80*	579 ± 54*
CD8hi/$CD38^+$ cells (%)	55.1 ± 2.8	73.2 ± 3.9*	71.2 ± 2.8*
CD8hi/$CD38^-$ cells (%)	44.4 ± 2.6	26.4 ± 3.9*	28.3 ± 3.5*
CD8hi/CD38lo cells (%)	46.6 ± 2.3	55.5 ± 2.7*	56.8 ± 2.3*
CD8hi/CD38med cells (%)	8.1 ± 0.7	15.0 ± 2.4	16.2 ± 3.6
CD8hi/CD38hi cells (%)	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.4

*$P < 0.05$ (patients vs. controls). Statistical analysis employed one-way ANOVA test. Data are presented as mean ± SE.

P987 Sequential computerized tomography (CT) assessment of thymic size modification in HIV-infected adult patients starting their first antiretroviral therapy (ART): relationship with virologic and immunological response

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Objective: To assess the relationship among first-line ART, thymic residual tissue, and thymic size evolution in a 12-month follow-up.

Methods: Ten consecutive HIV-infected patients (p) aged 23–58 years, underwent contrast-enhanced CT scan of thymus immediately before starting their first ART, including two nucleoside analogues (AZT-3TC or ddI-d4T), associated with either a protease inhibitor (nelfinavir or indinavir), or a non-nucleoside reverse trascriptase inhibitor (efavirenz or nevirapine). All p had an adherence >90% to the prescribed ART, and received a quarterly virologic

and immunological evaluation; in six cases a thymus CT scan was repeated 1 year after ART initiation. Thymic size was assessed by a 0–4 score proposed by McCune JM (J Clin Invest 1998; 101:2301),with 0 representing no appreciable thymic residuals, and grades 1–4 a progressively increased thymic size (minimal tissue at stages 1–2, and abundant tissue at score 3–4).

Results: While baseline thymic size did not correlate with p's age, gender, duration of HIV infection, diagnosis of AIDS, concurrent $CD4^+/CD8^+$ count, and HIV-RNA levels (a score 3 was recognized in two p, a score 2 in five p, a score 1 in two p, and a score 0 in one p), ART-related immune recovery and drop of viremia were associated with an increase or at least a maintenance of pre-treatment thymic score (three cases each), regardless of baseline laboratory assays, and composition of ART. In detail, when comparing the three p who did not show changes of thymic size after a 12-month ART (maintaining a score of 2 in two p, and a score of 1 in one p), the three p who experienced an increase of thymic volume (as expressed by a shift from score 2 to score 3 in two p, and from score 3 to score 4 in the third p), had a greater 1-year mean $CD4^+$ rise versus baseline levels (511 ± 61 vs. 296 ± 116, opposed to 441 ± 176 vs. 205 ± 167 cells/mL; $P = 0.04$), whereas virologic and immunological workout performed at the 3rd month of ART, and all temporal evolution of both $CD8^+$ count and viremia did not show significant differences between these two p groups.

Conclusion: Our preliminary experience confirms that thymopoiesis has a key role in the immune recovery following effective ART also in adults, from both a functional and a morphological point of view. Prospective studies involving large p samples and detailed immunological analysis are needed to investigate the significance of thymus in HIV-infected adults undergoing ART, and the complex relationship among virologic outcome, immune reconstitution, and cytokine network.

P988 Rhino-orbital mucormycosis secondary to diabetic ketoacidosis in a HIV-positive patient treated with protease inhibitors

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Objective: To describe a clinical case of rhinoorbital mucormycosis secondary to diabetic ketoacidosis in an HIV-positive patient treated with protease inhibitors.

Clinical case: A 42-year-old HIV-positive white heterosexual male was admitted to our hospital because of progressive asthenia, visual disturbances and polydipsia. He denied intravenous drug use. He was in treatment with indinavir, ritonavir, abacavir and lamivudine. On admission, he presented with clinical and laboratory signs of diabetic ketoacidosis. He was treated with intravenous insulin until normalization of plasma glucose and acidosis. On the 3rd day of hospital-stay, he developed fever and left-palpebral ptosis, and a standard radiograph evidenced maxillary sinusitis. Meticillin-sensitive *Staphylococcus aureus* grew from two blood cultures and from a nasal swab, and the patient was started i.v. amoxycillin plus clavulanate. On the 3rd day of therapy, the patient showed no amelioration of his clinical condition. A magnetic nuclear resonance of the brain showed a fluid collection in the left maxillary and ethmoidal sinuses and signs of orbital cellulitis. In the suspicion of mucormicosis, i.v. amphotericin-B was administered, and the patient was referred to an ENT specialist for sinus biopsy that confirmed the presence of necrotic tissue with hyphae characteristic of mucormycosis. Ten days later, an orbital exenteration plus maxillary ostheotomy was performed. After a prolonged course of amphotericin-B the patient was discharged, and a 3-month follow-up did not show progression of the fungal infection.

Discussion: Mucormycosis is rare in AIDS patients without specific risk factors such as diabetes or intravenous drug use. Treatment with protease inhibitors can induce ketoacidosis in diabetic patients, predisposing these patients to this severe and often fatal complication. Clinicians should be aware of this manifestation when managing diabetic HIV-infected patients.

P989 Lipid abnormalities and *Chlamydia pneumoniae* infection in HIV-positive patients: is there a relationship?

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Introduction: Lipid abnormalities are relatively common in HIV-positive patients, particularly after the introduction of HAART. *Chlamydia pneumoniae* (CP) is considered an important co-factor in the pathogenesis of atherosclerosis and seems 'per se' responsible of lipid abnormalities [1]. To note, the prevalence of CP infection and its role in inducing lipid alterations in HIV-positive patients have not been investigated yet.

Aim: To evaluate the prevalence of CP infection among HIV-infected patients and to assess the relationship with lipid abnormalities.

Materials and methods: Blood samples collected from 66 (M/F 49/17, mean age 41.2 ± 7.7) HIV-positive patients were tested for CP by nested-PCR. Out of the 66 patients tested 55 were on antiretroviral therapy (44/55 with protease inhibitors drugs). From each patients clinical, biochemical, immunological and virologic data were recorded at the time of sampling for CP testing.

Results: CP was detected in 18 (27.2%) of the 66 cases studied. Patients with and without CP were comparable in term of sex, mean age, and risk factors for HIV infection. Mean CD4 count was significantly lower in CP+ patients compared to CP– (348.8 ± 205 vs. 516 ± 278; $P = 0.02$), whereas viral load did not differ significantly (10341 ± 24961 vs. 6910 ± 16650, respectively). Patients on antiretroviral treatment had significant higher mean level of both cholesterol and triglycerides than untreated patients (192 ± 64.3 vs. 132 ± 49 e 212 ± 168.2 vs. 91 ± 64; $P < 0.05$). The relationship between lipid profile and CP is shown in the Table 1.

Table 1 The relationship between lipid profile and CP

	Clamydia P+	*Clamydia* P–	*P*
Cholesterol	193.6 ± 28.1	184 ± 68.9	NS
HDL	59.46 ± 20	61.4 ± 28	NS
LDL	96.3 ± 54	108 ± 61	NS
Triglycerides	196.2 ± 161	191.2 ± 162	NS

Conclusion: In HIV-positive patients, the prevalence of CP infection seems to be inversely related to the $CD4^+$ cell count. Furthermore, our data doesn't support a significant role of CP as cofactor of hyperlipidemia, which seems strictly related to antiretroviral therapy. The relatively high prevalence of CP infection together with the high prevalence of lipids abnormalities suggest that HIV-positive patients should be carefully evaluated and monitored as at risk for cardiovascular diseases.

P990 Efficacy and tolerability of pravastatin in the treatment of hyperlipidemia in HIV-infected patients receiving HAART

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Introduction: Laboratory and clinical abnormalities of lipid metabolism have been increasingly recognized after the advent of highly active antiretroviral therapy (HAART). Significant increases in plasma triglyceride and cholesterol levels have been reported especially in protease inhibitor (PI)-treated patients, and prolonged metabolic imbalances could significantly act on the long-term prognosis and outcome of HIV-infected persons.

Patients and methods: Sixteen HIV-infected patients on PI-based HAART since at least 12 months and presenting hypercholesterolemia (>280 mg/dL), with or without hypertriglyceridemia (>300 mg/dL) and lipodystrophy syndrome, of at least 6-month duration and unresponsive to a hypolipidemic diet and physical exercise, have been treated with a single daily dose of pravastatin (20 mg) for 12 months.

Results: Two patients were excluded from evaluation due to early drop-out. Ongoing antiretroviral treatment included ritonavir in five cases, indinavir in four, nelfinavir in two, and sequential PI administration in three; concomitant hypertriglyceridemia and lipodystrophy were observed in eight and five cases, respectively. Mean plasma triglyceride values tested significantly higher in all patients treated with ritonavir compared with other PI, while plasma cholesterol levels and lipodystrophy prevalence did not show any significant difference according to the administered PI-based regimen. At the close of 1-year follow-up of pravastatin therapy, a decrease of total cholesterol and triglyceride levels of 23.6 and 31.2% versus respective baseline values was observed; 6 out of 14 patients reached normal values for cholesterol and three out of eight for triglycerides. Mild gastroenteric symptoms were found in only one of the 14 treated patients, while no skeletal muscle and liver toxicity has been observed; adherence to pravastatin therapy proved always over 90% of daily doses for the overall follow-up.
Discussion: The statins showed beneficial effects in both reducing cholesterol and triglyceride levels in HIV-negative population; in HIV-positive persons, simvastatin, lovastatin and atorvastatin should be avoided because they are extensively metabolized by cytochrome P450 3A4, while pravastatin presents a lack of significant interaction with PI. In our study, pharmacological treatment with pravastatin proved certainly effective in the management of diet-resistant dyslipidemia, and was associated with a favorable tolerability and adherence profile.

P991 Meningeal co-infection with *Mycobacterium tuberculosis* and *Listeria monocytogenes* in a HIV-infected patient

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Objective: We report the case of an HIV-infected man with meningeal co-infection with *Mycobacterium tuberculosis* and *Listeria monocytogenes*.
Case-report: A 38-year-old Brazilian man presented to our hospital with a 36 h history of fever, headache, vomiting and diarrhea. He was a HIV-infected patient, diagnosed 3 months ago, because of generalized necrotic granulomatous lymphadenopathy, which after adequate work-up was attributed to the HIV infection. Of note, the Mantoux testing, lymph node acid-fast stain examination and blood cultures were negative for mycobacteria. His CD4 count was 166 cells/μL and viral load >50 000 copies/mL and was started on HAART with stavudine, lamivudine and indinavir with ritonavir as booster. He also received trimothoprim/sulfamethoxazole prophylaxis once daily. On examination the patient was severely ill with upper and lower meningeal signs. The lymphadenopathy persisted although much improved since 3 months ago. Chest X-ray was negative. MRI scan showed meningeal enhancement with no focal lesions. The lumbar puncture performed revealed 135 cells/mL, 60% of whom where lymphocytes, glucose <10 mg/dL and protein <1 mg/dL. The Gram stain showed rare Gram-positive bacilli and one acid-fast staining microorganism and the cultures were negative. The CSF PCR was positive for *Mycobacterium tuberculosis*. The 4a antibody titer against *Listeria monocytogenes* was 1 : 8 which was considered positive. The patient was started on quadruple antimycobacterial treatment with isoniazid, rifabutin, pyrazinamide and ethambutol; he also received prednisone for the first 3 days. He was started also on ampicillin and garamycin for listerial meningitis, as well as ceftriaxone and the patient responded by improving clinically and defervescing completely on the 10th day of treatment.
Conclusion: We describe this case as an example of rare meningeal co-infection with *Mycobacterium tuberculosis* and *Listeria monocytogenes* in an HIV-positive patient, who was treated effectively. As far as we know, there are no such previous reports in the literature up to date.

P992 Caspofungin in cryptococcal meningitis in an HIV(+) patient

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Introduction: We report a 32-year-old woman who was first diagnosed with HIV infection owing to meningitis with CD4 count 4 cells/μL and VL > 50 000 copies/mL. The CSF examination demonstrated increased number of cells (75–434, 95% lymphocytes) and a mild raise of protein, while the India ink stain proved the existence of *Cryptococcus*. *Cryptococcus* was also found on lymph-node biopsy. The cryptococcal antigen titer in CSF and serum was 1 : 8192 and 1 : 4096, respectively. The combination of liposomal amhotericin B (5–20 mg/kg/day) and 5-flucytosine (150 mg/kg/day) were used for 4 weeks to treat this generalized cryptococcal infection but unsuccessfully. Fluconazole 800 mg/day and itraconazole 400 mg/day were used instead, for 2 weeks, successively, without any therapeutic response, too. On the contrary, the patient's condition deteriorated. The cryptococcal antigen titer in CSF and serum remained stable, and the findings of the CSF examination did not really vary. There was an improvement only when caspofungin was given for 12 days (70 mg the 1st day and then 50 mg/day) although afterwards stopped because of thrombocytopenia. The clinical signs of meningitis declined within 5 days and the cryptococcal antigen titer decreased in serum and CSF to 1 : 1024 and 1 : 512, respectively, 3 weeks after caspofungin was administered. The *Cryptococcus* no longer was found in the serum or CSF.
Conclusion: Caspofungin is the first of a new class of fungicidal agents that has synergy in combination with liposomal amphotericin B and gives really safe results in specific cases of cryptococcal meningitis that are resistant to the usual therapy.

P993 Human T-cell lymphotropic virus (HTLV) antibody prevalence in HIV-1-infected individuals attending a sexual health clinic in South-east London

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Objectives: HIV and HTLV are retroviruses with similar biological features, e.g. route of transmission and targeting T lymphocytes. HTLV type 1 is associated with adult T-cell leukemia/lymphoma and tropical spastic paraparesis and HTLV-2, found mainly in drug users, is associated with neurological conditions. The epidemiology of HIV infection in the UK is changing. At our hospital, an increasing proportion of HIV patients are female and from diverse ethnic groups (38% born in sub-Saharan Africa and 4% in the Caribbean). This unlinked anonymised retrospective study aimed to determine the prevalence of HTLV in the local HIV-infected sexual-health clinic attendees.
Methods: Sera were collected from 777 HIV-1 positive adult (504 males, 273 females) attendees (January 2000–March 2001). Patients were anonymised for clinic number whilst data on ethnicity, country of birth, gender and age was retained. Duplicate samples were excluded. Pools containing five sera were tested using an enzyme linked immunoassay. Individual samples from reactive pools were re-tested using the same assay. Reactive samples were confirmed using a passive particle agglutination test and an immunoblot to discriminate between HTLV types.
Results: In total, 42% of the cohort were Black African (BA), 45% White (W), 8% Black Caribbean (BC), 1% Black other (BO) and 4% other ethnic groups. HTLV antibody was detected in 6 of 777 sera (0.77%). Two further sera were indeterminate by immunoblot.
Conclusion: To our knowledge, this is the first study to investigate the prevalence of HTLV–HIV co-infection in attendees of a UK sexual health clinic. HTLV antibody prevalence was double our antenatal population (0.39%). Four of six HTLV-1 co-infected patients were female reflecting greater efficiency of transmission from males to females. Recommendations on reduction of HIV transmission also apply to HTLV, but those co-infected should avoid breast feeding. Patient 5 demonstrates that transmission may occur outside recognized areas of high endemicity. Potential interactions between HIV and HTLV include the finding that CD4 counts may be higher in those co-infected but do not necessarily provide immunological benefit. This may affect clinical management as antiretroviral therapy or prophylaxis against opportunistic infections may be delayed. Moreover, the effect of

antiretroviral therapy on HTLV infection is being elucidated. Thus, consideration should be given to screening our local HIV population for HTLV co-infection.

Patient	Age	Gender	Ethnicity	Origin	HTLV type
1	46	F	BC	Grenada	1
2	40	F	BA	Ivory Coast	1
3	40	F	BA	Zaire	1
4	30	F	BA	Ivory Coast	
5	24	M	BO	UK	1
6	40	M	W	Italy	2
7	28	F	BC	Jamaica	Intermediate
8	36	F	W	UK	Intermediate

P994 Screening of HTLV-I/II in patients related to an organ transplantation program

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Objective: Analysis of the prevalence of HTLV-I/II infection in patients related to the organ transplantation program in the University Clinic Hospital of Zaragoza, Spain.
Methods: Presence of HTLV-I/II specific antibodies was analyzed in 250 patients related to the organ transplantation program from December 1998 to November 2001: 79 consecutive organ donors, 84 consecutive liver recipients, 34 discarded donors (for medical contraindication or family lack of consent) and 53 possible liver recipients in evaluation. Out of 250 patients, 179 were male and 71 female, aged between 14 and 78 (mean 50.8; SD 14.8). All patients were Spanish, except four male ones: a Rumanians liver recipient, a Moroccan possible donor (whose family denied consent), a Senegalese possible liver recipient (he was presenting HBV fulminant hepatitis, but grafting was contraindicated because of HIV infection) and a Senegalese in evaluation previous to kidney transplantation. HTLV specific antibodies were studied by using two different EIA: an indirect viral lysate-based HTLV I/II EIA (Abbott) until August 2000 and a recombinant and synthetic antigen sandwich EIA Murex HTLV I + II (Abbott Murex) later on.
Results: Two (0.8%) out of 250 patients were EIA-positive. Case 1 was a Spanish male liver recipients aged 61 years with an EIA index of 1.1. Case 2 was a Spanish male liver recipient aged 49 with an EIA index 1.2. Case 1 could not be reanalyzed by Western blot or PCR. Case 2 was tested with Western blot and PCR by the HTLV Spanish Study Group (Hospital Carlos III, Madrid). Western blot result was indeterminate, showing a weak antibody response to rgp21 and PCR was negative. Both cases were EIA-negative during the follow-up, 12 and 15 months after liver transplantation, respectively. Both cases were considered as HTLV-false positive.
Conclusions: HTLV-I/II infections do not seem a major problem in the transplantation program in Spain. Nevertheless, as 49 HTLV-I and 377 HTLV-II-infected patients have been registered by the HTLV Spanish Study Group until 2000 and as three cases of symptomatic HTLV-I infection in Spanish organ recipients, related with the same donor, have been also described, screening of organ donors and recipients must be considered.
Acknowledgement: To HTLV Spanish Study Group for their help with Western blot and PCR analysis.

P995 Epidemiological characteristics of AIDS in Kosovo

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In order to show epidemiological characteristics of AIDS in Kosova we have analyzed cases from 1986 until November 2001 using retrospective method. During this period of time, 42 persons have been affected while 13 of them died. The highest number of disease were register in the year 2001 (till November) 12 cases or 5.12 cases in 1 000 000 inhabitants. The highest number of deaths were register in the year 1999 two or 0.8 cases in 1 000 000 inhabitants. While the lowest number of cases were register in 1991, 1993 and 1998 with only one case. During the year 1988, 1989 and 1990 was not register any case of disease. During the period that we analyzed the highest number of cases were register in Prishtina municipality 7, Peja municipality 6 and Ferizaj 4. Overall, 65% were males and 35% females. The most affected age groups were 30–39 (37.5%), 40–49 (29.1%) and 20–29 (20.8%). According to the present data we could say that epidemiological situation in Kosova is not so alarming compared to other countries. Problem is not knowing the number of HIV carriers. However, since there are many foreign citizens coming to Kosova as well as many Kosovar's returning from foreign countries, the situation is expected to be worsen.

P996 Tuberculosis in HIV patients in a Spanish hospital

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Objectives: To know the epidemiology and evolutionary characteristic in patients with HIV infection that developed tuberculosis in the area of our hospital.
Materials and methods: We studied the retrospectives cases of tuberculosis in HIV patients from 1994 untill 2001 in Costa del Sol Hospital.
Results: Fifty-five patients with HIV infection were diagnosed of tuberculosis. Forty-seven patients (85.5%) were males and eight (14.8%) females. The risk was drug-abusers in 45 cases (81.8%), heterosexuals in six and two homosexuals. In two patients, the risk were unknown. In general, all were very inmunocompromised patients. Thirty-five patients (63.6%) had less of 200 CD4/mm^3 and were diagnosed of AIDS. In 20 patients (36.6%), the HIV infection were unknown and they were diagnosed of the two infections simultaneous. Twenty-five patients (45.4%) were diagnosed of pulmonary tuberculosis by bacteriologic examination of sputum. In three patients with pleural effusion by biopsy. Twenty-seven (49.6%) patients were diagnosed of disseminated tuberculosis (two patients had cultivators of cerebroespinal fluid positives). Eighteen patients (32.7%) the diagnosis was by lymph node examination. The evolution was: 38 patients (69%) recovered with treatment, 10 patients (18.1%) died and eight of this abandoned the treatment. Seven patients (12.7%) were lost. The patients that died were not taking antiretroviral treatment.
Conclusion:
1. Sixty-three percent the patients were very inmunocompromised (CD4 < 200/mm^3).
2. Fifty percent were extrapulmonary tuberculosis or disseminated tuberculosis cases.
3. Twenty patients (36.3%) with HIV infection and tuberculosis were simultaneous diagnosed.
4. Eighty percent of the patients that died, previous abandoned tuberculosis treatment.

P998 Pharmacokinetic evaluation of indinavir and indinavir/ritonavir containing antiretroviral regimens in a clinical setting

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Objective: To describe the pharmacokinetics of different regimens of indinavir (IDV) in HIV-1-infected subjects and to investigate the relationship between plasma concentrations and clinical outcome.
Methods: Twenty HIV-1-infected subjects receiving IDV (800 mg tid) as the only protease inhibitor ($n = 10$) or IDV (800 mg bid) in combination with RTV (100 mg bid) ($n = 10$) were recruited. Five blood samples (7 mL) from each patient were taken over the dosing interval (before drug intake in both groups and after 1, 2, 4, 8 h for IDV alone and after 2, 4, 8 and 12 h for IDV/RTV). Plasma drug concentrations were measured using liquid chromatography with tandem mass spectrometry detection.
Results: See Table 1.

Table 1

	IDV alone	IDV/RTV
Median $CD4^+$ (cell/mm^3) (range)	423 (313–635)	399 (130–710)
Undetectable VL	6/10	1/10
AUC (μg h/mL) (range)	30.2 (15.7–57.8)	28.8 (18.4–11.6)
Ctrough (ng/mL) (range)*	177 (81–496)	423 (223–4103)

*$P = 0.014$ (Mann–Whitney *U*-test).

None of the subjects treated with IDV/RTV had Ctrough below IDV MEC of 120 ng/mL No significant correlation was shown between plasma IDV concentrations and $CD4^+$ cell count for both groups ($P = 0.534$, IDV alone and $P = 0.916$, IDV/RTV; Pearson's correlation). IDV Ctrough showed a significant relationship with HIV-RNA values in patients on IDV alone ($P = 0.042$; Pearson's correlation).

Conclusions: Plasma IDV Ctrough showed wide inter-patient variability. Our results agree with previously published data where plasma IDV levels correlate with HIV-RNA and enhancement of IDV by RTV is often needed to maintain concentrations above IDV MEC.

Pediatric viruses

P999 Genotype of the wild measles viruses isolated in Iran

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Measles virus (MV) is an important human pathogen. Despite the encouraging progress towards control of measles in several areas of the world, it is still the cause of up to 1 million deaths each year. MV has one stereotype but wild-type isolates are known to be genetically heterogeneous and at least 15 distinct genetic clusters have been identified for MV by sequence comparisons of the nucleocapsid protein (N) gene. According to implemented measles control programs in Iran, mass vaccination will be initiated in 2002, so it is important to identify the genetic patterns of indigenous measles virus in Iran before mass vaccination campaigns are initiated. The comparisons of these data with the genetic data obtained from the wild viruses, which cause outbreaks after mass vaccination, would interpret the success of measles control programs in Iran. For achieving these purposes and isolating wild-MV, 220 throat-washing samples were selected and inoculated into the specific cell lines, such as B95 and Vero. Total seven wild-type of MV were isolated, which had significant CPE and positive IIF tests. The total RNA was extracted from positive cell culture and tested by RT-PCR. Specific primers amplified about 600 nucleotides of C-terminal of N gene. Sequencing of this variable region was performed for five measles viruses. Analyzing these data by the clustal routine of MegAlign program in DNA STAR package indicated that these strains are similar to each other and the most diversity was found in 1.3%. Comparison of these sequences with the sequence of Edmonston-wild-type (Ed-wt) suggested that these wild-type viruses are Edmonston-like and belong to Clade A. According to phylogenic tree, they form a separated group behind the Ed-wt. These molecular epidemiologic studies are an important marker for interpretation of the control programs. It will provide a means to describe the source of Measles viruses, assess the extent of virus circulation and outbreaks. Also they will become a powerful tool for evaluating strategies to control, eliminate and eventually eradicate measles.

P1000 Complications of varicella requiring hospitalization in previously healthy children

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Madrid, E

Objective: To describe hospital admissions for complicated varicella in previously healthy children.

Methods: A retrospective record review of patients between 1 month and 14 years hospitalized for complicated varicella between 1 January 1987 and 31 November 2001 was conducted in La Paz Children's Hospital, Madrid, Spain. Children with immunosuppressive/immunodeficient conditions or treated with immunosuppressants and newborn infants were not included in the study.

Results: During the study period, a total of 246 (134 males and 112 females) immunocompetent children were hospitalized for complicated varicella. Of these, 79 children presented two or more complications. Mean age was 3.6 years (0.2–14) and mean hospital stay was 7 days (1–50). Most frequent complications associated with varicella are presented in the Table.

Complication	*N* (%) patients	Mean age ± SD (years)	Mean hospital stay, days (range)
Skin/soft tissue infection	127 (51.6)	3.5 ± 2.8	7.4 (1–50)
Central nervous system	54 (21.9)	3.8 ± 3.1	7.0 (1–50)
Bacterial pneumonia	34 (13.8)	3.2 ± 3.0	9.2 (2–50)
Thrombocytopenia	18 (7.3)	5.6 ± 3.9	11.2 (1–38)
Ear–nose–throat	17 (6.9)	3.6 ± 2.9	5.5 (1–14)
Bronchitis	17 (6.9)	2.2 ± 1.8	5.9 (1–12)
Gastrointestinal	16 (6.5)	2.5 ± 3.7	5.0 (2–13)
Sepsis	14 (5.7)	2.5 ± 1.2	14 (6–50)
Osteoarticular	6 (2.4)	5.7 ± 2.5	18 (7–38)

Conclusions: Varicella may result in serious disease requiring hospitalization in otherwise healthy children. In this study, varicella complications that most frequently prompted hospitalization were secondary skin/soft tissue infections (51.6%) and neurologic complications (21.9%). These data provide further evidence that varicella may result in hospitalization in otherwise healthy children and may be used for cost–benefit analyses when universal varicella vaccination is considered.

P1001 *Picornaviridae* and central nervous system damages: a role for human Parechovirus type 1?

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Objectives: The study was engaged after the description of a case of encephalomyelitis observed in a male child aged 11 months diagnosed in Bahrain in 2001. Clinical specimens were sent to the laboratory and tested for virologic diagnosis. Classical viral etiologies (CMV, HSV, VZV and EV) were investigated in accordance with the described symptoms. After being negative, particularly when enterovirus RT-PCR was performed, the Human Parechovirus type 1 (HPEV1, formerly ECHOvirus 22) diagnosis was engaged.

Materials and methods: CSF samples, throat and rectal swabs, acute and convalescent sera were collected from the patient and sent to the laboratory. CSF and initial swabs were tested for virus isolation. Concomitantly, serologic analysis was achieved, and RT-PCR detection was performed on initial swabs and sera. HPEV1 isolation was carried out by inoculating five cell lines, including HRT. HPEV1 was identified with the conventional neutralization assay. Neutralizing antibody was titrated by neutralization in acute and convalescent sera. A sensitive and specific RT-PCR was developed to detect HPEV1 RNA in samples and HPEV1-infected cells. The RNA extraction was achieved with an isothyocianate-adapted method. The RT-PCRs using HPEV1-specific primers targeting the 5'NTR and VP1 regions were carried out with a one-step procedure. Each PCR product was directly sequenced and aligned in the EMBL data bank.

Results: Stool samples and CSF remained negative in virus isolation, whereas CPE was observed on HRT cells from initial throat swab. Neutralization test

allowed the HPEV1 serotyping. Serodiagnosis was negative (<4) with acute sera, while convalescent sera titrated 128. HPEV1 RNA was detected by RT-PCR from initial throat swab and acute serum as well as from HPEV1-infected cells. The sequence alignment of the PCR products confirmed the HPEV1 specificity.
Conclusion: Among the clinical symptoms, most classically associated with HPEV1 infections (respiratory infections, diarrhea or gastroenteritis), CNS damages have already been described in acute flaccid paralysis or aseptic meningitis. The first case of encephalomyelitis due to a recent acute HPEV1 infection in a child is reported here. In addition, the negative enterovirus detection by PCR confirmed the recent classification of HPEV1 as a member of a new *Picornaviridae* genus. This study emphasizes the importance of the HPEV1 diagnosis in cases of severe CNS infections.

P1002 The seroprevalence of IgG and IgM Parvo-B19 antibodies in Greece

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Objectives: Serologic survey was carried out to estimate the incidence of human Parvovirus-B19 infection among two groups: group A: healthy pregnant women during antenatal examination; group B: women who underwent laboratory tests to identify the cause of previous spontaneous abortions (more than two).
Materials and methods: Sera were collected from 259 pregnant women (group A) and 76 women (group B). Antibody levels for IgG and IgM antibodies against B19 were measured. Values of ^{3}3.5 IU/mL for IgG and ^{3}23 U/mL for IgM were considered positive.
Results: The seroprevalence of Parvovirus-B19 IgG and IgM antibodies was 41.7 and 1.9% for group A and 38.15 and 10.5% for group B, respectively. Negative or low positive IgG concentrations with negative IgM were found in 57.5 and 53.9% in groups A and B, respectively. Positive or low positive IgG values with negative or low positive IgM were detected in 42.1 and 35.5% in groups A and B, respectively. Acute infection with Parvovirus-B19 was confirmed in 1.9 and 10.5% in groups A and B, respectively.
Conclusions: Our results showed: (1) approximately 39.4% of all samples tested were from women associated with patterns of past B19 infection, while 56.7% were found susceptible; (2) specific IgM antibodies were detected in 1.9% of women of group A, but were significantly higher (10.5%) in women with more than two spontaneous abortions and was considered as recent B19 infection; (3) the markers of IgG and IgM antibodies should help the evaluation of the immune status and the discrimination of past or recent infection. In cases with borderline values of IgG or IgM antibodies, a second blood sample after 15 days and an ultrasonographic examination could be helpful.

P1003 Incidence of infections due to human PARVO B19-virus in adults

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Background: PARVO B19-virus, the causative agent of the 'fifth disease' or 'erythema infectiosum' in children, can also affect adults. Clinical manifestations of primary PARVO B19-virus infection vary greatly ('chameleon infection').
Objectives: *To determine the possible role of PARVO B19-virus as an etiologic agent of infections in adults during 18-month period (January 2000–June 2001).*
Materials and methods: Serum samples of 160 hospitalized patients and 35 outpatients (total number: 195), aged from 25 to 55 years, were tested for anti-PARVO B19-virus IgG- and/or IgM-antibodies (Abs). All serum samples were evaluated by an indirect immunofluorescence assay (IFA-BIOTRIN, Ireland) and an indirect immunoenzymatic assay (ELISA-SERION, Germany). Sera with IgG-Abs titers ^{3}1/64 (IFA) and >45 U/mL (ELISA) were considered as positive. Positive for IgM-Abs were sera with titers ^{3}1/16 (IFA) and >17 U/mL (ELISA). The criteria for the presence of recent infection were as follows:
a. high titer of IgG-Abs;
b. the presence of IgM-Abs;
c. seroconversion of IgG-Abs titer between two paired serum samples.
Results: A total of 106 of the 195 patients (54.35%) were positive for IgG-Abs, while 25 of them (12.82%) were found to have high titers of IgG-Abs. IgM-Abs were detected in seven of them (3.59%). Recent infection was confirmed in those seven patients (four outpatients, two patients from the Rheumatology Clinic, one from the Pathology Clinic). Symptoms included fever, insisting arthritis and long-lasting exanthema, although clinical manifestations varied from patient to patient.
Conclusion: The possibility of PARVO B19-virus infection must be considered in the differential diagnosis of febrile and exanthematous diseases in adults.

P1004 Viral infections in childhood bronchial asthma

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The study, carried out by us, of influence of a virus infection contamination on gravity of a bronchial asthma in 153 patients (children) in the age group from 1.5 to 15 years has revealed that the apposition of an acute respiratory infection contamination in 132 (90%) out of the 142 patients, taking place under observation, was accompanied by an exacerbation of a bronchial asthma. Higher frequency of exacerbations of a bronchial asthma was marked at stratification of an RS-virus infection contamination (50%), influenza (44%), parainfluenza (33%), blended virus infection contamination (46.7%). The gravity of a bronchial asthma at 60% of children was accompanied by stratification of often acute respiratory diseases. Eleven patients (children) had hepatitis B virus infection, and nine children had herpes viral infection. The immunologic researches have shown that in all the surveyed children, the origin of a virus infection descended at deficiency of an INF-γ of blood. Changes in the contents of cytokine was expressed in increase of the contents of IL-4, IL-5 and IL-8, and decrease in the level of IL-6 and IL-2, thus it was marked hyperproduction common IgE. We conclude that viral infection promotes allergic sensitization and influences gravity of a bronchial asthma in children.

P1005 G and P genotyping of human rotavirus strains isolated in Korea

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Objectives: The most prevalent rotavirus genotypes worldwide are reported to be P[8]G1, P[4]G2, P[8]G3, P[8]G4. But unusual genotypes have been recognized from several countries. To design a vaccine strategy and to evaluate success of candidate vaccines, it is essential to know the serotypes or genotypes of prevailing rotavirus strains in each country.
Methods: The diarrheal stool specimens from hospitalized pediatric patients were tested for rotavirus by enzyme immunoassay (Vidas Rotavirus, bioMerieux sa, France). EIA-positive stool specimens were analyzed by RT-PCR for group A rotavirus, and the group A-positive PCR products were genotyped by PCR for P and G types.
Results: Among the 65 EIA-positive fecal specimens, collected from 2000 to 2001, 33 were both G and P type. G2 genotype, constituting 62.9%, was the most prevalent type during the study period, and followed by G4 (11.4%), G3 and G1 (5.5% each). P typing revealed P4 as the most predominant type (62.9%), and followed by P6 (22.9%), P8 (11.4%), and P9 (2.9%). The most common combined P and G type was P[4]G2 constituting 62.9%, followed by 22.9% of P[6]G4, two cases of P[8]G1, and one case of P[8]G3, P[8]G4, P[9]G3 each.
Conclusion: The most common rotavirus strain in Korea during 2000–2001 period was P[4]G2, which shows changing pattern of strain prevalence compared to the G1 predominance (89%) in 1990 report from Korea.

P1006 Viral gastrointestinal infections among inpatients of an infectious diseases clinic in Pilsen, Czech Republic: first experiences with serotyping of rotaviruses

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Objectives: Rotaviruses are the most frequent etiologic agents among patients with diarrheal diseases. Epidemiologic data could be the basis for the start of vaccination. The role of other viral pathogens must be analyzed, too.
Methods: The importance of rotaviruses, adenoviruses, and astroviruses was analyzed among children inpatients during January 2000–October 2001. Detected rotaviruses were serotyped with monoclonal antibodies.
Results: During 2000–2001, 799 younger children were examined with gastrointestinal diseases. Viral etiology was observed among 33.5% patients. The most frequent agents were rotaviruses; a rotavirus etiology was proved among children 256× (32.0%), adenoviruses were detected 9× (1.1%), astroviruses 3× (0.4%). The incidence of rotavirus acute gastroenteritis among different age groups was as follows: children under 6 months: 17.2%; 7–12 months: 30.8%; 13–24 months: 46.1%; 25–36 months: 34.1%; older: 24.5%. The incidence rate during March and April was higher compared to the other months. Nosocomial infection was laboratory detected in 30 of the 454 infants repeatedly examined during the hospitalization (i.e. 6.6%). The most frequent agent was rotavirus, too. G-serotypization was investigated in 164 stool samples. The type was detected 131× (i.e. 79.9%). The most frequent serotypes were types G1 and G3 (40.2 and 20.8%, respectively).
Conclusion: The results indicate the need of vaccination against rotaviral infections in the Czech Republic, too. Distribution of serotypes is similar to other countries.
Grant support: IGA MZ Czech Republic No. 6036-3.

P1007 Outbreak of Norwalk virus gastroenteritis in a childcare center

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Objective: To describe an outbreak of Norwalk virus (NV) gastroenteritis in a childcare center.
Methods: A cohort study was conducted to describe the epidemiology of the outbreak and to determinate its cause. A case was defined by the presence of vomiting or diarrhea. Environmental health specialists inspected childcare center and the local restaurant, which supplied food items served at the lunch. Stool samples from cases and from the childcare and the restaurant staff were collected for bacterial and viral investigations.
Results: The gastroenteritis outbreak began in the childcare center on 15 June 2001, with new cases occurring after 3 days over the ensuing week. The first case ate lunch on the childcare center. The pattern of cases was consistent with person-to-person spread. In the center, 21 cases occurred (attack rate was 61%). Clinical infection was also reported in one employee of the restaurant who was not a food handler but shared the food served at the childcare center, and on 11 household contacts of children cases. Predominant symptoms were vomiting (89%) and diarrhea (54%). Taking lunch at the childcare center was not associated with illness. Fecal samples from 14 cases were positive for NV genogroup II using RT-PCR. Two food handlers of the restaurant that also shared food served at the center were positive for NV but had not reported illness, and three members of childcare center staff that did not take lunch at the center. Inspection of the restaurant revealed problems with food handling and preparation. In addition, the childcare center had inappropriate hygienic practices in hand washing, diapering and food handling.
Conclusion: Food-borne transmission probably occurred on the first cases but the person-to-person transmission spread NV among childcare assistants, staff and their families. Our report appears to be the first description outbreak of NV gastroenteritis in a Spanish childcare center.

P1008 Immunization against poliomyelitis in Kosova

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Introduction: Routine vaccination against poliomyelitis in Kosova is targeted to children 2–4 months administered simultaneously with DTP (three doses). First booster dose should be given to children 12–18 months together with DTP, second booster dose to children 6–7 years and third dose to children 12–13 years.
Objective: The aim of this study is to present routine vaccine coverage data against poliomyelitis with live polio vaccine during the last 10 years with approach to strengthening EPI program in Kosova.
Methods: Surveillance data and descriptive analysis of EPI.
Results: During the routine OPV3 coverage between 1991 and 2000, OPV3 was administered to 455 086 children in Kosova with average of 85%. During the year 2000, 92.5% of the children in average were completely covered with immunization. During 2001 (first 6 months), the average was 96%. Supplementary immunization activities SNIDs (mooping-up) in 2001 in the target groups was 69%.
Conclusions: Due to high immunization coverage against poliomyelitis, no cases have been registered in the last 4 years. It is foreseen that the transmission of wild polio virus is interrupted and the ground set of eradication of the disease by the year 2003, the goal set up by health authorities in Kosova. It is expected that the coverage of the population to be vaccinated will be 95%. A new immunization reporting form will be introduced to provide more realistic, timely and reliable data by age groups.

Bone and soft tissue infections

P1010 Interpretation of microbiological cultures from noninfected removed orthopedic devices: preliminary results

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Terrassa, E

Objective: To determine the frequency and significance of bacterial isolates cultured from removed orthopedic devices in patients without a suspected infection.
Methods: Five samples were obtained from each patient (two from osteosynthesis material, two from bone, and one from the interface). Samples were processed according to their characteristics (swab, homogeneizable sample, and solid sample). All patients had a peroperatory biopsy to exclude acute inflammation. None of the patients had a past or present history of osteoarticular infection and they were followed-up for 1 year postsurgery.
Results: To date, 48 patients have been included. In 25 cases (52%), one or more cultures have been positive. Sixteen patients had only one positive sample, four patients two positive samples, four patients three positive samples and in one patient all the five samples were positive. In three cases, the cultures were polymicrobial. The interface samples were more frequently positive (25% of cases) compared to bone and osteosynthesis material (16 and 14%). The most frequent bacterial isolates were coagulase-negative staphylococci (76%) and *Propionebacterium acnes*. Peroperative biopsies were negative for inflammation in all the cases except one. Currently, we have found no correlation between positive cultures and the site of or type of sample, the type of osteosynthesis material or the duration of surgery.
Conclusion: In our experience, more than half of the removed osteosynthesis material and the adjacent tissue are colonized by comensal cutaneous bacteria even in the absence of infection.

P1011 Soft tissue infections in injecting drug users in Scotland, 1996–2000

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Objectives: During the year 2000, Glasgow experienced an outbreak of infection among injecting drug users (IDUs) in which 23 died. It was the recommendation of the Outbreak Management Team that a study should be

undertaken to ascertain the underlying incidence and nature of soft tissue infections (STIs) among IDUs. The aim of this study was to estimate the incidence, trends in, and nature of STIs among IDUs over a 5-year period (1996–2000). These trends were compared with the general population admitted to hospital in Scotland with STI.
Methods: Data were collected using hospital discharge records returned to ISD Scotland. All the patients with a diagnosis of abscess and/or cellulitis (identified by ICD 10 codes) were selected. A further subsearch was carried out for patients with a history of opioid use (also identified by ICD 10 codes). The data were analyzed using SPSS 10.0 for Windows.
Results: STIs appear to be increasing, both in the general population and among IDUs. Among the general population with STI, there was a 60 : 40 male : female ratio, and a 66 : 34 male : female ratio in IDUs. The incidence of general patients presenting with STI increased with age. In 1996, the majority of IDUs presenting with STIs were between 21 and 35 years old. Today the age range is much wider; with the majority falling between 15 and 40 years old. Between 1996 and 2000, IDUs generally had a shorter hospital stay than the general patient. However, in 1999, IDUs still accounted for 981 hospital bed days. The main organisms causing STIs were *S. aureus*, group A streptococci and *S. milleri*. However, the data are incomplete, and it is therefore hypothesized that the incidence of STI was underestimated.
Conclusions: The incidence of STIs, in both the general population and in IDUs, is increasing year by year. The age range of IDUs developing STIs appears to be getting wider. This results in more patients being hospitalized, and thus utilizing more resources. This study further supports the recommendation of the Outbreak Team for better data accumulation and analysis. To investigate the true incidence of STI in IDUs, a comprehensive prospective study is essential. This would increase our understanding of the epidemiology and microbiology of such infections, thus providing a better basis for prevention and treatment.

P1012 Biofilm production in staphylococcal isolates from blood cultures and from the skin

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Objectives: To ascertain if the laboratory detection of the biofilm production is of clinical importance, i.e. if it correlates with the supposed etiological significance of the isolated strain in question.
Methods: The identical strains isolated at least twice from blood culture of patients with the clinical signs of sepsis were compared with the strains isolated from the skin and respiratory tract of relatively healthy persons. The biofilm production was demonstrated by means of two phenotypic methods (Christensen's method of staining the slime on the glass and the appearance of colonies on Congo red agar) and one genotypic method (the detection of ica-operon, which controls the production of slime).
Results: In a pilot experiment, 62 *Staphylococcus epidermidis* strains isolated from blood and its 61 strains isolated from the skin and the respiratory tract mucosa were compared. Of the 62 blood isolates, 30 (48%) were positive for biofilm production by the Christensen's method and 27 (44%) by the type of growth on Congo red agar. Results of 61 skin and respiratory strains were 9 (15%) and 12 (20%), respectively. Phenotypic and genotypic methods for the detection of biofilm production were preliminarily compared in 38 coagulase-negative staphylococcal strains isolated from blood culture. Ica-operon was detected in eight (21%) strains (in *S. epidermidis* only): in five out of nine slime-positive strains (the rest were two strains of *S. haemolyticus* and *S. hominis*) and in three out of 29 slime-negative ones.
Conclusions: *S. epidermidis* strains isolated from blood cultures produce biofilm more often than the isolates from skin and respiratory tract. Ica-operon can be detected in *S. epidermidis* only. It is present also in strains in which the production of the slime is phenotypically undetectable.
Acknowledgments: The study was supported by grant IGA 6818-3 from Ministry of Health of Czech Republic.

P1013 Adhesion of coagulase-negative staphylococci to biomaterials and biofilm formation

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Objectives: For many years, coagulase-negative staphylococci (CNS) was considered to be a nonpathogenic microorganism. The results of investigations carried out during the recent years have indicated contribution of CNS as etiologic factors of infection in particular clinical situations. Among the reasons for the increase in infections caused by CNS are both widespread diagnostic and therapeutic techniques of invasion. These bacteria can be introduced into a body together with the biomaterial being inoculated, ureteral catheter, vascular catheter and the equipment aiding breath. One of the most important mechanisms of virulence of these bacteria is their adhesion ability and biofilm formation. Biofilm protects bacteria against host's immunological mechanisms, decreases antibiotics and antibodies penetration, creates better nutritional conditions for coagulase-negative staphylococci. The aim of the study was to determine adhesion ability to biomaterial and biofilm formation for coagulase-negative staphylococci.
Methods: Researches have been performed on CNS strains isolated from various clinical materials derived from the patients hospitalized in the Intensive Care Unit. Adhesion of coagulase-negative staphylococci was analyzed according to Richards and cooperatives method assessing the value of substrate TTC (2,3,5-triphenyltetrazolium chloride) reduced to insoluble red formazan. In the studies, biomaterials made of various polymers, Foleys ureteral catheter (SL), Nelatos ureteral catheter (PCW) and vascular catheter (PTFE) were used.
Results: A total of 11% of the assessed strains showed reduction value of TTC at +4, 42% at +3, 20% at +2, and 15% at +1. Among these, 12% of the assessed strains did not reduce TTC.
Conclusion:
1 *Staphylococcus epidermidis* strains isolated from blood showed the best adhesive abilities (all the strains showed TTC reduction at +3 and +4).
2 The worst abilities were shown with *S. warnerii* and *S. chromogenes*.

P1014 Surgical site infections after hip prosthesis

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Objective: Based on CDC's National Nosocomial Infections Surveillance (NNIS) system reports, surgical site infections (SSIs) were the third most common frequently reported nosocomial infection accounting for 14–16% of all nosocomial infections (NI). The aim of this study is to know the rate of SSI after hip prosthesis in our hospital and to compare our results with the NNIS reports.
Methods: The hospital of Basurto where the study was carried out is a 800-bed primary and tertiary care teaching hospital that provides care in the urban area of Bilbao (Spain). Infection control team from January 1998 to December 2000 prospectively studied all patients operated on hip prosthesis (CIE-9 procedures 8151 or 8152). They were followed for a year after the end of the episode.
Results: A total of 620 patients were studied, 36 (45%) of them were male, and mean age was 73.4 years (SD = 12.8). Sixty-four patients (10.32%) had 72 NI: 28 UTI, 20 respiratory, 11 surgical site, 4 bacteremia, 2 phlebitis and 7 other locations. Cumulated incidence of infected patients was 10.32/100. Antibiotic prophylaxis was administered in 99.5% of the cases. The dosage, time, drug and duration of the prophylaxis were appropriated (100, 99.3, 99.2 and 96.1%, respectively). SSIs were present in 11 cases: superficial incisional SSIs (6), deep incisional SSIs (2) and organ/space SSIs (3). Cumulated incidence SSIs was 1.77/100. ASA index 1: 151 patients, 0.7% SSIs; ASA 2: 307 patients, 2.3% SSIs; ASA 3: 152 patients, 2.0% SSIs; ASA 4: 10 patients, 0% SSIs. NNIS score 0: 433 patients, 1.8% SSIs; score 1: 185 patients, 1.6% SSIs; score 2: 2 patients, 0% SSIs. Two patients with organ space infection had hip replacement. Mean hospital stay was 21.3 days (SD = 14) in patients with NI and 15.3 days (SD = 11.3) in patients without NI. Crude mortality was 1.9%: 9.4% with NI and 1.1% without NI.

Conclusions: Comparing our data with the NNIS report from December 1999, our hospital's rate is between 75 and 90%. Nosocomial infection is significantly higher in CIE-9 procedure 8152 than in procedure 8151.

P1015 Medical-conservative therapy of infected osteosynthesis

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Objectives: The conventional therapeutic approach of bone infection associated with osteosynthesis proposes that microbial eradication is most readily achieved by removal of the foreign material together with adequate antimicrobial therapy. This strategy usually requires implantation of external fixation device with additional discomfort to the patients (pts). Herewith, we report our experience with medical-conservative antimicrobial therapy without removal of the osteosynthesis until adequate bone callus deposition is documented by bone radiography scan.
Methods: Eighteen patients with infections associated with intramedullary nailing (8 pts), screw-plates (7 pts) or screws (3 pts) have been treated from 1997 to 2001. Osteosynthesis implantation sites were tibia (6 pts), femur (4 pts), humerus (2 pts), and others (6 pts). Diagnosis of infection was based on clinical-microbiological evidence and confirmed by 99m-TC HMPAO-labeled leukocytes scan studies.
Results: Offending pathogens were MRSA (9), MSSA (6), MSSA + *P. aeruginosa* (2), unknown (1). Most cases were initially treated with intravenous or intramuscular teicoplanin ± ciprofloxacin or rifampin, followed by oral antimicrobial therapy usually with ciprofloxacin or mynocicline plus rifampin. Mean duration of antimicrobial therapy was 27.6 weeks (range 10–84 weeks). Cure (improvement with no relapse after 6 months follow up) was established in 15 patients (83%); failure was observed in one patient who relapsed; two patients improved but had positive post-therapy 99m-TC HMPAO-labeled leukocyte scintigraphy examination, so they are still performing additional antimicrobial therapy (one out of these could not remove osteosynthetic material). No patient complained side-effects requiring antibiotic therapy discontinuation.
Conclusions: We concluded that medical-conservative therapy is feasible for osteosynthesis-associated bone infection.

P1016 Staphylococci in infections of hip prostheses and bacteremia

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Objectives: To follow in patients, the participation of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) in bacteremias and catheter sepses, and postsurgery infections of hip prostheses. To compare the phenotype of resistance to anti-infective drugs and occurrence of selected virulence factors of staphylococci isolated from both groups of patients.
Methods: Over the period of January 1999–June 2001, blood, vascular catheters and pus taken from wounds connected with hip prostheses were investigated. Isolated strains were identified biochemically. Susceptibility against selected drugs was tested by the disk diffusion test according to NCCLS, MIC was established by colorimetric method, and susceptibility to vancomycin was confirmed by the E-test. Beta-lactamase, coagulase, fibrinolysin, alpha-, beta-, delta-hemolysins, and DNase were tested by standard microbiological methods. Slime layer production was detected by use of Congo red agar plates.
Results: Three methicillin-resistant strains of *S. aureus* (MRSA) and 13 susceptible strains (MSSA) were isolated from blood and vascular catheters of the patients. All the MSSA were DNase-positive, MIC of vancomycin ranged from 1.5 to 2.0 mg/L. Out of 88 coagulase-negative strains, 65 (74%) were methicillin resistant (MRCoNS). Total 61 (94%) of MRCoNS produced delta-like toxin and 16 (25%) of them DNase. MIC to vancomycin ranged from 1.5 to 4.0 mg/L. Twelve MSSA and one MRSA (*mecA* gene not proved) were isolated from hip prostheses infections. Out of 25 CoNS strains, 16 (64%) were MR, DNase-negative, 12 (75%) of them produced delta-like toxins. MIC to vancomycin of MRCoNS ranged from 2.0 to 4.0 mg/L, and of MSCoNS from 0.75 to 2.0 mg/L. Ninety nine of 113 investigated CoNS were slime producers.
Conclusions: CoNS prevailed (85%) in the group of 104 staphylococcal strains isolated from bacteremias and vascular catheter sepses. Characteristic feature of these bacteria was the high percentage of methicillin resistance and delta-like toxin production. CoNS participated in 66% of staphylococcal hip prostheses infections; they did not produce DNase, and produced delta-like toxins in a lower percentage in comparison with the strains found in bacteremias. Total 88% of all investigated CoNS produced slime layer. From therapeutical point of view, the fact that MRCoNS showed higher MICs to vancomycin as MS strains is important. However, they still represent strains where therapy with vancomycin can be effective.

P1017 Osteoarticular infections complicating enterococcal endocarditis: report of a case and review of the literature

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We report a case of disk space infection complicating enterococcal endocarditis, and review eight additional cases reported in the literature from 1966 through 1998 for which clinical and treatment data were available. Seven of the nine patients were men. All except two were 60 years of age or older. Seven patients had disk space infections; one patient had septic arthritis of the knee, another had infection of the sacroiliac joint. The median interval between the onset of symptoms and the diagnosis of infection was 60 days (7 days–9 months). Fever was present in seven patients. Elevated erythrocyte sedimentation rate or C-reactive protein values were noted in all. Blood cultures grew *Enterococcus* in all nine cases; *Enterococcus fecalis* in five cases, speciation was not performed in four cases. Culture from the intervertebral space was obtained in only one case. In the rest, diagnosis of disk space infection was based on abnormalities on imaging studies. The lumbar spine was affected in all cases. With the exception of one patient, the heart valves involved were all native valves: two cases of mitral valve alone, four cases of aortic valve alone, and two cases of dual involvement of the mitral and aortic valves. Parenteral penicillin in combination with an aminoglycoside was used for treatment. Duration of treatment ranged between 28 and 45 days. Infected valves required replacement in at least three cases. Osteoarticular infection was considered cured in all cases. One patient died from dehiscence of a prosthetic valve.

Non-molecular diagnostic methods II

P1018 Comparison of two bedside urine culture devices containing chromogenic agar

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Objectives: To compare the performances of two recently developed bedside urine culture devices: Colorex Diaslide (Hy Laboratories Ltd, Park Tamar, Israel) and DipStreak (Novamed Ltd, Jerusalem, Israel). Both devices allow the quantitative sampling of urine which is being automatically streaked on conventional media, MacConkey on one side and different formulas of chromogenic agar in the other, thus enabling the presumptive identification of most uropathogens.
Methods: Eight hundred and fifty urine samples from patients with leucocyturia were included in this study. Samples were inoculated in both devices and 1 µL aliquotes were plated on sheep blood agar, MacConkey agar, and Columbia CNA agar as golden standard. Following 24-h incubation, colonies

were counted and presumptive identification by both chromogenic agars was compared to that obtained using Microscan panels (Dade-Behring, Sacramento, USA) and a Microscan WalkAway 96 system.
Results: The sensitivity of the devices in the detection of >100 000 cfu/mL and in the correct presumptive identification was 98.7 and 97.9% for Colorex Diaslide and 96.2 and 87.7% for DipStreak, respectively. When the threshold was lowered to include >10 000 cfu/mL, sensitivity values were 97.5 and 98.2% for Colorex Diaslide and 81.4 and 87.1% for DipStreak. Negative predictive value was calculated to be 99.0% for Colorex Diaslide and 92.8% for DipStreak. The sensitivity in detecting mixed cultures (three or more organisms present) was 96.7% for Colorex Diaslide and 75.0% for DipStreak. The overall full-agreement rates of quantitative results (categorized in four levels: negative, up to 10 000 cfu/mL, up to 100 000 cfu/mL and >100 000 cfu/mL) compared to the gold standard were 98.0 and 84.5% for Colorex Diaslide and DipStreak, respectively. Colorex Diaslide succeed in presumptively identifying 98.7% of all microorganisms while DipStreak identified 89.4%.
Conclusions: Both devices demonstrated excellent performance in quantifying and identifying uropathogens from urine specimens containing >100 000 cfu/mL compared to conventional methods. However, Colorex Diaslide proved to be superior in terms of sensitivity and accuracy with lower bacterial counts and mixed cultures.

P1019 Evaluation of Granada HT medium for detection of group B Streptococci (GBS) in pregnant women

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Objective: To evaluate a new commercial Granada agar (Medio Granada HT, Biomedics. Spain) for the detection of GBS in vaginal and rectal specimens from pregnant women.
Methods: A total of 600 samples including: 221 vaginal, 221 rectal and 11 vagino-rectal from women at 35–37 weeks' gestation, 55 endocervical and 55 vaginal from women with onset of labor or membrane rupture at <37 weeks and 37 vaginal at any time of pregnancy, were studied. All samples were placed in transpot medium (Modified Amies) and inoculated in selective Columbia agar (with 5% human blood, 15 µg/mL nalidixic acid and 15 µg/mL colistin), Granada agar plate prepared in our loboratory (proteose peptone 25 g, starch 20 g, MOPS 11 g, Na2HPO4 8.5 g, glucose 2.5 g, pyruvate 1 g, $MgSO_4$ 0.2 g, methotrexate 6 mg, crystal violet 0.2 mg, colistine 5 mg, metronidazole 10 mg, horse serum 50 mL, agar 10 g and water 1 L), Granada HT plate and Todd-Hewitt broth (with 5% horse serum and 15 µg/mL amikacin). After 18–24 h, Todd-Hewitt was subcultured in Columbia, Granada and Granada HT. Columbia plates were incubated in 5% CO_2 and Granada in anaerobiosis and examined at 24 and 48 h.
Results: Among the 324 pregnant women studied, 60 were colonized by GBS. Of the 600 samples processed, 93 were GBS-positive: 42 vaginal, 41 rectal, seven endocervical and three vagino-rectal. GBS were isolated in the following media: 64 in Columbia, 80 in Granada, 79 in Granada HT directly inoculated and 83 in Columbia, 89 in Granada and 89 in Granada HT after Todd-Hewitt enrichment, with sensitivities of 69, 86, 85, 89, 96 and 96%, respectively. For GBS detection in rectal samples, there were no significant differences between the two Granada media and before and after enrichment ($P=0.13$, $P=0.48$, $P=0.32$, $P=0.32$). In contrast, significant differences were found for vaginal samples ($P=0.01$, $P=0.01$, $P=1$, $P=1$). When vaginal and rectal samples were studied separately, 65% of women horbored GBS in both vagina and rectum, 2% only in vagina and 33% only in rectum.
Conclusions: The 18.5% of pregnant women were colonized by GBS. Granada agar is more sensitive than Columbia for GBS detection and has the advantage of providing results in 18–24 h because its characteristic red-orange pigment makes futher identification unnecessary. New commercial Granada HT was as sensitive as our Granada medium and has the advantage of easier prepatation.

P1020 Evaluation of the new 'Chromogenic *Salmonella* Agar' for the selective isolation of *Salmonella* spp. from stools

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Objectives: The purpose of this research was to evaluate suitability of the new 'Chromogenic *Salmonella* Agar', CSA (Biolife, Italy) in comparison with '*Salmonella–Shigella* Agar' SSA (Biolife, Italy) for the selective isolation of *Salmonella* spp. from stools.
Methods: One Gram of stool was put in 9 mL of Selenite-F enrichment medium (Biolife-Italy) at 37 °C for 24 h followed by streaking onto SSA as a referent medium and CSA as a test medium. After incubation at 37 °C/24 h, suspect colonies from both media were transfered onto Kligler Iron Agar, KIA (Biolife-Italy) for preliminary testing. Strains showing typical reactions on KIA were confirmed by biochemical tests using API 10S gallery (bioMerieux, France) followed by final identification with sera manifactured by Immunology Institut, Zagreb. Specificity and sensitivity was calculated and compared by McNamara test.
Results: During September to November 2000, total of 300 stools were examined. 45 out of 76 suspected colonies were confirmed to be *Salmonella* spp. on SSA (sensitivity 100%, specificity 88%). From the same number of stool samples, 45 out of 46 suspected colonies showing magenta colour on CSA, were confirmed to belong to genus *Salmonella* with one false positive reaction due to *Pseudomonas* spp. (sensitivity 100%, specificity 99%). In comparison these two media CSA showed better results in preliminary test ($\chi^2=28$, d.f. $=1$, $P<0.01$). Confirmation test gave the same results on both media. Serotyping confirmed all isolates as: somatic antigen O 1, 9, 12; flagellar antigen H 1 fase: gm – *Salmonella enterica* serotype *Enteritidis*.
Conclusion: Due to its good sensitivity and specificity, CSA can be recommended for the use for screening of human stools when *Salmonella* spp. are concerned and may help to reduce the work load when this medium is used for plating after enrichment.

P1021 Are out-of-hours positive blood cultures clinically relevant?

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Objective: Prompt and appropriate treatment of bacteraemia reduces mortality significantly. Recent automated continuous blood culture monitoring systems facilitate early intervention and the institution of appropriate therapy more quickly. We have evaluated the effect of out-of-hours blood culture monitoring on reporting and the start of more appropriate antibiotic treatment.
Methods: The laboratory rang the medical microbiologist at a specified time each night with the microscopy result of new blood cultures. The microbiologist then liased with medical and other staff regarding the relevance of these and recommended changes in antibiotics as appropriate. The following day each positive blood culture was followed up and an assessment was made as to their significance or otherwise. A significant positive blood culture was defined as the same organism grown from two or more blood cultures with appropriate clinical signs and symptoms or the isolation of a known pathogen from one positive blood culture with appropriate signs and symptoms where there was no other obvious cause of infection.
Results: A total of 150 positive blood cultures were phoned during the study period. 61 patients were already on appropriate therapy, the positive blood culture was considered a contaminant in 47 cases and treatment was reviewed in a further 42 cases. In 13 of these 42 cases, the patient was already on appropriate therapy but in 29 cases, inappropriate antibiotics were either stopped, rationalised and/or appropriate therapy started. In 8 of 18 MRSA patients, this was the first isolate of MRSA for that patient facilitating earlier treatment and patient isolation. One patient with vancomycin resistant enterococci was identified for the first time out of hours. Clinical liaison by the medical microbiologist took approximately 25 h over the study period.
Conclusion: The main benefits from out-of-hours blood cultures is that the laboratory results are available a day earlier and appropriate therapy can be started sooner in approximately 20% of cases.

P1022 Utility of the Gram stain for the diagnosis of organic fluid infections

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Objectives: To evaluate the performance of the Gram stain in the diagnosis of the organic fluid infections: Cerebrospinal fluid (CSF), Ascitic fluid (ASCF), Pleural fluid (PLEF) and Synovial fluid (SYNF).
Methods: During 6 months we analyzed 608 organic fluids. We processed the Gram stain following an established protocol: (1) leukocytes count of at least 10/mm^3 in CSF, 250/mm^3 in ASCF, 150/mm^3 in PLEF and 2000/mm^3 in SYNF with predominant polymorphonuclear; (2) when the physician asked for it. In case of CSF, we also processed when: (3) predominant lymphocytes with glucose below 40 mg/dL and protein above 50 mg/dL; (4) suspicion of sepsis or meningitis; and (5) immunodeficiency. The culture was carried out following standard techniques and was considered as the gold standard. We compared the Gram stain result to the culture result. The data were analyzed with the statistical program SPSS 10.0. We calculated the sensitivity (S), the specificity (SP), the positive predictive value (PPV) and the negative predictive value (NPV).
Results: We analyzed 608 organic fluids: 218 (35%) CSF, 198 (33%) ASCF, 140 (23%) PLEF and 52 (9%) SYNF. Only 230 (38%) reached the established protocol for realizing the Gram stain. All in all the results were: S = 75%, SP = 98.9%, PPV = 94.3%, and NPV = 94.3%. According to the fluid studied the results were: CSF (84, 100, 100, 97), ASCF (53, 100, 100, 85), PLEF (78, 97, 91, 93), and SYNF (100, 97, 83, 100), respectively.
Cconclusions: The Gram stain is a simple, rapid, accurate and inexpensive method to find out the etiological agent of organic fluid infections in approximately 75% of the cases. We have found good S, SP, PPV and NPV and we would point out the good sensitivity in the CSF and the poor sensitivity in the ASCF.

P1023 Elastase and alkaline protease activity of *Pseudomonas aeruginosa* strains: comparison of two methods

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Objective: *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause fatal infections in immunocompromised hosts. The virulence of *P. aeruginosa* is associated with the presence of various extracellular factors, like elastase and alkaline protease. These enzymes are suggested to contribute tissue destruction and assist bacterial invasion during infection. Therefore it seems likely that, determination of these virulence factors will be an important prognostic marker in the near future especially for follow up of cystic fibrosis patients, in order to start antimicrobial agents that are directly or indirectly inhibits microbial growth or virulence factor production. Herein, we suggested a simple test procedure to be used in routine laboratories for estimation of elastase and alkaline protease levels and compared them with quantitative methods in the literature.
Methods: Elastolytic activity of the strains was determined by (i). measurement: of zone diameter in elastin nutrient agar plates containing a nutrient agar base with 1% elastin (Sigma)-containing agar overlay. Supernatants (20 µL) were added to the wells punched in plates, after overnight incubation at 37 °C diameter in mm of the zone of clearing were measured; (ii). measurement: of the supernatant absorbance at 495 nm after mixing with elastin-congo red substrate. Alkaline protease activity of the strains were determined by (i).measurement: of zone diameter in skim milk agar plates as described above; (ii). measurement: of the supernatant absorbance at 595 nm after mixing with hide azure blue substrate. After each assay the experimental data which were the mean of two or three independent assays compared with corresponding standard curves established with standard elastase and protease (Sigma). Results were extrapolated according to the standart values.
Results and conclusion: The resulting values were in the range reported in the literature and differed from one strain to another (elastase: 0–1390 mg/mL, alkaline protease: 0–770 mg/mL). Linear relationships were found when assays compared in pairs and significant correlation coefficients were obtained ($r > 0.788$ for alkaline protease, $P < 0.0001$, $r > 0.926$ for elastase, $P < 0.0001$). The plate assay method can be applied to roughly quantify the level of these enzymes regardless the availability of technical equipment.

P1024 Prodigiosin producing *Serratia marcescens* in a tertiary care hospital

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Objective: The aim of this study was to analyze sensitivity, incidence and biological properties of prodigiosin producing *S. marcescens* isolated in our hospital over 5 years.
Materials and methods: The collection 527 *S. marcescens* isolates from 1996 to 2000 was analyzed. Production of pigment was examined on – Peptone Glycerol Agar (PGA) containing 1% glycerol. Sensivity to selected 11 agents was determined by the agar dilution method according to NCCLS guidelines: piperacillin, piperacillin/tazobactam, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, netilmicin and ciprofloxacin. Hemolitic activity was determined on Columbia Sheep Blood Agar and protease production on Skim Milk Agar. ESBL production was examined in routine double-disk synergy test (DDS).
Results: Prodigiosin producing *S. marcescens* was detected in 20 (4%) of isolates. They were recovered from respiratory tract (10), skin and soft tissues (4), drainages (2), blood (2), urine (1), and bile (1). Prodigiosin positive *S. marcescens* were most frequently isolated in surgical wards (7) medical (4) and neurological wards (4). The majority of strains were isolated in 1998 (9). Pigmented strains showed wide range of MICs to tested agents. All strains were sensitive to ceftazidime, cefepime, carbapenems, 85% to ciprofloxacin, and 80% to aminoglycosides. Only 13 (65%) isolates were sensitive to piperacillin. Among prodigiosin positive isolates 14 produced hemolysin and 18 proteases.
Conclusions: Pigmented *S. marcescens* are thought to be mostly isolated from environmental sources and only transiently presented in hospital setting. However such strains are rare, and probably not epidemiologically linked, they are no longer uniformly susceptible to antibiotics. Our study proves that they can persist in hospital environment gaining some resistant determinants and express virulence factors.

P1025 Performance of the Sensititre Gram-negative Identification panel (GNID) compared to API 20E/20NE, IDS RapID ONE/NF and the Vitek AMS GNI+

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Objectives: A clinical trial was conducted at Limerick Regional Hospital, Limerick, Eire, to predict the performance of the new Sensititre GNID (Gram-negative identification panel, Trek Diagnostic Systems Ltd, UK) in correctly identifying *Enterobacteriaceae* and non-*Enterobacteriaceae*. The GNID will replace the current AP80 AutoIdentification plate, with the release of the new Sensititre software.
Methods: Performance of GNID was compared to three commonly used bacterial identification systems: API 20E and 20NE (bioMerieux, France), IDS RapID ONE and RapID NF (Remel Inc. USA) and the Vitek AutoMicrobic System GNI+ (bioMerieux Vitek Systems Inc. Hazelwood, Mo.), all of which are used to identify common Gram-negative bacteria. Almost 100 clinical isolates were tested.
Results: GNID correctly identified 95% of *Enterobacteriaceae* isolates ($n = 56$) and 95% of non-*Enterobacteriaceae* isolates ($n = 37$) to species. API systems correctly identified 77% of *Enterobacteriaceae* isolates ($n = 52$) and 69% of non-*Enterobacteriaceae* isolates ($n = 26$). IDS RapID panels correctly identified 94% of *Enterobacteriaceae* isolates ($n = 16$) and 81% of non-*Enterobacteriaceae* isolates ($n = 16$). The Vitek GNI+ card correctly identified 100% of

Enterobacteriaceae isolates ($n = 7$) to species and 91% of non-*Enterobacteriaceae* isolates ($n = 11$).
Conclusions: The performance of GNID compared favourably and in most cases exceeded the performance of the other three systems tested in correctly identifying common, clinically significant gram-negative bacteria. The GNID performed equally well in identifying members of the *Enterobacteriaceae* and non-*Enterobacteriaceae*.

P1026 Identification and biotyping of stains of *Acinetobacter calcoaceticus–A. baumannii* complex

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Objectives: The aim of this work was to biotype the strains of *A. calcoaceticus–A. baumanii* complex that were identified at the HCU of Valladolid during the period october 1998-january 2000 and to study the biotype distribution.
Methods: The strains were presuntively identified as *Acinetobacter spp* by means of comercial systems and its belonging to *A. calcoaceticuts–A. baumanii* complex was studied by additional tests: growth at 41 °C and 44 °C and hemolysis. The strains that did not grow at 44 °C were identified to species level by means of the simplified scheme by Bouvet and Grimont. All the strains that were identified as *A. calcoaceticuts–A. baumanii* complex were biotyped according to the procedure by Bouvet and Grimont.
Results: Of all 504 isolated strains of *Acinetobacter*, 493 corresponding to 302 patients were identified as belonging to *A. calcoaceticus–A. baumanii* complex. Nine biotypes were identified 1, 2, 3, 5, 6, 8, 9, 14 and 18. The most frequent was biotype 1 (84.4%), followed by 2 (7.9%), 18 (4.1%), and 9 (3.7%). In all types of samples biotype 1 predominated, with percentages ranging from 73.8% in urine samples to 100% in CSF samples. 48.3% of strains of biotype 1 were isolated from the exudates and 27.8% from respiratory samples. Biotype 2 was observed in all types of samples except in CSF and 59.0% of strains of such biotype were isolated from the exudates. Biotype 9 was only encountered in exudates (88.9%) and respiratory samples (11.1%), whereas biotype 18 was predominantly isolated from exudates (50%) and urine samples (40%). Of all 459 strains of nosocomial origin, the predominant was biotype 1 in all months. Biotypes 2 and 18 were isolated almost every month, whereas biotype 9 was predominantly isolated during the 1st month of the studied period. Biotype 1 predominated in all areas of the hospital, with percentages between 44.4% and 84.3%. Biotypes 1 and 2 were mainly isolated at the surgery area and from intensive care patients, whereas biotype 18 was generally encountered in samples from surgery patients and medical patients. Finally, 46.7% of patients yielding isolation of biotype 9 came from the ambulatory area.
Conclusions: Nine biotypes of *A. calcoaceticuts–A. baumanii* complex have been identified. Biotype 1 predominated. Biotypes 1, 2 and 18 were mostly of hospital origin, whereas almost half of the isolation cases of biotype 9 came from ambulatory areas. During the studied period biotypes 1, 2 and 18 coexisted.

P1027 Biotyping of *Arcanobacterium haemolyticum* strains by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS)

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Objectives: *Arcanobacterium haemolyticum* is an aerobic Gram-positive rod that causes tonsillitis and wound infections. Rough and a smooth *A. haemolyticum* biotypes exist. The rough type predominates in the respiratory tract and the smooth type in wound infections. The chemical differences in the cell wall structure of the two types are not known. MALDI-TOF-MS was used to distinguish between the biotypes.
Methods: Five smooth and five rough type *A. haemolyticum* strains were analyzed in a blind fashion. The strains were cultured on Columbia blood agar plates for 48 h in 5% CO_2 at 35 °C and colonies were transferred to a MALDI target plate with a loop. Twelve replicates were sampled for each of the 10 strains in order to construct a database from the fingerprints obtained. Samples were then overlaid with alpha-cyano-4-hydroxycinnamic acid and the dried plates were analyzed. Mass spectral data was acquired over the m/z range 500–1000 Da. The results were processed using the MicrobeLynx software.
Results: Mass spectral fingerprints were obtained for all 10 strains. In order to determine the grouping of strains a database was made. Individual cultures were then compared against each entry in the database to obtain a root mean square value to measure the relationship of each strain to the other nine. A second comparison was made using a database search to determine the five closest matches for each strain. The strains could be correctly divided into the two biotypes using MALDI-TOF-MS only ($P < 0.001$).
Conclusion: The two *A. haemolyticum* biotypes have differencies in cell wall structure that can be demonstrated using MALDI-TOF-MS. The precise chemical differences of the the cell wall composition of the two biotypes remain to be elucidated.

P1028 Point of care inoculating: preliminary evalutation of Granada Medium Instant for early detection group B *streptococci*

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Objectives: Group B *Streptococcus* (GBS) is etiological agent of serious infections that affect newborn babies, children, adults. Recent data show the possibility to reduce the incidence of infections, expecially neonatal, through a carefull controlo of the woman during the pregnancy. We evaluated the Granada Medium Instant for early detection group B *Streptococcus* by rectal swabs of women 34th-38th week pregnant. Our laboratory makes also diagnostic test coming from peripherical hospitals and ambulatories; so that we have the necessity to collect specimens directly at the patient's bed, assuring the quality of the transport of the specimen and offering to the peripherical districts the opportunity to prepare the culture also during the closing hours of the laboratory. The GMI is contained in tubes of glass and the colonies of GBS grow with a red-orange colour characteristic of human isolates. In the Granada there are folates that increase the colonies colour intensity.
Methods: We examinated 200 subjects collecting the material with two rectal swabs. One swab was dipped directly in the tube containig GMI, while the other was used to next preparation in laboratory of the culture standard in Blood agar after enrichment and Selecttive Strepto agar. The Granada and Blood Agar were icubated under aerobic conditions at 35 °C. The direct reading of Granada, based on the red-orange colouration of the medium, was made after 18 h and in case of negativity extended till 48 h. For verifying the specificity of the colouration, the identification of GBS colonies was made with biochemical and serological tests.
Results: The preliminary results obtained in our study are showed in the Table.

	Blood agar	
Granada medium	Positive	Negative
Positive	40	1
Negative		159

Conclusion: On the ground of our experience the Granada Medium Instant answer to three actual important requisites:
1 It makes possible the execution at the patient's bed of inoculation on the culture medium (point of care inoculating).
2 It is a means able to screening during the pregnancy both the subjects carriers of GBS and the newborn babies (specificity 100%).
3 It allows an economical saving because it does not require more biochemical or serologic tests for the GBS identification.

P1029 Procalcitonin as a diagnostic marker in septic patients

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Objectives: To compare procalcitonin (PCT) plasma levels of septic patients with positive and negative blood culture.
Methods: A total of 49 patients were included: 25 patients with positive blood culture, 18 patients with negative blood culture and six patients without

sepsis. PCT were evaluated on the 1st day after onset of sepsis symptoms. If the result was positive, the next PCT was done after 24 h. If it was still positive, the next one was done also 24 h until gave negative outcome. If first PCT was negative, the control was done after 72 h. Procalcitonin level was measured by two methods (PCT-Q and LUMItest, BRAHMS Diagnostica, Berlin).
Results: Observed mortality was 10/49 (20.4%), PCT values were higher in patients with positive blood culture (11.24 ± SD 16.56 ng/mL) vs.
Patients: with negative blood culture (6.76 ± SD 10.75 ng/mL). Patients without sepsis had very low PCT levels (1.86 ± SD 10.25 ng/mL). The higher PCT concentration was found in patients with sepsis caused by Gram-positive comparing with Gram-negative.
Conclusions: PCT presents itself as a diagnostic parameter of sepsis. Patients with positive blood culture had significantly higher PCT level in comparison to patients with negative blood culture. Elevated PCT level revealed a complicated course and indicated worse prognosis. PCT correlated with the clinical outcome in sepsis.

P1030 Procalcitonin in patients with *Legionella pneumonia*

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Objective: Procalcitonin (PCT) is a specific marker of severe bacterial and fungal infections. The aim of the study was to determine PCT in patients with *Legionella pneumonia*.
Methods: First serum sample of 140 patients (121 male, 19 females; 51.73 mean years old and a 7-day-old-neonate) with laboratory confirmed *Legionella* infection (defined by seroconversion with indirect immunofluorescence assay and microagglutination test and/or by culture positivity) were examined. PCT was measured by immunoluminometric assay (Lumitest PCT Brahms Diagnostica).
Results: PCT values were positive (> 0.5 ng/mL) in 57.1% of the sera, collected from 2 to 45 days after symptoms onset. Positive sample rate was higher in early samples and decreased progressively in late sera. 69.8% of the samples collected in the first two weeks were positive against 29.5% in the following period ($P < 0.001$). Mean PCT values (11.76 ± 27.36 ng/mL; range 0.51–150.7) of patients sera in the first two weeks of disease were higher than those (3.47 ± 3.78 ng/mL; range 0.89–14.32) of samples collected in the following period. PCT was positive in 86.7% of culture positive Legionellosis cases; in these patients, mean positive PCT values (39.4 ± 54.67 ng/mL; range 1.56–150.7) of the sera, collected in the first two weeks, were significantly higher ($P < 0.001$) than those (5.68 ± 9.95 ng/mL; range 0.5–63.11) of culture negative subjects.
Conclusions: Procalcitonin can be a useful marker of bacterial infection, when serum samples are collected at the onset of symptoms. However, *Legionella pneumonia* is sometimes unrecognized and diagnosis may be delayed. In this case, PCT can represent a useful prognostic marker in patients with severe bacterial infection only in the first two weeks of disease.

P1031 Rapid presumptive diagnosis of bacterial meningitis with a new Procalcitonin method

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We assess a new rapid test for Procalcitonin determination (PCT-Q, BRAHMS) in order to differentiate between bacterial (BM) and aseptic meningitis (AM). The PCT-Q is a new method that determines procalcitonin, in 0.2 mL of serum or plasma samples, in a disposable device without need of other instrument. The results are read after 30 min and positive samples show colour intensity directly proportional to the PCT concentration. Using a reference card with a colour scale, it is possible to differentiate three PCT concentrations: mildly elevated PCT (0.5–2 ng/mL), elevated PCT (2–10 ng/mL) and highly elevated PCT (more than 10 ng/mL). In order to see the correlation of this semiquantitative test (PCT-Q) with the quantitative method (LUMItest PCT, BHRAMS), 75 serum samples with a range of PCT concentration of 0–100 ng/mL were measured by both methods. There was a close concordance rate (89.3%), with only two being positive with the quantitative method samples (with a low level PCT, 0.5–2 ng/mL) and negative with the semiquantitative test PCT-Q (two false negative). To differentiate BM from AM with PCT-Q, 26 sera of consecutive patients with acute meningitis (nine bacterial meningitis and 17 aseptic meningitis) were studied. All BM cases were confirmed by CSF or/and blood culture (seven *N. meningitidis*, one *S. pneumoniae* and one *H. influenzae*); All AM cases were culture negative. The mean of test routinely used to differentiate BM from AM as CSF glucose, CSF protein, CSF leukocytes count were different ($P < 0.01$) although there was overlapping in some samples between the two groups. With the PCT-Q all BM serum sample were positive (PCT > 0.5 ng/mL) and only one AM sample was positive in the 'mildly level'. Using as cut off the three concentrations obtainable by PCT-Q-test (0.5, 2 and 10 ng/mL) we achieved a diagnostic sensitivity of 100, 78 and 67% and a diagnostic specificity of 94, 100 and 100%, respectively. If these results are confirmed with more samples, a negative result with PCT-Q in meningitis patients would indicate a high probability of aseptic meningitis; however, if PCT-Q is more than 2 ng/mL there would be a high probability of bacterial meningitis. These results shown that PCT-Q-test may be useful in an emergency lab to differentiate aseptic from bacterial meningitis, especially when other test routinely used are inconclusive.

P1032 Procalcitonin as a marker of bacterial infection after cardiac surgery

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Objective: Cardiac surgery with cardiopulmonary bypass (CPB) generally leads to a systemic inflammatory response syndrome (SIRS) because of exposure of blood to nonphysiological surfaces and endotoxines release secondary to aortic clamping. Differential diagnosis between hemodynamic, respiratory or infectious complications is then difficult by usual markers. The objective of the study was to evaluate the reliability of procalcitonin (PCT) serum measurement as a marker of bacterial infection after cardiac surgery, distinguishing from unspecific SIRS.
Methods: Serum samples from 150 patients undergoing cardiac surgery were collected at the time of admission to Intensive Care Unit and 24 and 48 h later. Sixty-eight patients underwent valvular surgery, 69 coronary artery bypass with CPB and, 13, coronary artery bypass without CPB. A total of five patients suffered cardiogenic shock (and three of them died), and six patients showed microbiologically documented bacterial infection. PCT levels were also measured to a group of 10 patients undergoing surgery of the intestine with bacteremic peritonitis.
Results: PCT levels kept below 1 ng/mL on first determination in all patients without any complication, even those who needed inotropic support within the first hours. Thirty-two of the uncomplicated patients showed an increased PCT level at second postoperative day: mean 1.58 ng/mL (SD 0.6), decreasing below 1 ng/mL at third. No significant differences were found between the different kinds of surgery. No increased PCT levels were found related to duration of CPB, nor to time of aortic clamping nor to time of mechanical ventilation. Those patients who presented cardiogenic shock, showed slightly increased PCT levels (always below 5 ng/mL) mean 1.76 ng/mL (SD 1.95). Those patients who presented bacterial infection showed highly increased PCT levels: mean 16.02 ng/mL (SD 8.71). Patients undergoing surgery of the intestine showed a mean PCT value of 53.26 ng/mL (SD 124.1).
Conclusions: Unspecific SIRS after cardiac surgery does not increase significantly PCT levels. Increased PCT concentrations of more than 5 ng/mL after cardiac surgery highly suggest postoperative complication. PCT serum measurement can be useful as a predictive marker of bacterial infection after cardiac surgery.

P1033 Rapid identification of *Streptococcus pyogenes* by the use of a new commercial immunocard assay

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Purpose: To evaluate the performance of a new kit, the ImmunoCard Stat Strep A test (Meridian Diagnostics, Inc), for the rapid and qualitative detection of *Streptococcus pyogenes* directly from clinical specimens.
Methods and materials: A total of one hundred-20 throat swabs obtained during 3 months period from individual outpatients at the Department of ENT, have been included. All the specimens have been examined by the

ImmunoCard Strep A test in parallel by the conventional methods (culture, isolation and identification of β-haemolytic colonies).
Results: Forty-two of 44 specimens positive by culture for *Streptococcus pyogenes* were positive by the ImmunoCard Strep A test (sensitivity 95%). Only two specimens positive by the cultures were negative by the ImmunoCard. These specimens yielded few colonies after an exhausting search. No false-positive results were obtained from specimens negative by culture (specificity 100%).
Conclusions: The Immunocard Stat Strep A test is simple to perform, gives results within 10 min, and is characterized by high sensitivity and specificity. This test can detect *Streptococcus pyogenes* directly from throat swabs and allow physicians to diagnose and administer therapy immediately.

Antibiotic testing

P1034 Evaluation of phenotypic antimicrobial susceptibility testing of VanA and VanB type vancomycin resistance in enterococci

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Objectives: Many laboratories find it difficult to detect glycopeptide resistance in enterococci. The accuracy of disc susceptibility testing (DST) is highly dependent on the method used and improvement is warranted. We evaluated the disk diffusion method recommended by the Swedish Reference Group for Antibiotics (SRGA), a method using blood supplementation as well as 14 other disc/agar variants of the SRGA method.
Methods: A total of 72 epidemiologically and genetically different *E. faecalis* and *E. faecium* with known vancomycin resistance genotype (susceptible, *vanA* and *vanB* positive) established using the EVIGENE (TM) VRE Detection Kit, was subjected to DST for vancomycin and teicoplanin by three laboratories. 16 variants of the SRGA disk diffusion method were tested: with and without 5% horseblood supplementation, high (10^8 cfu/mL) and standard inocula (10^6 cfu/mL), incubation with air and 5% CO_2, respectively, and readings after 20 and 44 h, respectively. Furthermore, the methods were evaluated with respect to results obtained for: gentamicin, netilmicin, linezolid, ampicillin, trimethoprim and nitrofurantoin.
Results: All laboratories detected *vanA* positive strains and strains susceptible to vancomycin with high accuracy (99–100%). However, the number of misin-terpretations of *vanB* positive strains varied for each laboratory from 2.7 to 15.2%, with variations for each method used (0–36%). High inoculum and 44 h of incubation performed best, with a sensitivity of 98% and a specificity of 99%. The best method using 20 h of incubation and standard in-oculum was the variant using 5% horseblood supplementation and incubation with 5% CO_2. This method had a sensitivity of 91% and a specificity of 100%. Conflicting results between the SRGA and the alternative method was not observed when making DST for gentamicin, linezolid, ampicillin, trimethoprim and nitrofurantoin, whereas slight problems where registered for netilmicin.
Discussion: In the Scandinavian countries, the prevalence of VRE is well below 1%. In low prevalence areas, a gain obtained with an increase in sensitivity, such as seen here with a high inoculum and incubation for 44 h is lost with even a slight decrease in specificity. The loss of specificity results in a large proportion of false positives and thus a frequent need for confirmatory testing (genotyping).

P1035 Evaluation of different methods to detect extended spectrum β-lactamases (ESBL) producing *Serratia marcescens*

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Objectives: To compare different methods detecting ESBL production in *S. marcescens*. Production of AmpC β-lactamase can mask the concurrent production of ESBL making such strains difficult to detect and may leading to inappropriate patients' therapy.
Methods: Over 5 years we isolated 68 nonreplicate ESBL positive *S. marcescens* using double-disc synergy (DDS) test with cetazidime (CAZ), amoxicillin-clavulanate (AMC), cefotaxime (CTX) and cefepime (FEP). ESBL E-tests with CAZ alone and with clavulanic acid (TZ/TZL), CTX alone and with clavulanic acid (CT/CTL) were used and the results compared to the DDS method. MICs to piperacillin (PIP), piperacillin/tazobactam (TZP), ceftriaxon (CRO), CAZ and FEP were determined by agar dilution method according to NCCLS guidelines.
Results: ESBL E-tests were able to detect only 27 isolates using the manufacturer suggested MIC ratio TZ/TZL = 8. If the boundary ESBL × production MIC ratio was lowered to 5, 17 other isolates would be regarded ESBL positive. The median inhibition zone diameters for CAZ, CTX and FEP were 19, 10 and 16 mm, respectively. The $MIC_{50/90}$ for PIP were 128/>256 mg/L, TZP 1/32 mg/L, CRO 128/>128 mg/L, CAZ 4/32, FEP 16/128 mg/L.
Conclusion: DDS proved to be the most convenient method to detect ESBL-producing *S. marcescens*. Poor performance of the ESBL E-tests could be related to the presence of AmpC enzyme and therefore the E-tests MICs values above the test ranges. Our isolates demonstrated substrate preference for CTX/CRO. Increased FEP and CAZ MICs with concurrent lower MIC values for TZP could also suggest ESBL production in *S. marcescens*.

P1036 Flow cytometry assay is useful to identify heterogeneous and homogeneous vancomycin intermediate *Staphylococcus aureus* strains

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Objective: To explore the possibility of glycopeptide intermediate *S. aureus* isolates (GISA) detection by flow cytometry assay.
Methods: Five clinical heterogeneous GISA strains, including the Mu3, and two clinical homogenous GISA strains, including the Mu50, were study by Flow Cytometry with the EPICS XL (Coulter) and LSR (Becton-Dickinson) systems. Membrane potential was analyzed with rhodamine 123, DIOC5(3), and Bisoxonol fluorochromes in the absence or presence (1 h exposition, LB broth) of vancomycin (1 μg/mL). Results were compared with those obtained with two vancomycin susceptible strains (ATCC 43300 and 49476). GISA status was confirmed by the MH-vancomycin (6 μg/mL) supplemented agar screening test and population analysis.
Results: Relevant results (mean values of 10 assays) of membrane potential modification expressed as reduction (*) or increased (**) number of times of fluorescence mean intensity compared with the corresponding controls were as follows:

Vancomycin status	$DIOC_5$ (3)*	Bisoxonol**	Rhodamine-123**
Susceptible	4.60 ± 1.55	1.60 ± 0.12	1.47 ± 1.17
Heterogeneous GISA	1.72 ± 0.67	1.07 ± 0.15	0.66 ± 0.20
Homogeneous GISA	1.10 ± 0.60	1.06 ± 0.26	0.77 ± 0.60

Differences among hetero- and homo-geneous GISA and vancomycin susceptible strains were better detected with DIOC5(3) and to a lesser extend with Bisoxonol and Rhodamine 123.
Conclusion: Flow Cytometry assay was useful to rapidly identify hetero- and homo-geneous GISA populations.

P1037 Phoenix ESBL test as a reliable method for direct detection of extended-spectrum β-lactamase (ESBL)-producing members of the family Enterobacteriaceae

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Objectives: Production of plasmid-mediated extended-spectrum β-lactamases (ESBLs) has emerged as an important mechanism contributing to β-lactam resistance in members of the family Enterobacteriaceae. Microbiology laboratory plays a crucial role in revealing these ESBL-positive bacteria, thus rapid and direct methods for their detection are desirable now. In this study, we evaluated the reliability of a commercial method, the Phoenix ESBL test (Becton Dickinson, Sparks, MD, USA), for determining ESBL resistance in a wide number of clinical isolates of Enterobacteriaceae. Results were compared with those of the double-disk (DD) and E-test confirmatory assays.
Methods: A total of 510 clinical isolates of Enterobacteriaceae were included in the study. Isolates consisting of 88 *E. coli*, 158 *K. pneumoniae*, 18 *K. oxytoca*, 106 *P. mirabilis*, 26 *P. stuartii*, 12 *M. morganii*, 44 *E. aerogenes*, 23 *E. cloacae*, six *S. marcescens*, 21 *C. freundii*, eight *C. koseri* were recovered from patients hospitalized at the Catholic University Medical Center. Of these isolates, 319 were characterized as ESBL producers and the remaining 191 as other β-lactamases producers by means of double disk synergy test and determining MICs to cefpodoxime, ceftazidime, ceftriaxone, aztreonam, ceftazidime/clavulanic acid and cefotaxime/clavulanic acid using E-test. All of the 510 isolates were analyzed by Phoenix ESBL test that uses five cephalosporins alone or in combination with clavulanic acid, and a continuous monitoring of the growth. The results of Phoenix ESBL test were integrated into the antimicrobial susceptibility report through the BDXpert system. In addition, the 319 ESBL-producing strains were characterized by colony blot hybridization method using TEM and SHV genes as probes.
Results: Phoenix ESBL test was able to detect all of the 319 strains identified as ESBL producers by DD and E-test, whereas of the 191 ESBL-not producing strains, 189 strains were confirmed as negative, and two strains gave a positive result by Phoenix ESBL. The strains with false-positive results were two isolates of *K. oxytoca* overproducing K1-β-lactamase. Excellent agreement between the methods was thus observed, with an accuracy of 99.6%. The sensitivity and specificity of Phoenix ESBL test were of 100 and 98.9%, respectively.
Conclusions: Our data demonstrate that Phoenix ESBL can be considered a rapid and reliable method for routine laboratory testing of ESBL resistance.

P1038 Evaluation of the E-test for susceptibility testing of *Mycobacterium tuberculosis* to first-line antituberculous drugs

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Introduction: The E-test is a diffusion method consisting of a strip with a gradient of drug concentrations that permits easy and rapid reading of MICs. Although this method has shown excellent results for other bacteria, its yield for *M. tuberculosis* is still being studied.
Objectives: To evaluate E-test for susceptibility testing of *M. tuberculosis* to streptomycin, isoniazid, rifampicin and ethambutol.
Methods: We evaluated the E-test for susceptibility testing of *M. tuberculosis* to streptomycin (S), isoniazid (I), rifampicin (R) and ethambutol (E) against 102 clinical isolates of *M. tuberculosis* and the reference strain H37Rv. The proportions method (PM) was used as the reference method (NCCLS, M24-T2) and the following drug concentrations (μg/mL) were used: S (2 and 10), I (0.2 and 1), R (1) and E (5 and 10). E-test was performed according to the manufacturer's recommendations. For analysis purpose in PM, strains were considered as intermediate to one drug when resistant to the low concentration and susceptible to the high concentration, and susceptible or resistant when susceptible or resistant to both concentrations, respectively.
Results: Forty-three strains were intermediate or resistant to at least one drug using PM. According to the E-test results, optimal breakpoints (μg/mL) for this method were: S (low: 0.75; high: 8), I (low: 0.016; high: 0.06), R (1) and E (low: 0.19; high: 10). Agreements between both methods using these proposed breakpoints were: 96.1% (S), 99.0% (I), 100% (R) and 99.0% (E). Discordant strains for S were one PM-susceptible and E-test-intermediate, two PM-intermediate and E-test-susceptible and one PM-intermediate and E-test-resistant, for I one PM-intermediate and E-test-susceptible, and for E one PM-susceptible and E-test-intermediate.
Conclusions: E-test represents a rapid and valid alternative for the susceptibility testing of *M. tuberculosis* to first-line antituberculous drugs in the clinical laboratory routine.

P1039 Evaluation of methods for detection of extended spectrum β-lactamases (ESBL) in *E. coli* and *K. pneumoniae*. How reliable are the confirmatory methods?

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Some of the emerging mechanisms of resistance are of difficult laboratory detection. The emergence of these newer β-lactamases in Gram negative pathogens is consequence of abusive, and sometimes inappropriate use of extended-spectrum antibiotics. The detection of these new mechanisms of resistance requires special procedures and has been challenging for microbiologists. The extended-spectrum β-lactamases (ESBL) are very potent plasmid-mediated enzymes that are able to hydrolise all penicillins, cephalosporins, and monobactams. The ESBLs are spreading among members of Enterobacteriaceae, however, these enzymes are mostly encountered in *E. coli* and *K. pneumoniae*. There are several methods of ESBL detection, automated or not, all of them are based on the synergistic effect of β-lactamases inhibitors with cephalosporins (ceftazidime, cefotaxime, ceftriaxone), or monobactam (aztreonam). In this study, we evaluated four confirmatory methods: double-disk (tested simultaneously at 20 and 30 mm), E-test ESBL 'screen', Vitek GNS-650 card, and ATB BLSE panel for the ESBL detection in *E. coli* ($n = 269$) and *K. pneumoniae* ($n = 204$) isolated from several Brazilian hospitals. Among the confirmatory methods, the double-disk method presented the best results showing a sensitivity of 98%, followed by E-test (96.1%), Vitek (90.2%), and ATB (57.8%). The results of this study show that the double-disk method, when performed correctly and read by experienced personnel, can be a very sensitive and inexpensive method, being easily implemented in the laboratory.

P1040 Detection of extended spectrum β-lactamases (ESBLs) in Enterobacteriaceae with the Vitek ESBL and E-test ESBL

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Objectives: To compare the Vitek ESBL test and E-test ESBL in detection of ESBLs in clinical Enterobacteriaceae isolates.
Methods: Ninety-nine isolates of the seven different species of the family Enterobacteriaceae were used in the study. They were collected in 1998 from six hospitals in Poland. ESBL production was initially detected by the double-disc synergy (DDS) test and later confirmed by a combination of biochemical and molecular methods, including IEF, bioassay, PCR and sequencing of ESBL-encoding genes. Six of the isolates, all belonging to AmpC producer species, apart from ESBLs produced also AmpC β-lactamases in the depressed manner. The isolates were analyzed with the Vitek GNS-650 card containing the ESBL detection test and the E-test.
Results: Of the 99 strains examined (45 *K. pneumoniae*, 18 *E. coli*, 10 *S. marcescens*, 10 *E. cloacae*, nine *K. oxytoca*, six *C. freundii* and one *M. morganii*), 80 (80.8%) strains were found to produce ESBLs by Vitek, and 83 (83.8%) by the E-test ESBL. All strains with ESBLs and depressed AmpC cephalosporinases were defined as ESBL negative by the two approaches.
Conclusions: The Vitek ESBL test and the E-test ESBL are comparable and the results obtained are similar to those obtained by the double disk-test and they are useful in microbiological routine diagnostic.

P1041 Use of E-test for detection of phenotypical expression of hetero-glycopeptide-intermediate resistance in nosocomial coagulase negative Staphylococci (CoNS)

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Background: Recently E-test with High bacterial Inoculum (Et-HI) was found to be a reliable and sensitive method for the detection of phenotipical Hetero-Glycopeptide-Intermediate resistance (hGIS) in Staphylococci (Walsh T.R., JCM, July 2001).
Objective: To screen, with the E-test conventional inoculum (Et-CI) and proposed Et-HI, nosocomial bloodstream isolates of CoNS for the emergence of reduced susceptibility to glycopeptides.
Methods: The MICs to vancomycin (VA) and teicoplanin (TEI) was assessed by (i) Et-CI and Microdilutions Panels (MPs) (Microscan, Dade) (NCCLS proposed protocols and breakpoints applied) and (ii) Et-HI: after induction by an E-test strip placed in a Brain–Heart Infusion agar plate streaked out with two McFarland bacterial inoculum, the results were read after 24 and 48 h at 35 °C. Strains studied were 105 nonrepetitive oxacillin resistant CoNS recovered from blood cultures of different patients hospitalized in PICU (62%) and NICU (37%): 64 *S. epidermidis* (SE), 17 *S. haemolyticus* (SHAE), 12 *S. warneri* (SW), and 11 *S. hominis* (SHO). Species identification was carried out with coagulase test and API Staph System (BioMerieux).
Results: Et-CI and MPs agreed to categorize 100% of SE, SW and SHO as VA susceptible (VA-S), but one SHAE strain with Et-CI gave results VA-S and MPs VA-intermediate(I); for TEI the agreement was 100% on SHO to categorize TEI-Susceptible (S) and one strain of SE, SW and SHAE, respectively, resulted Et-CI TEI-I but MPs TEI-S. After 48 h Et-HI, strains resulted: (1) SE 23% VA-I (MICs 6–8 μg/mL) and 37% TEI-I (MICs 12–24) and 8% TEI-R (MICs ≥ 32); (2) SHAE 65% VA-I (MICs 6–8) and 41% TEI-I (MICs 12–16) and 35% TEI-R (MICs ≥ 128); (3) SW 33% VA-I (MICs 6) and 50% TEI-I (MICs 12–24) and 33% TEI-R (MICs > 64); (4) SHO 27% VA-I (MICs 6–8) and TEI-I (MICs 12–16). Colonies grown into the intermediate and/or resistance zone of the VA or TEI strips in Et-HI were re-tested with Et-CI after repeated subcultures in blood agar. Only SHAE and SW strains confirmed the results of reduced susceptibility found with Et-HI.
Conclusions: Our results confirm that conventional E-test is more accurate to MPs to asses the glycopeptides susceptibility of staphylococci. The E-test under induction appears to be a simple way for the detection and monitoring of subtle variations (heteroresistance) in VA and TEI susceptibility of CoNS recovered from blood stream infections in NICU and PICU.

P1042 Screening for extended spectrum β-lactamases by *double disk synergy test with different cephalosporins*

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Objectives: Jarlier et al. described double-disk synergy test (DDST) with cephalosporins and amoxicillin-clavulanic acid (AMC) disks for detection of ESBL. The aim of our study was to evaluate sensitivity of DDST with different cephalosporins to find suitable cephalosporins for DDST to be included in routine disk-diffusion antimicrobial sensitivity testing (AST).
Methods: (1) Thirty ESBL strains of *Klebsiella pneumoniae* (determined as ESBL strains by NCCLS phenotypic confirmatory tests with disks) were isolated in three Slovenian hospitals. Strains were tested by DDST with four cephalosporins: cephalotin (CF), ceftriaxone (CRO), ceftazidime (CAZ) and cefuroxime (CXM). Standard NCCLS disk-diffusion procedure was used for DDST. Distance between disks was 23 mm (center to center). Three results of DDST were possible: positive (enhancement of zones without any doubt), questionable (small enhancements), negative (no enhancement of zones between AMC and cephalosporin disk). (2) DDST with two cephalosporins, CRO and CF, was used in 200 consecutive clinical isolates of *Escherichia coli* and *K. pneumoniae*.
Results:
1 ESBL strains. Percentage of positive, questionable and false negative DDST results were: CF 100%, 0 and 0%; CRO 73, 20 and 7; CAZ 30, 50 and 20%; CXM 47, 37 and 16%, respectively. One ESBL strain was falsely negative by DDST with both, CRO and CAZ, but DDST with CF was positive.
2 Consecutive clinical strains. DDSTs were negative with both cephalosporins in 194 strains. DDST with CF only was positive in four strains (false positivity: NCCLS confirmatory ESBL tests with disks were negative). False positivity rate was 2% (four out of 200). Two strains were positive in DDST with both CF and CRO (ESBL strains, confirmed by NCCLS method).

Conclusions: DDST with CF and CRO can be part of routine AST for every strain. Only positioning of CF and CRO disks on each side of AMC disk and observation of synergy is necessary. DDST with CF has excellent sensitivity, but most of positive results will be falsely positive. When DDST with CRO is positive, ESBL are probably present. Clinicians can be informed immediately. Evaluation of the method in different epidemiological situations is needed.

P1043 An efficient method for the detection and differentiation of β-lactamases in Enterobacteriaceae

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Background: In recent years, an effort has been made to develop methods for β-lactamase detection with emphasis on extended-spectrum β-lactamases. But other β-lactamases also present a threat to therapy, especially plasmid and chromosomally encoded AmpC β-lactamases. Since different β-lactamases leave different options for therapy we developed a reliable phenotypic test that covers the important β-lactamases.
Methods: Susceptibility testing was performed by a microdilution procedure. To establish the differentiation method 87 strains with well characterized β-lactamases were tested against 22 β-lactams. The strains included 45 *E. coli*, 10 *K. pneumoniae*, eight *K. oxytoca*, 11 *E. cloacae*, six *C. freundii* and seven *S. marcescens*. For validation of the method the β-lactamases of 41 clinical isolates with a minimal inhibitory concentration (MIC) of cefotaxime ≥1 were then identified. PCR and sequencing methods used to confirm the identification results were standard.
Results: We succeeded in differentiating the following types of β-lactamases: original spectrum, inhibitor resistant/OXA, extended spectrum, inducible K1, hyperproduced K1, basal produced AmpC, inducible AmpC, hyperproduced/plasmid coded AmpC. The differentiation was made on the basis of MIC ranges towards a minimum of seven β-lactams (amoxicillin, amoxicillin/clavulanate, cefoxitin, cefpodoxime, aztreonam, cefotaxime, ceftazidime). Validation with 41 clinical isolates confirmed our results.
Conclusions: We developed a simple and efficient method for the detection and differentiation of β-lactamases in Enterobacteriaceae based on their MICs of 7 β-lactams. The identification results were confirmed by PCR and/or DNA sequencing. The required substances easily fit on a microdilution panel which allows automation in the clinical laboratory.

P1044 Intermethod agreement and preliminary quality control (QC) guidelines for susceptibility testing AZD2563 by disk diffusion and MIC methods

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Objectives: AZD2563 is a new oxazolidinone with favorable PK/PD features enabling once-daily dosing in projected clinical trails. To support the clinical susceptibility testing, method results were compared for the reference agar and broth tests, and the QC guidelines were determined for the NCCLS broth MIC and disk diffusion methods.
Methods: All tests or methods (M2-A7 and M7-A5, 2000) and QC study designs (M23-A2, 2001) were those published by the NCCLS. Agar dilution results for AZD2563 were compared to those of broth microdilution using 120 strains including 30 strains each of *S. pneumoniae*, other streptococci, staphylococci and enterococci. QC trials utilized eight participant laboratories and linezolid (LZD) as the drug class control. QC strains *S. aureus* (SA) ATCC 25923 and 29213, *E. faecalis* (EF) ATCC 29212 and *S. pneumoniae* (SP) ATCC 49619 were tested. Recent reports [ICAAC Abstr. D-167, 2001] of LZD disk QC range (SA) problems were also addressed.
Results: AZD2563 broth method MICs were identical to agar dilution results for 89.2% of comparisons (LZD control = 84.2%), the remaining broth results were generally lower by one two-fold dilution step. In the multicenter QC

trial 240 and 480 results were generated for MIC and disk tests, respectively. AZD2563 MIC modes were: SA at 2 mg/L, EF at 2 mg/L and SP at 1 mg/L (LZD at 4, 2, and 1 mg/L). Three or four dilution ranges (>95% of results in range) were proposed. AZD 30-μg disk zone diameters were generally 2–4 mm smaller than LZD zones, proposed ranges were 6–7 mm wide for each QC strain. SA zones for LZD required a 2–4 mm reduction in the lower range limit, pending action by NCCLS (vancomycin used as control).
Conclusions: AZD2563 susceptibility test results for NCCLS reference methods were demonstrated to be comparable. QC ranges for AZD2563 MIC and disk diffusion methods were established in preparation for clinical laboratory use of this promising, new oxazolidinone.

P1045 Validation of commercial dry-form panels (sensititre) for the susceptibility testing of AZD2563, a new long-acting oxazolidinone

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Objectives: The escalating problems of resistant Gram-positive cocci has necessitated the rapid development of new molecular classes of antimicrobials. AZD2563 is a novel, long-acting oxazolidinone being prepared for clinical trials at once-daily dosing. This report summarizes susceptibility testing, dry-form (DF) panel validations compared to reference NCCLS methods.
Methods: The susceptibility testing methods compared were Sensititre DF panels containing AZD2563 and linezolid (LZD; class control) vs. broth microdilution panels in reference frozen-form format (NCCLS, M7-A5) prepared by TREK Diagnostics (Westlake, OH). 462 strains were tested by M23-A2 guidelines using 111 *S. pneumoniae*, 105 staphylococci, 100 enterococci, 106 other streptococci and 40 selected Gram-negative organisms as oxazolidinone-resistant controls. Reproducibility was also determined (10 strains, 90 replicates).
Results: The validation trial used ±100 strains of four Gram-positive organism groups specified by NCCLS M23-A2. For AZD2563 and LZD tests, 462 determinations showed 382 (82.7%) and 399 (86.4%) with identical MICs for DF and reference methods, respectively. All DF MICs were ±1 log2 dilution of reference results for AZD2563 and LZD. Reproducibility tests used 10 strains tested 3× daily × 3 days (90 determinations). AZD2563 DF MIC reproducibility was 100% ± 1 log dilution analyzed within same day and between day results (87/90 and 76/90 results were identical, respectively). The same acceptable DF validation results were produced by a second laboratory, TREK Diagnostics.
Conclusions: The commercial DF panels for AZD2563 produced by Sensititre were validated as producing reproducible results comparable to the NCCLS reference method. These panels appear acceptable for use by AZD2563 clinical investigators worldwide and to generate values identical to those of NCCLS M7-A5 procedures.

P1047 Antimicrobial susceptibility testing for 10 5-Fluoroquinolones using the Phoenix™ system

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Objectives: In a performance evaluation of the Phoenix Automated System (BD Biosciences, Sparks, USA) for identification (ID) and antimicrobial susceptibility testing (AST), 10,5-fluoroquinolones were under investigation. Individual drug results were compared to the NCCLS reference broth microdilution method, and are presented for both Gram-positive and Gram-negative bacteria, derived from fresh clinical single patient isolates.
Methods: A total of 365 Gram-positive strains (235 staphylococci, 130 enterococci) and 384 Gram-negative strains (295 *Enterobacteriaceae*, 89 Non-fermenters) were tested. For AST the following 5-fluoroquinolones were evaluated in parallel with the NCCLS reference broth microdilution method: ciprofloxacin, gatifloxacin, grepafloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin, pefloxacin. Discrepant results were repeated in duplicate, all data were evaluated as defined by the ASM guidelines, additionally the distribution of the minimal inhibitory concentration (MIC) values were documented for each individual drug.
Results: The essential agreement (EA), category agreement (CA), very major error rate (VME) and major error rate (ME) for all 5-fluoroquinolones combined was 96.7, 98.0, 1.3 and 0.1% for Gram-positive isolates, and 97.9, 99.2, 0.0 and 0.04% for Gram-negative isolates, respectively. The observed resistance rates for Gram-positive cocci and Gram-negative rods were: ciprofloxacin 38.6, 18.1%, gatifloxacin 3.9, 12.2%, grepafloxacin 37.7, 16.0%, levofloxacin 29.1, 15.6%, lomefloxacin 38.6, 20.2%, moxifloxacin 2.2, 14.4%, norfloxacin 36.8, 16.5%, ofloxacin 38.2, 18.2%, trovafloxacin 12.2, 17.8%, respectively, and pefloxacin 41.5% for Gram-positive cocci.
Conclusions: The Phoenix™ System provides highly reliable AST results for 5-fluoroquinolones in both Gram-positive and Gram-negative isolates. The MIC distribution differed substantially between old and new Quinolones for Gram-positive strains, whereas for Gram-negative strains only slight differences were seen.

P1048 Reliability of E-test in the determination of in vitro susceptibility to *A. otitidis*

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Objectives: To determinate the reliability of E-test to study the in vitro susceptibility to *Alloiococcus otitidis*.
Methods: Isolations of *A. otitidis* were identified with standard methods and confirmed by PCR. Six antibiotics were tested: amoxicillin, cefotaxime, cefuroxime, azithromycin, tetracycline and cotrimoxazole. Agar dilution method was performed using Mueller–Hinton agar (OXOID) supplemented with 5% defibrinated horse blood. Concentrations were tested (prepared from twofold dilutions) ranging from 0.06 to 16 μg/mL and plates were incubated for 72 h at 37 °C in 10% CO_2 atmosphere. E-test was developed with the same medium and incubated under the same conditions.
Results: The results are showed in the following table

	Agar dilution		E-test		Resistance (%)	
	CMI_{50}	CMI_{90}	CMI_{50}	CMI_{90}	Agar dilution	E-test
Amoxicillin	0.06	0.5	0.03	0.23	0	0
Cefotaxime	0.5	1	0.25	0.5	4.8	5
Cefuroxime	0.12	0.5	0.12	1.5	9.5	10
Azithromycin	0.5	4	2	256	19	60
Tetracycline	0.12	0.25	0.5	0.75	0	0
Cotrimoxazole	4/76	4/76	0.006/0.0024	0.38/7.22	80.9	0

Conclusions: *Alloiococcus otitidis* shows an excellent susceptibility to β-lactams and tetracycline. However, very major discrepances were found when azithromycin and cotrimoxazole were tested. Therefore, E-test is not a reliable method for testing *A. otitidis* to azithromycin and cotrimoxazole.

P1049 Evaluation of the VIGI@ct software for detection of nosocomial infections and multidrug resistant bacteria

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An evaluation of the VIGI@ct software, a new program being developed by Bio-Mrieux (Marcy-l'Etoile France), was performed. This software is designed to detect nosocomial infections (NI), to analyze data on the sisceptibility patterns of bacteria including multidrug resistant bacteria (MBR), and to generate epidemiology statistics. The study was performed for a period of 2 months. The VIGI@ct software was connected to the Laboratory Information System (LIS) and to a VITEK and a VITEK2 instrument. All identification and susceptibility information was received automatically from the two instruments and was analyzed by the VIGI@ct software. During the 2-month study, we analyzed cultures from urine, central venous catheter, blood, sputum and bronchial aspirates from the intensive care and hematology wards to detect nosocomial infections. The software was set to detect suspected nosocomial infections using specific criteria. MBR were detected using predetermined resistance profiles. Results were analyzed for multiresistant bacteria from all wards in the hospital. Epidemiology statistics

were also determined for all data. The VIGI@ct allows fast detection of suspected NI and MRB and for information to be rapidly transmitted to the ward. When a NI or MRB was suspected, alarms were generated and sent to the wards in real time to allow physicians for rapid confirmation, determination of appropriate therapy and infection control measures. Epidemiology statistics were also generated to study trends in resistance patterns and verify changes in susceptibility patterns. In conclusion, VIGI@act software is easy to use, provides alarms on dangerous situations in real-time, provides a means to communicate quickly and efficiently with the ward and the physician, and provides an additional security measure for microbiology results.

P1050 Comparison of three methods for determination of antibiotic resistance among clinical isolates of *Pseudomonas aeruginosa* in Ankara, Turkey

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Objectives: To determine the antibiotic resistance among clinical isolates of *Pseudomonas aeruginosa* from pediatrics departments of two university hospitals by disc diffusion, microdilution, and BIOMIC, and to compare the three methods.
Methods: In vitro resistance against various antimicrobials of 150 *Pseudomonas aeruginosa* strains (57 strains from Ankara University Hospital and 93 strains from Hacettepe University Hospital) were investigated by disc diffusion, microdilution, and BIOMIC methods, and the results were evaluated according to the NCCLS criteria, between May 1999 and May 2000.
Results: Antibiotic resistance of 150 *Pseudomonas aeruginosa* strains by BIOMIC is summarized in the Table. The chi-square test did not show a significant difference in resistance between Ankara University strains and Hacettepe University strains. There was no significant difference regarding antibiotic resistance among disc diffusion, microdilution and BIOMIC. All three methods had almost the same sensitivity to detect resistance of strains to antimicrobials.
Conclusion: It is concluded that although disc diffusion test is the easiest method to perform, BIOMIC is also an easy technique, and is useful since it provides MIC values as well (Table 1).

Table 1 Antibiotic resistance of 150 *Pseudomonas aeruginosa* strains by BIOMIC

Antibiotic	Resistance (%)	MIC_{50} (μg/mL)	MIC_{90}) (μg/mL)	Range (μg/mL)
Gentamicin	60.0	42	42	<0.22–>41
Amikacin	42.0	33	156	<0.79–>155
Ceftazidime	39.3	6.7	127	<0.56–>126
Cefepim	18.0	6.5	42	<0.54–>79
Cefoperazon	46.7	43	405	<0.47–>404
Ciprofloxacin	34.7	0.45	20	<0.11–>19
Imipenem	12.7	1	9.9	<0.56–>21
Piperacillin	44.0	39	609	<0.72–>828
Piperacillin/tazobactam	32.0	4.5	256	<0.17–>770

P1051 New method for normalized interpretation of antimicrobial resistance from disk test results for surveillance purposes

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Objectives: To evaluate a new calibration method for disc diffusion antibiotic susceptibility tests which relies solely on zone diameter values generated in the individual laboratory for each antibiotic-bacterial species combination as the internal calibrator.
Methods: The high-zone side of histogram distributions was analyzed by moving averages to determine the peak position, then the accumulated percentages of isolates for the high-zone diameter values were calculated and converted into probit values. The normal distribution of the ideal population of susceptible strains was determined using the least squares method for probit values against zone diameters. Zone diameter values were obtained from Karolinska hospital (KS), from the Växjö hospital (VX), and from Buenos Aires hospitals. The method requires a well standardized method but is independent of differences in MIC-limits and zone breakpoints and does not require the use of reference strains.
Results: Normalized interpretation of antimicrobial resistance was first tested on 3582 test results for control strains *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 at KS and VX using SRGA standardization. When the true means were compared with the calculated means a very high degree of correlation was seen (correlation coefficient $r = 0.998$), confirming the validity of the new method. Histograms of inhibition zone diameter values for antibiotics and clinical isolates of the two bacterial species, *S. aureus* and *E. coli* (114 217 test results) at the two laboratories were then analyzed. The ideal distribution of susceptible strains was calculated and isolates showing inhibition zones below the 3 SD limit were then taken as resistant. The results showed an almost complete agreement with the interpretations in the laboratories. Difficulties were seen for *E. coli* and mecillinam. The method was then tested on results from other laboratories which were using the NCCLS standard for disc diffusion tests and the results proved the promising potential for surveillance.
Conclusions: The normalized resistance interpretation offers a new approach to surveillance where existing inhibition zone diameter results from disc tests in clinical laboratories can be used for calibration of the test and calculation of resistance levels.

P1052 Ability of automated ID/MIC systems to detect ESBL-producing *Enterobacteriaceae*: comparison with DDST and E-test

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Objectives: Extended-spectrum β-lactamase (ESBL) – producing strains of *Enterobacteriaceae* are being isolated with increasing frequency worldwide and compromise antimicrobial therapy of infections caused by them. The objective of our study was to evaluate the ability of automated systems we use for routine testing to detect ESBLs, comparing their performance with DDST and E-test.
Methods: We tested 61 isolates (50 *E. coli* and 11 *Klebsiella* sp.) characterized as ESBL positive (+) by at least one of the automated ID/MIC systems used in our laboratory during the period 1 June 1999 to 31 October 2001, according to each system's expert rules. PASCO: cefpodoxime (CPD) > 1 mg/L or/and ceftazidime (CAZ) > 1 mg/L, MICROSCAN: ceftriaxone (CRO) ≥ 4 mg/L or/and CAZ ≥ 2 mg/L and WIDER: cefotaxime (CTX) > 1 mg/L or/and CAZ > 1 mg/L or CAZ ≥ 16 mg/L and ceftazidime + clavulanic acid (CAZ/CL) ≤ 1/4 mg/L. These isolates were further tested by: (1) Double Disk Synergy Test with CTX, CAZ and aztreonam (ATM) placed on 25 mm from an amoxicillin/clavulanic acid (AMC) disk and (2) E-test ESBL strips with CTX (CT/CTL) and CAZ (TZ/TZL). Four QC strains were used as positive and negative controls.
Results: The sensitivity of the antimicrobials used by each system for ESBL screening was: (1) PASCO: CPD 3/17 (17.6%), CAZ 0/2 (0%), CPD + CAZ 7/23 (30.4%) (2) MICROSCAN: CAZ 0/1 (0%), CRO 2/2 (100%), CAZ + CRO 3/6 (50%) (3) WIDER: CTX 2/4 (50%), CAZ 0/6 (0%), CTX + CAZ 4/16 (25%), CAZ + CAZ/CL 17/18 (94.4%). There was 100% agreement between DDST and E-test.
Conclusions: The ability of automated systems to detect ESBLs depends on the antimicrobials used by their expert rules; one or two 3rd generation cephalosporins are insufficient. Newer systems using rules that include a cephalosporin alone and in combination with clavulanic acid (e.g. CAZ + CAZ/CL) seem to have high sensitivity (94.4% in our study). These systems could eliminate the need for time-consuming manual tests like DDST and E-test.

P1053 Bactometer: alternative method for testing antimicrobial preservation efficacy

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Objectives: Antimicrobial preservatives have been added to pharmaceutical preparations to prevent proliferation or to limit microbial contamination. It should be demonstrate that preservatives provide adequate protection. The standard procedure – challenge test – recommended by pharmacopeia is time-consuming and require usually a few days of incubation. New alternative

technique based on impedimetric procedure makes possible to reduce this time to several hours. The growing of bacteria in Bactometer (bioMerieux, Vitek System, USA) cause a change of media conductivity. The time required by bacterial population to create a detectable changes in electrical conductivity is proportional to the initial number of cells.
Methods: The test is based on challenge the preparation with prescribed inoculum of microorganisms e.g. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Inoculated preparation was incubated at a prescribed temperature, followed by collection of samples at specified intervals and counting the viable microorganism in the sample by plate count in standard procedure or inoculation Bactometer module in alternative method. The samples for classical and alternative method were prepared in the same way and for Bactometer reading were serially diluted and inoculated in duplicate to several cells of module. Logaritmical reduction was calculated in comparison to initial microorganisms count estimated by plate method. In impedimetric method, the growth or lack of growth in cells with diluted sample was the base to calculation. The study was conducted with some pharmaceutical products e.g. ophthalmic preparation and nasal spray.
Results: All pharmaceutical preparations were tested in duplicate. As expected, in the most cases of ophthalmic drug testing, the reduction 99 and 99.9% of bacterial population was observed during 6 and 24 h, respectively, and 90% reduction of fungi in 7 days. For nasal spray the same reduction of bacteria was seen in the 2nd and 7th days, respectively, and fungi 14 days from the beginning of test. Logaritmical reduction calculated for plate method was similar to that obtained in alternative method. The results meet the requirements of pharmacopeia. The studies of efficacy of antimicrobial preservation indicated a positive correlation between standard plate count results and impedance reading.

P1054 Reassessment of cafaclor breakpoints to *Haemophilus influenzae*

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Background: Previously we reported on cefaclor instability in vitro, suggesting that *S. pneumoniae* MICs determined by NCCLS methodology are consequently falsely high. The objective of this study was to ascertain if standard methods accurately measure cefaclor MICs to *H. influenzae*.
Methods: The stabilities of cefaclor and cefuroxime were established over 24 h. Eight isolates of *H. influenzae* were tested, including one ATCC and one NCTC strain. Standard MICs were carried out by NCCLS broth microdilution. Kill curves were carried out by determining cfu/mL over 6 h. Antibiotic concentrations were expressed as multiples of the MIC (range 0.1–0.9 × MIC), as determined by NCCLS methodology. Cefuroxime was used as a comparator.
Results: After 24 h cefaclor had lost 88% activity and cefuroxime 34%. Cefaclor MICs by NCCLS methodology were 2 × 1, 3 × 2, 4 and 8 mg/L. Cefuroxime MICs were 5 × 0.5, 2 × 1 and 1 × 2 mg/L. By kill curve, the 'bacteriostatic' MIC was defined as the concentration which showed no significant growth and no significant kill over the test period, taking into account cefaclor instability. On average, the cefaclor bacteriostatic MIC was 0.2 × MIC determined by NCCLS methodology. The mean cefaclor NCCLS MIC was thus 2.875 mg/L and the mean bacteriostatic MIC was 1.725 mg/L. The cefuroxime bacteriostatic MIC was an average of 0.6× the MIC by NCCLS methodology. Thus, the mean MIC by NCCLS methodology was 0.812 mg/L and the mean bacteriostatic MIC was 0.488 mg/L.
Conclusions: Cefaclor MICs by NCCLS methodology were overestimated due to chemical instability over 18–24 h. The bacteriostatic MICs by kill curve were found to be an average of 0.2 × MIC by NCCLS methodology. Allowing for this overestimate of NCCLS MICs, the cefaclor pharmacodynamic breakpoint could appropriately be redefined as 2 or 4 mg/L.

Enterobacteriaceae in vitro studies

P1055 In vitro susceptibility trends of piperacillin/tazobactam in a tertiary care hospital in Athens, Greece

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Objectives: Prospective study was conducted in order to evaluate the antibacterial performance of piperacillin/tazobactam (pip/tazo) compared to other antibiotics, after 5 years of use. The trends of bacterial resistance and the impact of antibiotic consumption on it were also investigated.
Methods: During a 6-month period (November 2000–April 2001), 314 consecutive samples from hospitalized patients were collected. The cultures were performed by conventional methods. The bacteria studied were *Enterobacteriaceae, Haemophilus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., methicillin-sensitive *Staphylococci* and *Streptococcus* spp. Antimicrobial susceptibility was evaluated by MIC values determination, using microdilution method (PASCO MIC/ID Gram (–) and Gram (+) panels, Difco Laboratories) for: pip/tazo, ceftriaxone, ceftazidime, cefepime, cefotaxime, imipenem, ciprofloxacin, amox/clav, gentamycin, oxacillin and ampicillin. Susceptibility interpretation was based on current NCCLS guidelines. Comparative study of susceptibility patterns was carried out, and the results were also compared to those published in 1996. Furthermore, the resistance incidence versus hospital antibiotic consumption was evaluated.
Results: A total of 339 bacterial strains were isolated. Fifteen percent of the specimens were obtained from ICU patients. Urine and blood were the most frequent type of samples. The predominant pathogen was *E. coli* (23%) followed by *P. aeruginosa* (17%), *K. pneumoniae* (12%) and *S. aureus* (8%). *Acinetobacter* spp. (27%) and *P. aeruginosa* (25%) were the main pathogens isolated from ICU patients. The susceptibility of all *Enterobacteriaceae* strains to pip/tazo and Imipenem remained high (82–97 and 100%, respectively). Susceptibility to other β-lactams was also high except Amox/Clav, whereas *Enterobacter* spp.'s susceptibility proved to be lower. Susceptibility to ciprofloxacin ranged from 64 to 96%. All staphylococci tested were susceptible to pip/tazo and Imipenem. Enterococcus susceptibility to pip/tazo was 95%. The susceptibility of *P. aeruginosa* to pip/tazo, ceftazidime and imipenem was extremely high (96–98%). According to hospital pharmacy data, pip/tazo remains a β-lactam with high consumption. Any significant difference in susceptibility to pip/tazo between 1996 and 2001, despite its high consumption, was not observed.
Conclusions: Pip/tazo retains its initial high effectiveness to significant pathogens after 5 years of hospital use as first-choice antibiotic.

P1056 Antibiotic susceptibility study of 409 clinical strains of *E. coli* isolated during 2000 at an institute of infectious diseases, Bucharest, Romania

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Objective: To characterize the antibiotic (AB) susceptibility of all *E. coli* strains isolated from clinically significant specimens obtained from patients hospitalized at our institute, from 1st of January to 31st of December 2000.
Methods: Retrospective analysis of the 409 results of *E. coli* disk-diffusion antibiograms performed at our microbiology laboratory. The AB taken into study were: ampicillin (A), gentamicin (G), amikacin (AK), amoxicillin/clavulanate (A/C), nalidixic acid (NA), norfloxacin (NF), ciprofloxacin (CF), ofloxacin (OF), nitrofurantoin (NT), co-trimoxazole (CTX), colistin (CO), fosfomycin (FM), second-generation cephalosporins (2GC), third-generation cephalosporins (3GC) and imipenem (IM). The results were expressed using the 1999 NCCLS standards, as sensitive (S), intermediate (I) and resistant (R) strains.
Results: The 409 *E. coli* strains taken into study were isolated mainly from urine cultures (83%) and from sputum (6%), naso-pharynx swabs (6%), pus (2%), CSF (0.5%), blood (0.5%) and other pathological products (2%). The AB susceptibility to *E. coli* in our study, in decreasing order, was as follows: IM (100%), FM (99%), G (84%), CO (82%), 3GC (79.7%), OF and CF (77.5%), NA (73.9%), AK (72.7%), 2GC (44.3%), A/C (32.8%); the lowest sensitivity rates observed, involved A (21.4%), NF (13.5%), CTX (6.2%).

Conclusions: The *E. coli* strains isolated at our hospital during year 2000 showed practically the loss of sensitivity to co-trimoxazole and nitrofurantoin and a very low sensitivity to ampicillin. The strain I and R to amoxicillin/clavulanate have reached a worrisome level of 67.2% but A/C was still effective in certain clinical situations owing to the high percentage of I strains (48.5%) that responded to an increase of the daily dosage of the drug. Globally, the *E. coli* strains preserved a good enough sensitivity to nalidixic acid, and a very good sensitivity to fluorquinolones, aminoglycosides and third-generation cephalosporins. Our study did not detect any resistant strain of *E. coli* to imipenem, colistin and fosfomycin.

P1057 Antimicrobial activity of β-lactam antibiotics against *Proteus mirabilis* strains isolated in Poland between 1997 and 2000

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Objective: To study the resistance to β-lactams in *P. mirabilis* in Poland over the last 4 years.
Methods: A total of 350 *P. mirabilis* strains collected by the National Reference Center for Antibiotics in the Sera and Vaccines Central Research Laboratory during 1997–2000 were examined. The strains were isolated by more than 20 microbiology laboratories in Poland from a variety of clinical specimens, mostly from urine samples. Susceptibility to the following antibiotics was tested: ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefoxitin, cefotaxime, ceftazidime, cefepime, aztreonam, imipenem and meropenem. MICs of the antimicrobials were determined by the agar dilution method and interpreted according to the NCCLS recommendations.
Results: The highest percentage of *P. mirabilis* resistance was observed for ampicillin (70%), followed by piperacillin and amoxicillin/clavulanic acid (50%), cefoxitin, cefotaxime, ceftazidime (40%) and piperacillin/tazobactam (20%). The rate of the *P. mirabilis* resistance to most of the antibiotics mentioned above increased by about 30% between 1997 and 2000. Resistance to piperacillin/tazobactam raised nearly by 11% during the same period. Cefepime, aztreonam, meropenem and imipenem were the most active antimicrobials. However, the imipenem MICs were relatively high for about 50% of susceptible strains (MICs, 2–4 mg/L).
Conclusions: Resistance of *P. mirabilis* to many β-lactam antibiotics that are commonly used in the empirical therapy is very frequent in Poland. Among these, only cefepime, aztreonam and carbapenems showed high antimicrobial activity in vitro. The increase of *P. mirabilis* resistance to β-lactams over the last years seems to be an emerging problem in Poland.

P1058 Antimicrobial susceptibility of *Enterobacteriaceae* involved in urinary tract infections in Crete, Greece

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Objective: To investigate the prevalence of *Enterobacteriaceae* causing urinary tract infections and to determine their in vitro susceptibility to antimicrobial agents.
Methods: We studied 1105 consecutive strains of *Enterobacteriaceae* isolated from patients with urinary tract infections in the period from January to December 2000. Urine cultures were performed by standard method and the isolates were identified to the species level using the API 20E system (BioMerieux). Susceptibility testing to antimicrobials was done by the disk diffusion method based on the NCCLS guidelines. Double-disk diffusion method was performed for the detection of extended-spectrum β-lactamases (ESBL).
Results: *E. coli* was the predominant species representing 76.6% of the isolates, followed by *Proteus* spp. (8.6%), *Klebsiella* spp. (8.4%), *Enterobacter* spp. (3.7%), and other species of the family *Enterobacteriaceae* (*Citrobacter* spp., *Morganella morganii*, *Providencia* spp., *Serratia marcescens*, *Salmonella* spp.) (2.7%). The most common urinary pathogens *E. coli*, *Proteus* spp., and *Klebsiella* spp. showed the following susceptibility rates (%) to antimicrobial agents tested: ampicillin 56.3/69.5/0.0; amoxicillin/clavulanate 62.9/79.0/66.7; cefuroxime 79.8/85.3/65.6; cefotaxime 98.6/98.9/82.8; gentamicin 94.8/92.6/81.7; cotrimoxazole 67.4/85.3/60.2; nitrofurantoin 96.3/6.3/49.5; nalidixic acid 90.8/79.0/89.2; norfloxacin 93.5/98.0/92.5; ciprofloxacin 93.3/95.8/94.6. ESBL producers were found among *E. coli* isolates (1.3%), among *Klebsiella* spp. (16.1%), and among *Enterobacter* spp. (9.8%).
Conclusions: *E. coli* is the most common uropathogen in our hospital. Local surveillance data that provides information about antimicrobial susceptibility of pathogens causing UTIs are necessary in order to select the appropriate antibiotic treatment.

P1059 Occurrence and transferability of β-lactam and aminoglycoside resistance in clinical isolates of *Enterobacteriaceae*

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Objectives: To study the occurrence of resistance to 15 antimicrobial agents: ampicillin (AMPI), cefoxitine (CFOX), cefotaxime (CTAX), ceftazidime (CTAZ), ceftriaxone (CIAX), cefepime (CEPI), azthreonam (AZTR), meropenem (MERP), gentamicin (GEN), tobramycin (TOB), netilmicin (NET), amikacin (AMI), isepamicin (ISE), ofloxacin (OFL), ciprofloxacin (CIP), transferability of β-lactam and aminoglycoside resistance and production of β-lactamases in a set of 20 clinical isolates of *Enterobacteriaceae* – five *Klebsiella pneumoniae* (25%), five *Enterobacter cloacae* (25%), one *Enterobacter aerogenes* (5%), three *Citrobacter freundii* (15%), three *Escherichia coli* (15%), and three *Serratia marcescens* (15%). The isolates were obtained in the years 1999–2000 in Hospital Ruzinov, Bratislava from skin infections and were selected as resistant to β-lactams.
Methods: The level of resistance was determined by standard method NCCLS 2000. Production of β-lactamases was detected by nitrocephin method. ESBL producers were identified by double-diffusion test. Transferability of β-lactam and aminoglycoside resistance was studied by bacterial conjugation.
Results: A total of 16 isolates were resistant to AMPI (80%), 19 to CFOX (95%), 15 to CTAX (75%), 16 to CTAZ (80%), 13 to CIAX (65%), 13 to CEPI (65%), 19 to AZTR (95%) and 7 to MERP (35%), 17 to GEN (85%), 12 to TOB (60%), 2 to NET (10%), 11 to AMI (55%), 7 to ISE (35%), 11 to OFL (55%) and 7 to CIP (35%). Thirteen isolates (65%) produced β-lactamases and 11 isolates (55%) were identified as producers of ESBL. Resistance to all β-lactam and aminoglycoside antibiotics, with only one exception of MERP, was transferable by bacterial conjugation to the recipient strain *Escherichia coli* K-12 3110.
Conclusions: Occurrence of resistance to all β-lactam antibiotics tested and the production of β-lactamases and ESBL were very frequent. The most efficient β-lactam was the carbapenem MERP. The occurrence of resistance to aminoglycosides and fluoroquinolones was very high, too. The most efficient amonoglycoside was NET. Thirty-five percent resistance to ISE is interesting, as this aminoglycoside has not been used in clinical practice in Slovakia yet. The transferability of resistance to β-lactams together with aminoglycoside resistance by bacterial conjugation points to its plasmid determination and to the alarming situation in the spread of resistance in the mentioned hospital, so the further more detailed studies are indispensable.

P1060 The PEARLS study: determining incidence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant *E. faecium* (VREF), and methicillin-resistant *S. aureus* (MRSA) in 16 centers from 10 European countries

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Background: Resistant isolates found in the hospital environment continue to increase and are of great concern. This study is part of an ongoing study examining resistance determinants in pathogens. The current incidence of ESBL producers in selected Enterobacteriaceae, VREF and MRSA were measured in 16 investigative sites from 10 European countries. The information gathered from The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) study will be useful in determining selective pressures that influence the increasing incidence of resistance.

Methods: Each site collected consecutive isolates consisting of the following: 50 *Enterococcus faecium* (EF), 50 *Enterobacter cloacae* (EC), 50 *Enterobacter aerogenes* (EA), 75 *Escherichia coli* (EsC), 75 *Klebsiella pneumoniae* (KP), 25 *Acinetobacter* spp. (As), 25 *Pseudomonas aeruginosa* (PA), 25 *Serratia marcescens* (SM), 25 *Staphylococcus aureus* (SA). All isolates were tested in a central reference laboratory using broth microdilution following manufacturer's instructions and NCCLS guidelines.
Results: The overall incidence of VREF and MRSA was 8.8 and 35.2%, respectively. Following are the ESBL rates for Enterobacteriaceae: EC (23.2%), EA (27.6%), EsC (4.3%), KP (18.9%) and SM (37.7%). The total ESBL rate for all Enterobacteriaceae was 16.3 and 23.7% without EsC included. The highest rates of ESBL occurrence per country without EsC included were Portugal (32.0%); Italy (26.4%); Austria (26.6%); Greece (25.0%); Spain (23.0%); France (21.8%); Germany (19.7%); the Netherlands (17.8%); Switzerland (8.3%) and Belgium (0.0%). The highest occurrence of VREF was in Portugal (44.4%) and the lowest with no occurrences of VREF in France, Greece and the Netherlands. MRSA rates varied with a high of 87.5% in Portugal, whereas the Netherlands had no occurrences of MRSA.
Conclusion: Resistant rates are affected by many factors including patient population, prior antimicrobial therapy, formulary changes and regional differences, among other factors. The rates of these three determinants of resistance are consistent with current literature for VREF, MRSA and ESBL in Europe but vary greatly by region. These data will serve as a baseline for determining selective pressures that influence resistance rates.

P1061 A 2001 multicenter, multicountry surveillance study identifying antibiotic resistance to extended-spectrum β-lactamase-producing *Enterobacteriaceae*, vancomycin resistant *E. faecium* and methicillin-resistant *S. aureus* isolates: the PEARLS study

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Background: The increase of extended-spectrum β-lactamase (ESBL), vancomycin-resistant *Enterococcus faecium* (VREF) and methicillin resistant *Staphylococcus aureus* (MRSA) isolates worldwide has been reported in recent studies. The impact of high cephalosporin use may significantly affect the increased incidence of these organisms. The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) Study compares susceptibility results of various antimicrobials in Europe, the Middle East and South Africa.
Methods: A total of 3850 isolates were evaluated from 33 investigative sites in: Austria; Belgium; Egypt; France; Germany; Greece; Italy; Lebanon; Portugal; Saudi Arabia; South Africa; Spain; Switzerland; the Netherlands and Turkey. Each investigator collected the following selected species: 50 *Enterococcus faecium* (EF); 50 *Enterobacter cloacae* (EC); 50 *Enterobacter aerogenes* (EA); 75 *Escherichia coli* (EsC); 75 *Klebsiella pneumoniae* (KP); 25 *Acinetobacter* spp. (As); 25 *Pseudomonas aeruginosa* (PA); 25 *Serratia marcescens* (SM); 25 *Staphylococcus aureus* (SA). The following antimicrobials: amoxicillin/clavulanic acid (A/C); cefepime (Cfp); cefotaxime (Cft); ceftazidime (Cfz); ceftriaxone (Ctx); ciprofloxacin (Cip); gentamicin (Gtm); imipenem (Imp); levofloxacin (Lev); piperacillin/tazobactam (P/T) and vancomycin (Vcm) were evaluated following manufacturer's instructions and NCCLS guidelines.
Results: ESBL *Enterobacteriaceae* isolates, including EsC, showed the lowest level of resistance to Imp at 3.1%. Average cephalosporin resistant rates for ESBL *Enterobacteriaceae*, including EsC, ranged from 5.0 to 30.0% and varied from country to country. Among the β-lactams/β-lactamase inhibitors (i.e. P/T and A/C) the resistant rates averaged 18.4% vs. 73.8%. Lev and Cip were at 37.9 and 11.17% susceptible, respectively, for non-VREF. Cfz had the highest percent resistance to MRSA at 93.3%. Vcm demonstrated the highest percent susceptible to MSSA at 99.6% and MRSA at 99.3%.
Conclusions: Imp, P/T and some cephalosporins demonstrated good activity against *Enterobacteriaceae* including ESBL producers. None of the antimicrobials tested did well against VREF. All antimicrobials tested had high resistance percentages to MRSA except Vcm. More studies are needed in order to monitor the impact-specific antimicrobials may have in the hospital environment.

P1062 Emergence of multiresistant Gram-negative bacteria in the nursing-home setting

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Objective: The epidemiology, clinical presentation, and natural history of many infections are unique in the nursing home setting. In this study, our aim was to revise the emergence of extended-spectrum β-lactamase (ESBL)-producing strains of the family *Enterobacteriaceae* among the population of a nursing home in Beirut.
Material and methods: In this retrospective study, all the microbiologic data of the Saint George nursing-home population consisting of 57 males and female was collected and compiled. The antibiotic susceptibility testing (AST) of the isolated germs was examined and recorded for ESBL production. These strains were identified by using the standard techniques and/or the API 20E system (BioMérieux, Marcy Etoile, France) and AST were performed as recommended by the NCCLS. ESBL production was detected by the double-disc method and confirmed by synergy between third generation cephalosporins synergy with clavulanate. The isolates included were those collected from different patients or those from the same patient with different susceptibilities.
Results and discussion: Among the 19 males and 38 females who form the population of the nursing home, 30 (52.63%) had suffered at least one symptomatic or asymptomatic UTI during the past 18 months. Five of these patients (16.6%) were infected with ESBL-producing strain of *Enterobacteriaceae*. Results are shown in Table 1.

Table 1 Frequency (%) of the different isolated strains of ESBL-producing *Enterobacteriaceae*

	K. pneumoniae	*E. coli*	*C. freundii*
Frequency (%) ESBL among *Enterobacteriaceae*	5 (12.8)	2 (5.1)	2 (5.1)
Total number of *Enterobacteriaceae*	39	39	39
Frequency (%) ESBL among the genus	5 (41.7)	2 (11.1)	2 (100)
Total number	12	18	2

The isolated germs were: 2 *Klebsiella pneumoniae*, 18 *Escherichia coli*, 7 *Proteus* spp., 2 *Citrobacter freundii*, 1 *Burkholderia cepacia*, 1 *Acinetobacter baumanii*, 1 *Pseudomonas* spp., and 6 *Enterococcus faecalis*. Our results show an important percentage of ESBL-producing strains showing similar pattern to those isolated in the Saint George Hospital located in the proximity of the nursing home and that are increasing yearly. The challenge for the future is to minimize infection in the nursing home, while limiting the emergence and spread of antimicrobial resistance with optimal cost-effective care. There is an urgent need for clinical studies to evaluate strategies for the prevention and management of infections in the nursing-home patient.

P1063 Cefepime in vitro activity against Gram-negative rods

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Objectives: To determine cefepime (FEP) in vitro activity against *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated over 6 months.
Methods: Antibiotic sensitivity was done by disc-diffusion method according to NCCLS guidelines. Non-replicate bacterial isolates were obtained from clinical material collected from January 2001 to June 2001. ESBL production was determined by double-disc method.
Results: During the study period, 1675 isolates from 20 species were obtained. Majority of them were as follows: *Escherichia coli* 556, *Pseudomonas aeruginosa* 391, *Enterobacter cloacae* 174, *Klebsiella pneumoniae* 169, *Proteus mirabilis* 140, *A. baumannii* 73. FEP was most active against *E. coli* and *P. mirabilis* – 99%, *E. cloacae* – 94%, *K. pneumoniae* – 89%. The highest

resistance was noted in *A. baumannii* – 59% and *P. aeruginosa* – 9%. These species were also intermediate susceptible to FEP 11 and 21%, respectively. ESBL phenotype was found in 117 isolates, 66 of them being *K. pneumoniae*. Seventy-four percent of ESBL-producing isolates were sensitive to FEP, 19% intermediate and 7% resistant.
Conclusion: Overall sensitivity of bacteria to FEP reached 87% independent of the species, Ninety-six percent of *Enterobacteriaceae* and 63% of nonfermenting rods were sensitive. Because ESBL positive isolates constituted 10% of *Enterobacteriaceae*, FEP should be, then reported as resistant regardless of the in vitro result. Urinary tract isolates can be the only exception. FEP was least active against *A. baumannii*.

P1064 Synergistic action of ceftazidime with serum in the bactericidal action on *E. coli* isolated from blood of patients with bacteremia

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Objectives: Purpose of this work was determination and comparison of the minimal bactericidal concentration (MBC) of ceftazidime (CAZ) in dependence of cooperation or without cooperation with bactericidal factors of normal human serum (NHS) against *E. coli* strains isolated from blood of patients with bacteremia.
Methods: Following *E. coli* strains were studied: sensitive to the bactericidal action of NHS, resistant to NHS and strains for which the NHS was bacteriostatic. All studied strains were susceptible on CAZ. Values of MBC were determined for CAZ alone and CAZ combined with NHS.
Results: For killing of *E. coli* strains sensitive to the bactericidal action of NHS, the MBC was 32 times smaller for the CAZ combined with NHS in comparison to the bactericidal action CAZ alone. On the other hand, the value of MBC of CAZ alone or CAZ combined with NHS was exactly the same for strains resistant on NHS or for strains for which the action of NHS was bacteriostatic.
Conclusions: It is shown that the cooperation between CAZ and NHS in the bactericidal action against *E. coli* strains sensitive to the action of NHS. Moreover, the cooperation between the antibiotic and NHS in the bactericidal action results in 32 times less than the value of MBC of antibiotic.

P1065 Cooperation of cefotaxime with serum in the bactericidal action on *Klebsiella pneumoniae* and *Enterobacter cloacae* strains isolated from blood

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Objectives: Purpose of this work was determination and comparison of minimal the bactericidal concentration (MBC) of cefotaxime (CTX) in dependence of cooperation or without cooperation with bactericidal factors of normal human serum (NHS) against *Klebsiella pneumoniae* and *Enterobacter cloacae* isolated from blood of patients with bacteremia.
Methods: Following *K. pneumoniae* and *E. cloacae* strains were studied: sensitive to the bactericidal action of NHS, resistant to NHS and strains for which the NHS was bacteriostatic. Moreover, all studied strains were susceptible on CTX. Values of MBC were determined for CTX alone and CTX combined with NHS.
Results: For killing of *K. pneumoniae* strains sensitive to the bactericidal action of NHS the MBC was eight times smaller for the CTX combined with NHS than for the CTX without NHS. Similarly, for killing of *E. cloacae* strains sensitive to the bactericidal action of NHS the MBC was 32 times smaller for the CTX combined with NHS than for the CTX alone. On the other hand, the amount of MBC of CTX with or without NHS was exactly the same for *K. pneumoniae* and *E. cloacae* strains resistant on NHS or for strains for which the action of NHS was bacteriostatic.
Conclusions: Cooperation of CTX and NHS in bactericidal action against *K. pneumoniae* and *E. cloacae* strains sensitive to the action of NHS results in significant decrease of MBC of the antibiotic.

P1066 Influence of moxifloxacin on endotoxin release of *Escherichia coli*

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Objectives: To examine whether moxifloxacin, a new fourth-generation fluoroquinolone, causes endotoxin (LPS) release from *Escherichia coli* and to compare its degree of LPS-releasing capacity with that of the drugs belonging to other antibiotic classes. We used imipenem as a representative of drugs known to cause low-LPS release, and ceftazidime as a drug known to induce large amounts of LPS.
Methods: *Escherichia coli* O18:K1:H7 was grown in Mueller–Hinton broth in the absence and presence of 2×, 10× and 50×. The minimal inhibitory concentration (MIC) of moxifloxacin, imipenem and ceftazidime. At 0, 2, 4, 6, 8 and 24 h, bacterial concentrations in the suspension were determined by plate counts, and supernatant samples were taken for determination of free (soluble) LPS. The LPS was quantified by enzyme-linked immunosorbent assay using two monoclonal antibodies specific for different epitopes of the side-chain region of *E. coli* O18 LPS. The biological activity of antibiotic-induced LPS was examined by measuring the release of tumor necrosis factor alpha (TNFα) from a CD14-positive monocytic tumor cell line, MonoMac6, in response to LPS challenge.
Results: MICs (mg/L) were 0.06 mg/L for moxifloxacin, 0.25 mg/L for imipenem, and 0.125 mg/L for ceftazidime. The time course of bacterial killing was comparable between the three drugs. LPS concentrations were significantly lower in cultures containing moxifloxacin or imipenem compared to the ceftazidime and controls. For example, at 10 × MIC and 4 h exposure time, LPS (ng/mL; mean + SEM) concentrations were 73 + 11.9 for moxifloxacin, 35.2 + 4.4 for imipenem, 590 + 183.1 for ceftazidime and 7866.7 + 2150.8 for controls. Corresponding TNF levels (pg/mL) were 50.5 + 3.5 for moxifloxacin, 44 + 3.6 for imipenem, 292 + 78.7 for ceftazidime, and 712 + 129.5 for controls.
Conclusion: Moxifloxacin induced significantly less endotoxin release compared to ceftazidime-containing and control cultures, and similarly, low levels compared to imipenem. Low-endotoxin release may be regarded as a favorable property regarding the future use of parenteral moxifloxacin in abdominal infections.

P1068 Effect of subinhibitory concentrations of gentamicin and tetracyclin on hemolytic activity of uropathogenic *Escherichia coli*

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Objectives: Hemolysins are a major virulance factor for some pathogenic strains of *Escherichia coli*. The aim of this study was to evaluate of effect of subinhibitory concentration of gentamicin and tetracyclin on hemolytic activity of uropathoganic *E. coli*.
Methods: We isolated desirable strains from patients with urinary tract infections. The hamolytic activity of these strains was measured by a standard method. By using this method, the effects of iron and calcium on hemolytic activity of the strains were investigated. Minimum inhibitory concentrations (MIC) of gentamicin and tetracyclin for these strains were determined and the effect of subMIC of gentamicin and tetracyclin (1/2, 1/4 and 1/8), alone or in combination, on the hemolytic activity was evaluated.
Results: The results showed that after elimination of free iron ions of the medium by caco3 hemolytic activity was reduced. Addition of calcium in the medium to concentration of 10 mM, induced a six-fold increase in hemolytic activity of the strains. Experiments showed that only subMICs of tetracyclin significantly reduced the hemolytic activity.
Conclusion: This study shows that tetracyclin (1/2 & 1/4) can significantly reduced hemolytic activity. Also, the combination of subMICs of gentamicin and tetracyclin (1/8 MIC) reduce the hemolytic activity.

Mechanisms of resistance in Gram-negative bacteria

P1069 Assessing neutrality in an in vitro competition model of paired mutator–nonmutator *P. aeruginosa* clinical isolates from cystic fibrosis patients

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Objective: A simple model to investigate the factors favoring selection of mutator strains in cystic fibrosis patients is proposed. The objective of this work is to establish the conditions for neutrality in the absence of selection of different mixtures of paired isolates from the same patient exhibiting either a mutator (M) or nonmutator (NM) phenotype.
Methods: Three paired M and NM *P. aeruginosa* strains from three different patients were selected. Mutation frequencies were determined on 300 μg/mL rifampin LB-agar plates. Clonal relatedness among clinical isolates was determined by using PFGE–*Xba*I technique. One colony from each isolate grown on LB-agar plate was suspended in 5 mL of LB-broth and incubated overnight at 35 °C under strong agitation. Cultures were adjusted to the same OD (when necessary), and colony counts were performed for inoculum control. Simultaneously, an aliquot of individual cultures was mixed rendering, in the final volume, the following proportions (M : NM): 10 : 90; 25 : 75; 50 : 50; 75 : 25; 90 : 10. Then, 100 μg/mL of each mixture were placed onto a nitrocellulose filter which in turn was placed on the surface of an LB-agar plate. After 18 h at 35 °C, the filter was removed, re-suspended in 1 m of saline, vortexed and the resulting suspension was diluted for plating on LB-agar plates. After overnight incubation, 100 colonies were re-streaked on LB-agar plates with and without 300 μg/mL of rifampin to analyze if initial proportions were maintained.
Results: Initial proportions of paired M and NM *P. aeruginosa* strains in the different assayed mixtures were maintained in repeated independent experiments. However, in one pair of strains tested, a prolonged (48 h) incubation period was necessary to ascertain this observation owing to the slower growth of the M partner strain.
Conclusions: These conditions should allow the evaluation of the in vitro behavior of M and NM *P. aeruginosa* strains when competing in different proportions under various environmental challenges.

P1070 Aminoglycoside resistance mechanisms in *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolated from different hospitals in Bratislava and western Slovakia

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Multiple antibiotic resistance in pathogens has become a major problem in nosocomial infections in many countries. Aminoglycoside antibiotics remain a mainstay in the treatment of serious bacterial infections and in combination therapy they are commonly recommended to treat infections owing to the noninducible Gram-negative pathogens. The main mechanisms of aminoglycoside resistance are production of aminoglycoside-modifying enzymes (AGME) and permeability changes. In our study, we followed production of AGME, transferability of resistance and occurrence of plasmid DNA in 42 ceftazidime-resistant clinical isolates of *Enterobacteriaceae*, which were high resistant also to of five aminoglycosides (57–94%). The second group was presented by 22 gentamicin (GEN), tobramycin (TOB) and netilmicin (NET) – resistant clinical isolates of *Pseudomonas aeruginosa* which were also resistant of amikacin (AMI) and isepamicin (ISE). Isolates were originating from different hospitals in Bratislava and western Slovakia. In all the *Enterobacteriaceae* isolates tested aminoglycoside resistance was owing to the production of AGME. In the *Pseudomonas* isolates, only two cases of non enzymatic mechanism of resistance were observed. Presence of AGME was examined directly by a radioenzymatic assay. The most frequently encountered combination in *Enterobacteriaceae* isolates was the production of AAC(6′)-I (82%), causing resistance to AMI, TOB and NET, with APH(2″) (73%), conferring resistance to GEN and TOB. In Pseudomonas isolates presence of AAC(6′)-III, causing resistance to GEN, TOB, NET, AMI and ISE in 77%, APH(2″), conferring resistance to GEN and TOB in 64% and APH(3′)-VI, causing resistance to AMI and ISE in 55% of isolates. A frequency of resistance transfer by bacterial conjugation to recipient *Escherichia coli* 3110 was found in limits of 10-2-10-9 in *Enterobacteriaceae* isolates and 10-2-10-7 in *Pseudomonas* isolates. The aminoglycoside resistance was transferable by bacterial conjugation with exception of ISE as transconjugants donors were ISE-sensitive, although presence of ISE-inactivating enzyme APH(3′)-VI has been detected in them (*Pseudomonas*). In donors and transconjugants plasmid DNA in the range of 36–57 MDa (*Enterobacteriaceae*) and 55–75 MDa (*Pseudomonas*) was observed. Resistance to ISE which has not been introduced into therapy in Slovakia yet is interesting and evidently another substrate of this enzyme – AMI, was responsible for dissemination of this resistance mechanism.

P1071 A clinical strain of *Burkholderia pseudomallei* lacking a macrolide-aminoglycoside efflux-pump

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B. pseudomallei is the causative agent of melioidosis. Typically, these bacteria are multidrug resistant, including aminoglycoside resistance, which is owing to an efflux mechanism. In the laboratory, mutant strains lacking this efflux pump, thus being aminoglycoside susceptible, have been generated. Interestingly, these strains are also susceptible to macrolide. Very rarely, those susceptible phenotypes also appear in clinical strains. (three out of >3000 isolates in one report). Here, an aminoglycoside and macrolide susceptible strain is presented, which was isolated together with two other strains of *B. pseudomallei* from sputum of a patient with cystic fibrosis. The history of the patient has been published earlier. He has undergone multiple courses of antibiotic therapy for relapsing pulmonary melioidosis. Far into his course, three different phenotypes, a mucoid, a rough and a small colony variant of *B. pseudomallei* were isolated from sputum. The small-colony variant exhibited gentamicin susceptibility. Lack of an efflux pump was suspected as the underlying mechanism and MICs for other aminoglycosides and macrolides were tested by the E-test method. MICs (in mg/L) of the small colony variant for erythromycin A (8), clarithromycin (4), gentamicin (0.25), streptomycin (4), netilmicin (1), tobramycin (0.5), and amikacin (2) were well below the MIC of the other phenotypes. The strains were identical by molecular fingerprinting. The lack of the efflux pump has not been confirmed by molecular testing. Different phenotypes of *Pseudomonas aeruginosa* with different resistance patterns in CF patients are a well-recognized phenomenon. This has been explained by heavy antibiotic use in this population with subsequent forming of resistant subpopulations. Also, small colony variants have been described, being usually more resistant than the parent strains. This is also true for described small colony variants of *B. pseudomallei*. Our case shows, that also more susceptible subpopulations may be generated in vivo. It is important to test all phenotypes of strains occurring under therapy or in relapsing disease to choose the most adequate regimen of antibiotics and to detect unusual resistance patterns.

P1072 Evidence for additional regulatory system for the MexXY efflux pump in aminoglycoside-resistant *Pseudomonas aeruginosa* strains

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Objectives: The *Pseudomonas aeruginosa* efflux pump MexX–MexY pumps out aminoglycosides, fluoroquinolones and other antibiotics. It is negatively regulated by *mexZ*. This efflux pump lacks an outer membrane protein, but can use OprM from the MexA–MexB–OprM pump. Because resistance to aminoglycosides and other antibiotics is common among strains from cystic fibrosis (CF) patients, we have studied the role of MexXY pump in aminoglycoside resistance in CF strains.
Methods: Twelve aminoglycoside resistant strains (MIC tobramycin 4–16 mg/L, amikacin 32 to >256 mg/L) were used. Strain PAO1 and two aminoglycoside sensitive CF strains were used as control. Genomic DNA was amplified by PCR for *mexZ* and the operator region between *mexZ* and *mexX* and sequenced in ABI 310 Genetic Analyzer. Total RNA was purified from the *Pseudomonas* cells. Single-strand cDNA was prepared from RNA by reverse transcriptase. The amount of cDNA for the pump protein *mexY*

was determined quantitatively by real-time PCR in the LightCycler using SYBR Green. As a standard for the amount of cDNA in each preparation, we used the *parC* gene (topoisomerase i.v. subunit C).
Results: Mutations were found in 9 of 12 resistant in *mexZ*, leading to frameshift or amino acid substitutions. A mutation was found in one of the sensitive CF strains (Leu4 to Pro), probably not significant. Mutations were also found in the operator region in four strains. The amount of mRNA for MexY (pump protein) was significantly higher (>100 × PAO1) in three of the resistant strains but also in one strain, fully sensitive to aminoglycosides, ciprofloxacin and other anti-*Pseudomonas* antibiotics. We found no mutations in *mexZ* or operator region in one resistant strain with over-expression of *mexY*.
Conclusion: Overproduction of the efflux pump MexXY cannot be the only explanation for resistance to aminoglycosides in *Pseudomonas* strains from CF patients, because increased mRNA for the pump protein MexY was detected in three of 12 strains only. The sensitive strain with over-expression of MexY may have a nonfunctional efflux pump. Also, because no mutations in *mexZ* or operator region were found for one strain with over-expression of *mexY*, we must assume that there are additional regulatory functions for the *mexX–mexY* genes.

P1073 Partial characterization of a transposon containing the *tetA* gene in an *Acinetobacter baumannii* clinical isolate

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Objective: The objective of this study was to analyze the molecular mechanisms of resistance to tetracycline in one clinical isolate of *A. baumannii*.
Methods: To achieve this aim, a genomic library of the afore-mentioned strain was obtained. Briefly, genomic DNA was relieved and partially digested with an enzyme of a high frequency of cut (*Sau*3AI). Fragments ranging from 4 to 9 kb were cloned into an expression vector (pBSK) which was further transformed in DH5-α *Escherichia coli* strain, susceptible to a great variety of antibiotics. Resistant strains were selected with Mueller–Hinton plates supplied with 18 mg/L of tetracycline. The resistant clone was characterized and the whole insert was then sequenced. Moreover, the MICs of tetracycline in both the wild-type and the transformed *E. coli* strains were performed by E-test.
Results: One clone resistant to tetracycline was obtained. The clone contained an insert of 5859 bp which was thoroughly characterized. It was found that the resistance to this antibiotic was owing to the *tetA* gene which encodes for a tetracycline efflux pump. Together with the *tetA* (1199 bp), *tetR* (650 bp), the *tetA* repressor, was cloned. Moreover, the partial sequence (2019 bp of a total of 2972 bp) of a transposase gene, *tnpA*, and 1340 bp corresponding to an IS similar to that of *Salmonella typhi* (IS4321) which included an IR and another transposase, were found. The activity of the gene responsible for tetracycline resistance in the *E. coli* strain was demonstrated by calculating the MIC of this antimicrobial agent. Whereas the MIC of tetracycline in the wild-type strain was 0.75 mg/L, the MIC in the transformed strain was 256 mg/L.
Conclusion: In this work, the presence of the *tetA* gene (encoding for an efflux pump, and responsible for tetracycline resistance) is located in a transposon carried by an *A. baumannii* clinical isolate. Moreover, owing to the high homology, almost of 100%, among the genes found within the insert and those described in other Gram-negative bacteria, an horizontal transference is suggested.

P1074 Chloramphenicol sensitivity of some *Escherichia coli* cat-expressing strains

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Objectives: *Escherichia coli* CM2555 strain was recently shown to be sensitive to chloramphenicol when expressing cat, the chloramphenicol resistance gene. A functional resistance protein, chloramphenicol acetyltransferase (CAT), was synthesized in this strain. The strain phenotype seemed to be linked to the CAT mechanism of action – acetylation of chloramphenicol with a simultaneous de-acetylation of acetyl coenzyme A (Acetyl CoA). In the course of genetic mapping experiments we discovered that the CM2555 strain carries a nonsense mutation in *acrA*, a gene encoding a component of *acrAB*–TolC efflux system. The goal of this study was to investigate the impact of the mutation on chloramphenicol sensitivity of the cat-carrying CM2555 strain.
Methods: *acrAB* and *acrEF* deletion strains were generated using P1 generalized transduction. Plasmids carrying the *acrA*, *acrE* and *acrEF* genes (analogs of *acrA* and *acrAB*, respectively) were constructed from pUC19 cloning vector. Intracellular acetyl coenzyme A levels were determined by a spectrophotometric assay.
Results: Introduction of *acrA*, *acrE* and *acrEF* genes on multiple copy plasmids into the cat-carrying CM2555 strain reversed the sensitivity phenotype. Earlier we have shown that the level of Acetyl CoA decreases in cat-carrying CM2555 strain after the addition of chloramphenicol to the growth medium. When the strain carried efflux pump genes on a plasmid, the decrease in Acetyl CoA levels was not observed. The *acrAB* deletion rendered CM732 (CM2555 parental strain) and C600 cat-expressing strains sensitive to chloramphenicol. The sensitivity phenotype was accompanied by a decrease in levels of Acetyl CoA in the presence of chloramphenicol. The phenotype was reversed by introduction of *acrEF* on multicopy plasmid. The deletion did not affect cat-mediated chloramphenicol resistance of MG1655 and MC1061 strains. Deleting *acrEF* had no effect on the resistance phenotype of either strain.
Conclusions: The *acrA* gene product seems to affect the chloramphenicol sensitivity of CAT producing CM2555 strain by influencing intracellular Acetyl CoA levels. The deletion of *acrAB* might sensitize some other cat-carrying *E. coli* strains, such as C600, to the antibiotic. In this case, over-expression of both efflux-pump components is necessary to reverse the phenotype. Further investigation is needed to determine molecular basis of this phenomenon.

P1075 Physiological alterations associated with fusidic acid resistance cost

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Objectives: To explain the reduced virulence of fusidic acid-resistant (*FusR*) mutants. To elucidate the costs associated with bacterial resistance to fusidic acid in vivo. To create a synthetic medium that mimics the in vivo conditions relevant for the cost of fusidic acid resistance in *S. typhimurium*.
Methods: Fitness measurements (growth rates, competitive ability and survival) of *FusR* mutants in synthetic media. Fitness measurements in vivo in B6 wild-type and n-fox mice. Quantification of catalase and ppGpp levels in *FusR* mutants.
Results: Fusidic acid resistance is caused by mutations in a component of the protein synthesis machinery, elongation factor-G (EF-G). These mutations reduce the affinity of the antibiotic for its target. Observed phenotypes of *FusR* mutants of *S. typhimurium* include avirulence in a mouse infection model. In this study, hydrogen peroxide is identified as the major factor that imposes this fitness cost on *FusR* mutants in vivo. Co-sensitivity to superoxide or nitric oxide is not observed. Catalase levels are reduced in *FusR* mutants and this can account for their reduced survival in the presence of hydrogen peroxide in vitro and in macrophage.
Conclusions: The results suggest a direct link between *FusR* mutations and transcriptional regulation of catalase levels. This transcriptional effect is mediated via a ribosomally synthesized global transcription factor ppGpp. The reduced levels of catalase produced in *FusR* bacteria can explain their decreased ability to survive and proliferate in the intracellular environment in which they are exposed to an oxidative burst that generates hydrogen peroxide.

P1076 Analysis of *penA* gene in *Neisseria meningitidis* isolates with reduced susceptibility to penicillin

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Objectives: The aim of this study was to analyze the *penA* sequence, which encodes the penicillin-binding protein 2 (PBP2), in 28 *N. meningitidis* clinical isolates, 24 with decreased susceptibility to penicillin (MIC > 0.06 μg/mL, *penI*) and four susceptible (MIC < 0.06 μg/mL, *penS*) used as control, in order to assess the relationship between alterations in the gene and reduced susceptibility to penicillin.

Methods: Meningoccoccal strains sent to the Reference Laboratory of the Istituto Superiore di Sanità were serotyped and tested for susceptibility to penicillin by E-test. MICs between 0.06 and 1 μg/mL indicated strains with decreased susceptibility to penicillin. The *penA* gene was amplified by PCR using already known oligonucleotides. The 2.1 Kb amplicons were analyzed by RFLP using different restriction enzymes and were sequenced.
Results: Among the 24 *penI N. meningitidis* isolates, 12 showed nucleotide alterations, in particular in the region coding for the transpeptidase domain of PBP2, caused by horizontal transfer of DNA from other commensal *Neisseriae*. In particular, *N. cinerea* was the most frequently found (8/12); *N. perflava/sicca* was recovered at the 3'-end of the gene and in four strains *N. flavescens* was found. In one strain, without translocations, a duplication of 54 nucleotides was found at the 3'-end of the gene. These polymorphism were analyzed by investigating restriction endonuclease patterns of the *penA* gene. The *penI* strains showed nine different patterns whereas two patterns were found among the *pens* as well as among the *penI* strains without any DNA translocations.
Conclusions: Modifications in PBP2 that may affect the affinity for penicillin result from changes in *penA* gene. This gene has been already reported to be polymorphic in meningococcal *penI* strains. This study on 24 strains with decreased susceptibility to penicillin suggests that the *penI* phenotype does not correspond to a single *penA* genotype. A decreased susceptibility to penicillin is only partially related to translocations in the gene from corresponding DNA regions of commensal *Neisseriae*. The polymorphism of this gene is not sufficient to characterize all the *penI* meningococci and other targets should be further investigated.

In vitro activity of various drugs

P1077 Moxifloxacin: determination of the in vitro activity against bacterial isolates from the skin and soft tissues to assess its value for clinical use

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Objectives: Aim of the project was to determine the range of pathogens in wound swabs from skin and soft tissues on a patient basis, to ascertain the in vitro susceptibilities of the isolated bacteria and to assess the value of moxifloxacin for clinical use.
Methods: Bacterial isolates from 100 wound swabs (skin and soft tissues, nonsterile, patients >15 years) were tested for resistance with the microdilution-method. Additional Moxifloxacin susceptibility-testing with E-test. Online collection of clinical data to assess the infectious status and the renal function of the patients.
Results: Isolation of 179 bacterial strains (*S. aureus* 26%/$n=47$, *S. aureus* (MRSA) 3% ($n=5$), coagulase-negative staphylococci (CNS) 13% ($n=24$), enterococci 16% ($n=29$), streptococci 7% ($n=12$), corynebacteria 3% ($n=6$), *Enterobacteriaceae* 20% ($n=35$), *Pseudomonas* spp. 8% ($n=14$), *Acinetobacter* spp. 4% ($n=7$); strictly anaerobic organisms were not considered within this study). The overall susceptibility for moxifloxacin was 89% as well for the Gram-positive as for the Gram-negative strains (95% excluding *Pseudomonas* spp.). A total of 53 swabs showed growth of 1 strain, 47 of at least 2 strains (up to 5). Wounds from which only one strain was isolated were more often associated with *S. aureus* than wounds with at least two strains, i.e. foot or decubital wounds with mean values of 2.4 strains per swab. A total of 43 isolates (18 cases) were from these two locations. 38 (88%) of them showed susceptibility against moxifloxacin. Patients were 17–93 years old, about the half (52%) had a reduced renal function. A total of 64 of them needed systemic antibiotic treatment.
Conclusions: Considering the suitable properties of moxifloxacin as wide range of efficacy, high oral bioavailability and very high tissue-penetration infections of the skin and soft tissues seem to be another area for use of moxifloxacin. This is confirmed by the results of the present susceptibility tests for leading staphylococci, streptococci and *Enterobacteriaceae*. Even chronic wounds like 'diabetic feet' or decubiti, often starting point of systemic infections, seem to be an indication for a moxifloxacin-regime in antibiotic therapy.

P1078 Streptidine is released from streptomycin by enzymatic hydrolysis in the human serum

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Objectives: It has been speculated in the past whether streptomycin (STP) acts by complete or broken down the damaged hair cells of the inner ear. Therefore, its hydrolysis by human-serum components was investigated by incubating STP in it, and separating the resulting compounds by high performance liquid chromatography.
Methods: In vitro enzymatic hydrolysis was performed by incubation of STP 0.1 mM in serum at 37 °C for 24 h. The serum was then deproteinized with 20% trichloroacetic acid, and the supernatant was analyzed using a paired-ion, reversed-phase chromatography in combination with a diode detector.
Results: We found that after in vitro incubation of STP in the presence of human serum, the presence of a metabolite that matched the peak of the streptidine (STD) portion of the molecule of STP linked to it via and α- 1,4-glycosidic bond was observed. The initial concentration of STP before incubation was of 0.1 mM and after incubation this concentration decreased to 0.079 mM, the STD signal from a standard curve was 8–8.5 μM. Control samples consisted of 0.1 mM of STP solutions, in phosphate buffer (50 mM, pH 6.8), 20% trichloroacetic acid solution and the supernatant of deproteinized serum. The three solutions were incubated in the same conditions as the experimental sample. In all the controls, were found 0.099 + 0.002 mM of STP. Except for the buffer controls, in which a concentration of 2 μM of STD was detected.
Conclusions: Aminoglycoside antibiotics, such as STP, can be vulnerable to suffer some metabolic changes in contact with blood. This fact refutes the idea that these kinds of antibiotics are eliminated from the organism without suffering any kind of degradation, and stresses the notion that its metabolites instead of the complete structure, may be the actual ototoxic compounds. With this discovery, new frontiers of research in the vestibular toxic effects of STP are opening.

P1079 In vitro activity of moxifloxacin against bacteria isolated from odontogenic abscesses

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Objectives: Odontogenic abscesses are caused mainly by mixed infections owing to facultative anaerobes and anaerobes. Moxifloxacin (MXF) is a new 8-methoxyquinolone with a broad activity against a wide range of microorganisms including streptococci and anaerobes. The aim of our study was to analyze the in vitro activity of moxifloxacin in odontogenic pathogens in order to estimate the hypothesized role of MXF in treatment of odontogenic abscesses.
Methods: In a prospective study we evaluated the antimicrobial susceptibility of all pathogens isolated from 41 swabs of 37 patients with odontogenic abscesses. The minimal inhibitory concentrations (MICs) of all bacterial isolates for penicillin (PEN), amoxycillin/clavulanic acid (AMX/CLA), clindamycin (CLI), doxycycline (DOX), levofloxacin (LVX), and MXF were determined with E-test. Additionally, as a pharmacodynamic parameter the ratio of the peak serum level C_{max} to the MIC was calculated for all antibiotics tested. C_{max}/MIC values of >8 are predictive for clinical efficacy especially among fluorochinolones.
Results: In total, 90 bacterial pathogens were isolated of which 87 pathogens (51 facultative anaerobes and 36 anaerobes) could be subcultivated for MIC determination. The most prevalent bacteria were different viridans streptococci with 38 isolates and *Prevotella* sp. with 31 isolates. Considering all bacterial isolates the lowest MIC_{90} values were obtained for MXF and AMX/CLA (0.5 mg/L each) followed by LVX (2 mg/L), PEN (8 mg/L), DOX (16 mg/L), and CLI (256 mg/L). According to recent NCCLS breakpoint criteria 98% of the isolates were susceptible to MXF and LVX. Comparable activity was observed for AMX/CLA with 95% susceptible isolates, whereas a

lower activity was observed for DOX, CLI, and PEN with only 76, 75, and 69% susceptible isolates. The highest C_{max}/MIC_{90} values as predictive for clinical cure were calculated for MXF and AMX/CLA (nine and seven) in contrast to the lower C_{max}/MIC_{90} values for LVX, PEN, DOX, and CLI (2.9, 0.8, 0.2, and <0.1).
Conclusions: Moxifloxacin provides superior in vitro activity and promising pharmacodynamic properties in odontogenic pathogens compared to usually employed antibiotics. Thus, moxifloxacin may be an alternative for antimicrobial therapy of odontogenic abscesses that should be evaluated in clinical trials.

P1080 Antimicrobial susceptibility and causative agents of hospital- and community-acquired urinary tract infections (UTI)

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Objectives: To establish the causative agents of hospital acquired and community acquired UTI and the antimicrobial susceptibility of isolated urinary pathogens.
Methods: Between November 2000 and October 2001, all urine isolates from hospitalized patients in Latvian Infectology Center and Hospital of Traumatology and Orthopedics were collected and also from community acquired patients. Cultures of urine samples were performed by isolation method of CHROM agar Orientation. Isolates were identified to the species level using the SCEPTOR and Crystal systems. Antimicrobial susceptibility tests to more then 20 antibiotics were performed using the disk diffusion and SCEPTOR MIC method. β-Lactamase activity was tested using cefinase disks.
Results: A total of 1015 isolates from urine cultures were analyzed. There were three groups of causative agents: (1) Gram-positive cocci- 496, from them *E. faecalis* – 233, *E. faecium* – 38, *E. durans* – 1, *S. epidermidis* group – 137, *S. saprophyticus* – 45, *S. aureus* – 20, *S. sciuri* – 3, *S. simulaus* – 2, *Streptococcus agalactiae* – 15, *Streptococcus canis* – 2; (2) Gram-negative rods: the family *Enterobacteriaceae* – 452, from them *E. coli* – 314, *Klebsiella–Enterobacter–Serratia* (KES) group – 92, *Citrobacter freundii* – 13, *Proteus vulgaris* – 8, *Proteus mirabilis* – 17, *Providencia rettgeri* – 3, *Morganella morganii* – 4, *Hafnia alvei* – 1; (3) Gram-negative nonfermenters – 67 from them: *P. aeruginosa* – 37, *P. putida* – 6, *Acinetobacter baumanii* – 14, *Acinetobacter lwoffi* – 10. The antimicrobial susceptibility of UTI causative agents: 254 strains (40.1%) of hospital patients and 56 strains (14.7%) of community patients were polyresistant. In hospitalized patients strains polyresistance was mainly registered of genus *Pseudomonas* – 86.2%, of KES group – 55.1%, of genus *Staphylococcus* – 35.2%. In community acquired UTI patinets, polyresistant strains prevailed in genus *Pseudomonas* – 85.7% in KES group – 34.8%, in genus *Staphylococcus* – 29.5%.
Conclusion: The most common pathogen isolates from urine cultures in both the examined two hospitals and community acquired UTI were *Escherichia coli, Enterococcus faecalis*, coagulase negative *Staphylococci* and KES group. Polyresistance in the hospitals was higher than in community acquired UTI, except genus *Pseudomonas*.

P1081 In vitro activity of piperacillin/tazobactam and comparator antibiotics against bacteria from hospitalized patients in the British Isles

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Objectives: Piperacillin/tazobactam is a β-lactam/β-lactamase inhibitor combination active versus clinically important Gram-negative and Gram-positive bacteria. We assessed its comparative in vitro activity versus contemporary isolates from hospitalized patients in the British Isles. Results were compared with those of a similar survey run in 1991, immediately before launch of piperacillin/tazobactam.
Methods: Twenty-seven British and Irish hospitals each collected up to 200 consecutive, clinically significant isolates. The organisms were identified by standard laboratory methods or with API kits, according to the species. Disc-susceptibility tests were performed at the collecting laboratories by the British Society for Antimicrobial Chemotherapy's method. For quality control, a 5% sample of the isolates, plus those with key resistance, were subjected to MIC tests centrally.
Results: Compared with the 1991 survey and based on the first 2500 isolates, *S. aureus* was more prevalent (36% of all isolates cf. 25%). Much of this increase reflected the dramatic emergence of MRSA (16.4% of all isolates in 2001, cf. 0.7% in 1991). *P. aeruginosa* was also more prevalent (9.3% in 2001 cf. 6.2% in 1991), whilst *E. coli* was less so (17.7% in 2001 cf. 26.6% in 1991). As in 1991, >95% of the isolates of *P. aeruginosa, Bacteroides* spp., *Streptococci, Pneumococci* and *E. faecalis* were susceptible to piperacillin/tazobactam, as were 87% of *Acinetobacter* spp. and >85% of Enterobacteriaceae. Nevertheless, more resistance to piperacillin/tazobactam was seen in *E. coli* than in 1991 (11% cf. 4%) and *Klebsiellae* (16% cf. 5%), but not in the *Citrobacter, Enterobacter, Morganella, Serratia* group (17% in both years). Amongst the comparators meropenem remained the most potent against enterobacteria, with near universal activity. Ceftazidime and gentamicin were similarly active as in 1991, but the activity of ciprofloxacin had been compromised vs. enterobacteria (86% susceptible in 2001 cf. 96% in 1991).
Conclusions: The main difference between the surveys in 1991 and 2001 was the dramatic rise in of *S. aureus* in general and MRSA in particular. This indicates a major shift in the patterns of infection. Other changes in resistance were subtler. Although there was some increase in piperacillin/tazobactam resistance amongst *E. coli* and *Klebsiellae*, the combination remains a potent antibiotic with a broader spectrum than any other noncarbapenem agent.

P1082 Checkerboard and time-kill methods for analysis of polymyxin B with second antibiotic combinations

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Objective: Polymyxin B with other antibiotic combined in some composed topical preparations exists on Polish market. Such multiantibiotic drugs might be active against different microorganisms: Gram-positive as well as Gram-negative. In this situation different targets in microbial cells are under focus and antibiotics could change their physical structure and biochemical properties e.g. ribosomes – aminoglycosides and tetracyclines as well as cell membrane – peptide antibiotics. Checkerboard and time-kill methods are the most widely used techniques to assess interaction between two agents. The aim of this study was to analyze the activity interactions of polymyxin B with the other antibiotics (neomycin, framycetin and oxytetracycline) combinations on standard bacterial strains used for the drug control and on some clinical isolates.
Methods: Using checkerboard technique, serial dilutions of two antibiotic standards were prepared and combinations of six concentrations proportional to the MIC values (from 1/4 × MIC to 2 × MIC) of the both drugs were analyzed.
Standard strains: *B. bronchiseptica* ATCC 4617, *M. luteus* ATCC 9341, *S. aureus* ATCC 6538P, *B. subtilis* ATCC 6633, *B. cereus* ATCC 11778 and *B. pumilus* ATCC 14884 as well as clinical isolates of *E. coli, P. aeruginosa, K. pneumoniae, Salmonella* sp., and *S. aureus* (MRSA and MSSA) were under investigation. Fractional Inhibitory Concentration (FIC) indexes for combinations of two antibiotics were also calculated from the checkerboard data.
Results: Investigating all standard strains, synergism for 33.3% of combinations was observed. However, additive effects were the predominant (47%), in three cases indifference effect was also noticed. No inhibition effect was detected when polymyxin B with each of three antibiotics was analyzed. In case of standard strains, *S. aureus, B. cereus* and *M. luteus* synergy was observed in each polymyxin B combinations. The lowest – 0.12–0.18 FIC values were present in case of polymyxin B and oxytetracycline combinations. Time-kill method was used for synergy analysis of polymyxin B with neomycin, framycetin and oxytetracycline combinations, against *M. luteus* ATCC 9431 strain. Effect of antibiotics combined in concentrations: 1 × MIC, 0.5 × MIC, and concentrations calculated from FIC indexes,

after 1, 2, 4, 8, and 24 h incubation on micrococcal growth, was analyzed. Synergy effect detected by checkerboard method was confirmed by time-kill assay.

P1083 Interaction of neomycin and the other antibiotic combined in multiantibiotic drugs

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Objective: Antimicrobial combinations are used most frequently to provide broad-spectrum empirical coverage in the treatment of bacterial infections. However, combination of two antibiotics may not influence their activity, may lead to synergy or antagonism in the activity. Neomycin is combined with one of the following antibiotics: ampicillin, procaine penicillin, polymyxin, lincomycin, oxytetracycline, erythromycin, gramicidin and bacitracin in some human and veterinary multiantibiotic drugs marked in Poland. The aim of this study was to analyze the activity interaction of neomycin with other antibiotic combination on standard bacterial strains used for drug control and on some clinical isolates.
Methods: Checkerboard technique was used. Serial dilutions of two drugs were performed using six antibiotic concentrations proportional to MICs (from 1/4 × MIC to 2 × MIC) of the drugs being tested.
Standard strains: *B. bronchiseptica* ATCC 4617, *M. luteus* ATCC 9341, *S. aureus* ATCC 6538P, *B. subtilis* ATCC 6633, *B. cereus* ATCC 11778, and *B. pumilus* ATCC 14884 as well as clinical isolates of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Salmonella* sp., and *S. aureus* (MRSA and MSSA) were under investigation. Fractional inhibitory concentration (FIC) indexes for combinations of two antibiotics were also calculated from the checkerboard data.
Results: Analyzing growth of particular bacterial strains in the presence of different neomycin concentrations combined with each of eight other antibiotics, additive effect was observed in 57.4%, indifference in 29.8% and synergy in 12.8% of combinations. Greatest synergy was observed when aminoglycoside – neomycin was combined with peptide antibiotics – polymyxin B (FIC = 0.31) and gramicidine (FIC = 0.19) and analyzed with the application of *M. luteus* strain. Also, in case of *B. cereus*, synergy was observed in neomycin/bacitracin (FIC = 0.31) and neomycin/gramicidin (FIC = 0.5) combinations. Analyzing results of all standard strains, lack of interaction or only additive effect was observed, when neomycin was combined with ampicillin, procaine penicillin, oxytetracycline and erythromycin. No any synergy was noticed when *B. bronchiseptica* and *B. pumilus* strains were analyzed. In case of neomycin and other antibiotic combinations against clinical isolates, no synergy effect was observed. Most often additive interaction was noticed. In few cases (resistant strains), determination of MIC and calculation of FIC values showed to be impossible.

P1084 In vitro susceptibility of *Bartonella* spp. against telithromycin and other newer antimicrobial compounds

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Macrolides are first-line antibiotics in treating diseases caused by *Bartonella* spp., based on clinical grounds. Only limited susceptibility data are available, owing to the fastidious nature of these microorganisms. We tested the MICs of 31 strains of *Bartonella* spp. (21 *B. henselae*, 2 *B. quintana*, and one of each *B. elizabethae*, *B. tribocorum*, *B. alsatica*, *B. vinsonii* spp. *vinsonii*, *B. vinsonii* spp. *arupensis*, *B. schoenbuchii*, *B. doshiae* and *B. grahamii*) against telithromycin, a new ketolide antibiotic, four macrolides (erythromycin, roxithromycin, clarithromycin, azithromycin), three fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), five aminoglycosides and rifampin. Agar-dilution was performed on chocolate-agar with a final inoculum of 10^6 cfu/spot, incubated at 37 °C in 5% CO_2. Results were read after 3–4 days of incubation and were as shown in the table above. Telithromycin was the most active agent exhibiting the lowest MICs. All strains had macrolide MICs lower than 0.25 mg/L. Moxifloxacin was the most potent fluoroquinolon tested. Cat-scratch disease is the most frequent clinical presentation of *Bartonella* infections and is usually a self limiting disease. In immunocompromised patients (especially HIV-patients) infections caused by these agents may persist for a longer time and a lifelong treatment or prophylaxis may be indicated. Although the clinical value ofin vitrosusceptibility testing for *Bartonella* strains remains uncertain, these data may be useful for therapeutic interventions in these diseases as well as for monitoring development of resistance in some isolates.

		MIC (mg/L)													
Antibioticum	*n*	<0.002	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
Telithromycin	29	28				1										
Errythromycin	26			2	5	7	4	4	4							
Roxithromycin	30			3	2	4	6	9	6							
Clarithromycin	31	19		3	5	4										
Azithromycin	30				8	15	4	3								
Gentamicin	25									1	1	13	6	3	1	
Tobramycin	26									1	1	1	10	11	2	
Amikacin	29												8	14	6	1
Streptomycin	26								1			1	10	7	7	
Netilmicin	26									3	16	6		1		
Ciprofloxacin	27									2	7	16	2			
Levofloxacin	24									1	1	21	1			
Moxifloxacin	29								1	5	19	4				
Rifampin	30				14	7	8	1								

P1085 Antibacterial properties of propolis (bee glue) against aerobic filamentous bacteria (*Nocardia*)

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One of the most useful products of the beehive is 'propolis', a resinous substance that is collected by bees from buds. Propolis has been used in ancient time for the treatment of malignant tumors and injuries. These observations attracted the attentions to the antibacterial properties of this substance. The effect of propolis on bacteria could be related to its high percentage of flavonoid content and also the presence of caffeine acid esters. In regard to important role of infectious disease in treating the life of people, and also inaccessibility to useful drugs with minor side-effects, we decided to consider the ability of ethanol extract of propolis in preventing the growth of bacteria. For this purpose, we chose two strains of *Nocardia* (*N. asteroides* and *N. brasiliensis*) as original bacteria and other six species (*P. aeroginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *E. cloaceae*, and *S. flexenery*) as elective organisms. Because of the influence of propolis in the treatment of both cutaneous and respiratory disorders, the effication of propolis on these bacteria has been studied. After preparing seven concentrations of ethanol extract of propolis in 80% Methanol, and culturing the organisms in their specific culture media, we tempted the antibacterial properties of propolis by means of four methods of distribution in agar (Drop-plate, Disk-plate, well-plate and cylinder-plate). After the distribution of propolis extract in agar, the diameter of the clear zone formed has been measured, which is related to the ability of extract to prevent the growth of bacteria. In according to the results, which is insert in tables, *N. asteroides* and *N. brosiliensis*, has nearly shown the same susceptibility to various concentrations of propolis extract, and the complete clear zones reveal that this effect has been completed. As for other bacteria, the effect of propolis was complete in two cases (*S. aureus* and *E. coli*) and for the other four bacteria the effect was the same and also less than the two mentioned before. In addition, we came to this conclusion that zones formed by Amikacin 50 mg/mL in each four routs of distribution in agar was equal to that of 5% concentration of propolis, and that the potency of propolis is 80% of Amikacin potency. (Which is the most effective antibiotic against *Nocardia*).

Pharmacokinetics/Pharmacodynamics

P1086 Studies of the selection of resistant *Streptococcus pneumoniae* by benzylpenicillin in an in vitro kinetic model

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Introduction: Little is known about the selective effect of different antibiotic concentrations on a mixed culture of bacteria with different degrees of susceptibilities. Dosing regimens should be optimized to obtain maximal efficacy and reduce the risk for emergence of resistance.
Objectives: The aim of the study was to investigate the selective effect of different concentrations of benzylpenicillin (Pc) in a culture of *Streptococcus pneumoniae* containing 90% of sensitive (PSP) (MIC = 0.015 mg/L), 9% of intermediate (PIP) (MIC = 0.25 mg/L) and 1% of resistant (R) PRP (MIC = 4 mg/L) using an in vitro kinetic model.
Methods: The culture was exposed to Pc at C_{max} of 0.33, 1.5, 2.9, 10.2 and 53.5 mg/L, respectively, in order to obtain different T > MIC for the included strains. Samples were withdrawn at 0, 3, 6, 9, 12 and 24 h and seeded on blood agar plates containing penicillinase, 0.062 mg/L Pc and 1 mg/L Pc.
Results: Maximal growth was obtained at 9 h and all controls had by then increased 3 log 10 cfu. At 24 h, PIP had decreased from 10 to 1% of the total amount and PRP from 1 to 0.1%. At C_{max} of 0.33 mg/L, T > MIC were 67, 6 and 0% for PSP, PIP and PRP, respectively. At this concentration, PSP and PIP disappeared after 6 h. However, a re-growth of PIP was seen at 24 h. At C_{max} of 1.5 mg/L, T > MIC for PSP, PIP and PRP were 100, 38 and 0%. PSP disappeared after 3 h and PIP after 6 h. At this concentration, PRP increased 7.2 log 10 cfu/mL at 24 h. At C_{max} 2.9 mg/L, T > MICs were 100, 54 and 0%, respectively. PSP and PIP disappeared after 6 h, whereas PRP increased steadily during the 24 h. At 10.2 mg/L, T > MIC were 100, 83 and 18%. All strains disappeared at 6 h but a re-growth was noted at 24 h for PRP. At the highest concentration with T > MIC of 100, 100 and 56%, respectively, all strains disappeared already at 3 h.
Conclusion: A selection of the non-susceptible strains of *S. pneumoniae* occurred in the in vitro kinetic model when the bacterial population was challenged with Pc except for the highest concentration. In our experiments T > MIC of approximately 38–56% was required to obtain efficacy against the populations with decreased susceptibility to penicillin. Sub-optimal antibiotic dosing regimens may be a risk factor for the emergence of resistance in the normal flora or at infection sites with pre-existing resistant subpopulations.

P1087 Investigation of the effects of ranitidine and sucralfate on the bioavailability of ABT-773 in healthy volunteers

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Objectives: ABT-773 is a new ketolide antibiotic which has potent activity against penicillin- and macrolide-resistant Gram-positive bacteria. Concomitant administration of ABT-773 with ranitidine or sucralfate might lead to delayed or reduced absorption in the upper gastrointestinal tract, through insolubility at increased pH (ranitidine) or formation of chelate complexes (sucralfate).
Methods: The pharmacokinetics (PK) and interaction of ABT-773 and its metabolite M-1 were assessed in a crossover trial with 12 healthy volunteers (age: 32 ± 5.7 years, weight: 79.0 ± 11.2 kg, creatinine clearance: 121.5 ± 25.5 mL/min/1.73 m^2) receiving 150 mg of ABT-773 alone or concomitantly with ranitidine or sucralfate. Plasma samples obtained at different time points were analyzed by liquid chromatography–mass spectrometry. Urine recovery was measured by validated bioassay. Statistical and pharmacokinetic analysis including estimation of the 90% confidence interval was performed by WinNonlin Pro 2.0.
Results: The PK data of a single oral dose of ABT-773 alone were as follows (arithmetic means ± SD): C_{max}: 318 ± 161 ng/mL at 1.79 ± 0.5 h (T_{max}); $t_{1/2}$: 5.66 ± 00.77 h; AUC0-Ë: 1662 ± 907 ng h/mL; total urine recovery (24 h): 13.67 ± 4.7%. The C_{max} and AUC were statistically significantly reduced by 25.7 and 15.8%, respectively, and T_{max} and $t_{1/2}$ were prolonged, owing to a delayed and decreased absorption when ABT-773 was co-administered with ranitidine. Statistical analysis revealed no impact of sucralfate on the bioavailability of ABT-773.
Conclusion: In conclusion, in our study there was a significant interaction between ABT-773 and ranitidine but not between ABT-773 and sucralfate.

P1088 Examination of antimicrobial activity of selected nonantibiotic drugs

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Objective: A variety of pharmaceutical preparations, which are applied for the treatment of noninfectious diseases, have shown in vitro some antimicrobial activity. These drugs are called 'nonantibiotics'. The aim of this study was to detect and characterize the antimicrobial activity of nonantibiotic drugs selected from the preparations analyzed during the state control performed in the Drug Institute in Poland.
Methods: About 100 pharmaceutical preparations were randomly chosen from different groups of drugs. The surveillance study was performed on standard microbial strains used for drugs control: *S. aureus* ATCC 6538P, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, and *C. albicans* ATCC 10231. The sterile blotting-paper discs were soaked with 10% (v/v or w/v) solution of tested drug in 0.08 M phosphate buffer pH 7 and placed onto Mueller–Hinton 2 Agar plates, inoculated with standardized cells suspension (0.5 GMC Farmland scale) of suitable strain. The inhibition of bacterial growth was observed as a halo around the disc containing the tested compound. MIC was calculated for the most interesting cases.
Results: It was shown, that the drugs listed below inhibited growth of at least one of the examined strains: acyclovir (awirol 100 mg), butorphanole (butamidor 10 mg/mL, amp.), emadastine (emadine 0.05% eye drops), fluvastatine (lescol 40 mg), ketamine (ketamidor 10%, amp.), clodroniate sodium (sindronat 400 mg, caps.), levocabastine (histimet 0.5 mg/mL, eye drops), oxaprosine (reumax 600 mg), oxymethazoline (nasivin 0.05%, nose drops), proxymetacaine (alcaine 0.5%, eye drops), rutozide with ascorbic acid (cerutin 20 + 200 mg), tegaserole (zelmac 50 mg), telmitarzane (pritor 20 mg), tramadole (tramundin 100 mg), tropicamide (tropicamidum 1%, eye drops). *Staphylococcus aureus* was susceptible to most of the drugs listed above. Levocabastine and emadastine inhibited growth of this microorganism in concentration 0.02 mg/mL and tegaserole – 0.08 mg/mL. Fluvastatine showed activity against *S. aureus* and *C. albicans* in concentration 0.4 mg/mL, oxymetazoline showed activity against *S. aureus* and *E. coli* (MICs: 0.005 and 0.025 mg/mL, respectively). Growth of *E. coli* was also inhibited by butorphanole (MIC – 0.9 mg/mL) and oxymethazoline (MIC – 0.025 mg/mL). *Pseudomonas aeruginosa* was sensitive to oxaprozine, clondroniate sodium and tramadole (MICs: 60, 63, and 43 mg/mL, respectively).

P1089 Pharmacodynamic studies in an in vitro kinetic model of a new pharmacokinetically enhanced formulation of amoxicillin against *Streptococcus pneumoniae* with different susceptibilities

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Objectives: The efficacy of amoxicillin (AMX) is mainly dependent on the time that the free concentration stays above the MIC (T > MIC). However, the exact fraction of the dosage interval during which this concentration should be exceeded for optimal efficacy is not known. The aim of the present investigation was to study the pharmacodynamic effects of a new formulation of AMX against different strains of *Streptococcus pneumoniae* in an in vitro kinetic model.
Methods: Four clinical isolates of *S. pneumoniae* with MICs of 1, 2, 4 and 8 mg/L, respectively, were exposed to AMX simulating the concentrations obtained in humans of the new formulation of AMX containing 1125 mg (immediate release) and 875 mg (slow release). Doses were given at 0 and 12 h. Bacterial counts were followed for 24 h.

Results: The T > MIC values for the investigated strains were 75, 64, 56 and 48%, respectively. AMX exerted a complete bactericidal effect after the first dose against the strain with a MIC of 1 mg/L. Against the strain with a MIC of 2 mg/L, re-growth was noted after the first dose but no re-growth was seen after the second dose. For the strains with MICs of 4 and 8 mg/L and T > MIC values of 56 and 48%, respectively, re-growth was seen after both the first and second dose. However, at 24 h the log(10) cfu/mL of the strain with a MIC of 4 mg/L was only 10^2–10^3 cfu/mL compared with 10^6–10^7 cfu/mL for the strain with a MIC of 8 mg/L.
Conclusion: In this model, the new formulation of AMX with a prolonged T > MIC was effective against *S. pneumoniae* with MICs up to 2 mg/L when given twice daily and resulted in significant killing of strains with a MIC of 4 mg/L. Further studies of this formulation of AMX in animals and clinical studies are warranted.

P1090 The demonstration of in vitro antagonism among fusidic acid and quinolones

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Objectives: Fusidic acid is an antibiotic which is active against staphylococci and some other bacterial pathogens. It is used in the treatment of staphylococcal infections, usually in combination with other agents. Report of the effects of antimicrobial combinations containing fusidic acid have been somewhat inconsistent. The aim of our study was to indicate the in vitro antagonism among fusidic acid and quinolones, and to be warning for clinical studies.
Methods: In our study, 26 *Staphylococcus* strains (15 coagulase-negative staphylococci and 11 coagulase-positive staphylococci) isolated from various clinical samples were used. All of these strains were found sensitive to fusidic acid, levofloxacin, ciprofloxacin, ofloxacin and moxifloxacin in sensitivity test carried out with the method of disc diffusion. In vitro antagonism among fusidic acid and quinolones was investigated. After detecting the inhibition zone-diameter of these five antibiotics for each strains, interaction among them was investigated with disc-approximation test. Quinolones and fusidic acid discs were placed on Mueller–Hinton agar plaques such a way that the distance between the disc would be 2 mm shorter than the total of both zone diameters. The fact that there was narrowness at the side of fusidic acid in zone diameter round quinolones was evaluated as in vitro antagonism.
Results: In all the staphylococci strains investigated, zone diameter of quinolones in Mueller–Hinton agar plaque had been narrowed at the side of fusidic acid. In all of 26 strains, quinolones and fusidic acid were antagonist in vitro.
Conclusion: Fusidic acid is used commonly in combined treatments of staphylococcal infections. Spectrum of quinolones in clinical use is more common. Fusidic acid and quinolones be used usually in combination with other agents in the treatments of chronic osteomyelitis and some other infections. Unfortunately, there are in vitro antagonism among fusidic acid and quinolones. The reason for this antagonistic effect and its clinical implications are not known. So, we are opinion of that clinicians should be more careful in combined treatment with quinolones and fusidic acid.

P1091 Microbiological outcomes and pharmacokinetics of oritavancin in patients with Gram-positive bacteremia

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Objectives: To evaluate the multiple-dose efficacy and pharmacokinetics (PK) of oritavancin (LY), a glycopeptide antibiotic with bactericidal activity against Gram-positive (G+) pathogens, including resistant strains, in patients with G+ bacteremia; and to examine the relationship between PK and efficacy endpoints.
Methods: Patients: ($n = 27$) were 18 years or older, weighed at least 37 kg and must have had true or probable G+ bacteremia from blood specimens obtained within 4 days prior to enrollment. Each patient received one of three dose regimens (loading dose/daily maintenance doses): 3/2 mg/kg ($n = 5$), 4/3 mg/kg ($n = 5$), or 5/4 mg/kg ($n = 17$) for 7–10 days. Bacteriologic outcome was measured at the primary efficacy endpoint (first follow-up visit, 5 days after end of therapy). Efficacy analyzes were based on 10 qualified patients from the LY 5/4 group who met pre-specified criteria. Plasma samples collected over approximately 60 days were assayed for LY concentration using LC/MS/MS; all plasma concentration data were analyzed using population PK modeling. Relevant clinical and demographic patient factors (e.g. age, weight, and dose) were evaluated for their influence on the disposition of LY. The relationship between plasma exposure and clinical endpoints was evaluated.
Results: Favorable bacteriologic response was observed in 9 of 10 qualified patients in the LY 5/4 group. Plasma exposure appeared to be a predictor of response; bacteriologic failure may be associated with subtherapeutic exposure to LY. The 27 patients contributed 313 plasma concentrations for PK analysis. The LY CL, Vc, and Vp estimates for a typical bacteremia patient were 0.841 L/h, 6.50 L, and 22.0 L, respectively. The inter-patient coefficients of variation on CL and Vc were approximately 40%. Plasma $t_{1/2}$ estimated during a dosing period was approximately 40 h. No patient factors appeared to significantly influence the disposition of LY; however, an effect of weight could not be excluded. No serious adverse events were observed. No relationship was identifiable between adverse events and plasma exposure.
Conclusions: Oritavancin 5/4 mg/kg for 7–10 days was effective and well tolerated in 9/10 patients with G+ bacteremia. Similar or higher doses may be efficacious in patients with G+ bacteremia. Based on this small exploratory analysis, no dosage adjustment for LY appears to be necessary owing to patient factors. Pharmacokinetics was linear in the dose range tested.

Respiratory pathogens: in vitro studies

P1092 Macrolide and ketolide susceptibility of *S. pneumoniae* isolates collected in Austria during 2000–2001

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Background: Macrolide resistance in pneumococci is increasing in many European countries. Telithromycin is the first member of the ketolide family of antimicrobials with activity against pneumococci including those with acquired resistance to erythromycin.
Objective: To investigate the activity of telithromycin and various macrolides on *S. pneumoniae* strains collected in Austria.
Methods: A total of 401 strains of *S. pneumoniae* isolated during the last year from children (206 strains) and adults (181 strains) from different regions of the country were tested for susceptibility to telithromycin, erythromycin (E), roxithromycin (R), clarithromycin (C), azithromycin (A), lindamycin (Cl), penicillin (P), and tetracycline (T) using an agar dilution method. E-resistant strains were further investigated for the presence of macrolide resistance genes (*mef*, *erm* and *ermTR*) using a PCR-based method.
Results: Overall the P, E, Cl, T resistance was 10, 10, 4 and 10%, respectively, with higher prevalence in children (16, 10, 5 and 14%) and lower prevalence in adults (5, 7, 4 and 7%). (In a similar investigation on strains collected during 1999–2000 the P, E and T resistance was 6, 10 and 7%). The respective MIC_{50} and MIC_{90} of the antibiotics tested were: telithromycin <0.015 and 0.03 mg/L; E 0.03 and 1 mg/L; R 0.06 and 2 mg/L; C <0.03 and 0.5 mg/L; A 0.12 and 2 mg/L; Cl < 0.06 and <0.06 mg/L. Resistance to E was exhibited by 43 strains, 21 of them harboring *erm* and 22 *mef* genes. Telithromycin showed the lowest MICs and retained its good activity also on strains containing erythromycin-resistance genes; the range of MICs for those strains was from <0.015 to 0.5 mg/L.
Conclusions: The macrolide resistance prevalence of pneumococci isolated in 2000–2001 was similar to that found in 1999–2000. Telithromycin was highly active against isolates of *S. pneumoniae* collected during the last 2 years in Austria.

P1093 The influence of the *erm* and *mef* genes on telithromycin resistance in *Streptococcus pneumoniae*

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Objectives: To investigate the effects of the presence of *erm* and *mef* genes on the ability to select for telithromycin resistance in *Streptococcus pneumoniae.*
Methods: The *S. pneumoniae* strains chosen for investigation were 02J1095 containing the *erm* gene, 02J1175 with the *mef* gene and a macrolide-sensitive strain NCTC 13593. Mutants were selected from plates containing telithromycin at the MIC or twice the MIC and subcultured twice on agar plates containing telithromycin. This procedure was repeated for each generation until telithromycin resistance occurred or successive generations resulted in the same MIC. The *erm* and *mef* genes from the parent and the mutant strains were amplified using PCR and sequenced to investigate changes in the gene sequences from parent to mutant.
Results: The strains 02J1095 and 02J1175 were both sensitive to telithromycin although they were resistant to macrolides owing to the presence of the *erm* and *mef* genes, respectively. Telithromycin did not select for resistance in the macrolide-sensitive NCTC 13593 strain after four generations of mutation. However, resistance to telithromycin occurred in both 02J1095 and 02J1175 mutants. Second generation 02J1095 mutants had high-level telithromycin resistance whereas the highest telithromycin MIC was 8 mg/L after four mutant generations of 02J1175. There were no nucleotide differences between the *erm* genes from the parent (02J1095) and the corresponding mutant strains. The *mef* genes from each of the four mutant 02J1175 generations also showed no changes to the parent 02J1175 *mef* gene.
Conclusions: Therefore, these results suggest that telithromycin does not select for resistance in strains lacking the *erm* or *mef* genes, but will select for resistance in strains containing either gene. Thus in order to select for telithromycin resistance the strain must first be macrolide resistant owing to an *erm* or *mef* gene. Also, high level resistance to telithromycin is selected with fewer generations in *S. pneumoniae* containing the *erm* gene rather than the *mef* gene.

Strain	Telithromycin MIC (mg/L)	Mutation frequency
02J1095 (*erm*)	0.06	Parent
J I	1	1×10^{-3}
J II	32	1×10^{-6}
02J1175 (*mef*)	0.5	Parent
M I	2	2×10^{-4}
M II	4	3×10^{-5}
M III	8	3×10^{-6}
M IV	8	3×10^{-5}
NCTC 13593	0.016	Parent
N I	0.032	2×10^{-2}
N II	0.12	3×10^{-2}
N III	0.5	6×10^{-1}
N IV	0.5	2×10^{-2}

P1094 Intracellular survival kinetics of *Streptococcus pneumoniae* in J774 cells and human blood monocytes: effect of moxifloxacin

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Introduction: As a member of the fluoroquinolones, moxifloxacin and exhibits antibacterial activity against several Gram-positive species and is known to concentrate intracellularly. In this connection, it seemed appropriate to determine whether the drug displayed intracellular bioactivity against various strains of *Streptococcus pneumoniae* with differing antibiotic susceptibilities and different capsular polysaccharide serotypes.
Methods: A number of clinical isolates with raised MICs towards penicillin, ciprofloxacin and/or erythromycin were selected. Their susceptibility to complement mediated phagocytosis by activated J774 macrophages was measured. J774-cells were pre-activated with phorbolmyristate acetate (0.16 μM) and interferon gamma (10 units) and freshly isolated blood monocytes were isolated from human blood donations using Histopaque 1077 (Sigma) with greater than 95% purity and viability. Phagocytosis was assessed using light or fluorescent microscopy (with a vital dye) followed by measurement of bacterial viability by culture techniques. The kinetics of intracellular killing varied amongst the test strains but was not correlated with their antibiotic susceptibility pattern.
Results: Addition of moxifloxacin at concentrations ranging from 1/2 to 4 × MIC resulted in enhanced killing of all the bacterial strains notwithstanding their resistance to penicillin, erythromycin or ciprofloxacin. Moxifloxacin pretreated cells induced at least a 50% loss of viability within 2 h (J774 cell lines) or 1 h (monocytes) as compared to control cells to which no moxifloxacin had been added. Intracellular staining with fluorescent vital dye clearly demonstrated that the loss of viability occurred intracellularly and that any increase in intracellular killing could be attributed to treatment with moxifloxacin.
Conclusion: The results demonstrate that moxifloxacin is bioactive intracellularly against antibiotic-resistant strains of *Streptococcus pneumoniae*, thereby augmenting innate cellular defense mechanisms.

P1095 Susceptibility to selected antibiotics of *Streptococcus pneumoniae* strains isolated from patients with respiratory tract infections

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Objectives: In recent years a number of studies have addressed the frequency of antibiotic resistance among respiratory pathogens, including *Streptococcus pneumoniae*, and an increased resistance has been reported for commonly used antibiotics. The aim of our study was to evaluate the susceptibility to selected antibiotics among *Streptococcus pneumoniae* strains isolated from patients suffering from respiratory tract infections. A total of 52 *Streptococcus pneumoniae* strains, isolated from patients with sinusitis, bronchitis, and pharyngitis, have been examined.
Methods: Susceptibility to penicillin, erythromycin, tetracycline, and vancomycin has been evaluated using Sceptor *Streptococcus* MIC/ID panels (Becton Dickinson) according to manufacturer's instructions.
Results: In our study, we have observed high penicillin ($S=23$, $I=26$, $R=3$), tetracycline ($S=23$, $I=13$, $R=16$), and erythromycin ($S=33$, $I=13$, $R=6$), resistance rates among *Streptococcus pneumoniae* strains. A total of 12 strains have been fully or moderately resistant to erythromycin and tetracycline – 8 of them have been also fully or intermediately resistant to penicillin. As a total, 19 strains have appeared to be multiresistant and have been fully or intermediately resistant to two or three antibiotics (penicillin, erythromycin, tetracycline). All examined strains have been susceptible to vancomycin. All strains have been also susceptible to optochine.
Conclusions: Increasing resistance of *Streptococcus pneumoniae* strains to commonly used antibiotics indicates the necessity of performing antimicrobial susceptibility test in every case of infection with this pathogen.

P1096 In vitro bactericidal activity of new fluoroquinolones against vancomycin-tolerant pneumococci

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Objectives: The incidence of penicillin-resistant and multiple-resistant pneumococci has increased worldwide at an alarming rate. A further threat is represented by the recent descriptions of vancomycin tolerant strains. The aim of this study was to evaluate the in vitro activity of new fluoroquinolones against vancomycin tolerant *S. pneumoniae* strains.
Methods: The bactericidal activity of levofloxacin, gemifloxacin, moxifloxacin, trovafloxacin, clinafloxacin and vancomycin was assessed by the time-kill curve method described by Novak et al. (Nature 339: 590–593, 1999) in nine multiresistant, vancomycin tolerant *S. pneumoniae* strains previously described (Marchese et al. 40th ICAAC, 2000 (Abstract 1778)). The reference-sensitive strain R6 was used as control.
Results: Time-kill tests confirmed tolerance to vancomycin and showed a pronounced bactericidal activity of all fluoroquinolones tested. In particular, after 4 h of exposure the initial inoculum was reduced by clinafloxacin from 99.9 to >99.999%, by levofloxacin and trovafloxacin from 99.9 to 99.999% and by gemifloxacin and moxifloxacin from 99.3 to 99.99% depending on the

strain. After 6 h of exposure, the initial inoculum was reduced to 0.01 or to <0.001% for all fluoroquinolones and no re-growth was observed after further incubation.
Conclusion: New fluoroquinolones active against Gram-positive pathogens retain bactericidal activity against multiresistant vancomycin-tolerant pneumococci. Approved molecules of this class endowed with appropriate pharmacokinetic properties may represent effective therapeutic options for serious pneumococcal infections, including meningitis, sustained by these difficult-to-treat strains.

P1097 Antibiotic sensitivity of *Streptococcus pneumoniae* isolates from Hungary

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Objective: *Streptococcus pneumoniae* is the major cause of community-acquired pneumonia and has become increasingly difficult to treat as it has acquired resistance to penicillin and other antibiotics. Hungary and Spain have reported the highest incidences of penicillin resistance in Europe, and therefore they are each a demanding environment for testing the efficacy of new antibacterials. This study examines recent Hungarian isolates to determine their sensitivity to the new antibacterials.
Methods: One hundred and twenty non-repeated *Streptococcus pneumoniae* strains were collected by the diagnostic laboratories of the Semmelweis University in Budapest between January and June 2000. The strains were transported to the UK and their identity confirmed. Antibiotic disc-diffusion tests were performed on 13 antibiotics and their sensitivity determined, where appropriate, according to NCCLS guidelines. Sensitivity to antibiotics not listed in the guidelines was determined by comparison with established antibiotics or disc contents of the BSAC guidelines were used.
Results: The rates of resistance and intermediate sensitivity (where appropriate) were extremely high to oxacillin, clindamycin and cotrimoxazole. No intermediate resistance was found for the macrolides though the resistance rates were high at around 36%. Amoxiclav, imipenem and cefotaxime sensitivity was high. Vancomycin tolerance has recently been observed in the US and Sweden, and five strains in this cohort showed evidence of the same in Hungary. Full sensitivity to levofloxacin and ciprofloxacin was found in only approximately two-thirds of the population but a much higher proportion were fully sensitive to moxifloxacin.
Conclusions: Against this very exacting population of bacteria moxifloxacin, amoxiclav and telithromycin showed a remarkably high level of efficacy.

		Percentage		
Antibiotic	Disc content (μg)	Sensitive	Intermediate	Resistant
Oxacillin	1	36.4		63.6
Imipenem	10	99.2		0.8
Amoxiclav	3	94.1		5.9
Cefotaxime	5	99.2		0.8
Vancomycin	30	95.8	4.2	
Erythromycin	15	63.2	0	36.8
Clarithromycin	15	63.2	0.8	36.0
Levofloxacin	5	59.7	35.5	4.8
Ciprofloxacin	5	68.9	23.8	7.4
Moxifloxacin	1	88.6		11.4
Clindamycin	2	27.6	39.7	32.8
Co-trimoxazole	25	7.4	14.8	77.9
Telithromycin	15	91.0	7.4	1.6

P1098 Bactericidal activity of moxifloxacin and other antibiotics against *Streptococcus pneumoniae*

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Objectives: To evaluate the in vitro bactericidal activity of moxifloxacin (MXF) against SP strains with different resistance phenotypes: penicillin S, penicillin R, amoxicillin R, erythromycin R (ERY-R)- *ermB* -low and high level, ERY-R *mefE*.
Methods: 12 SP strains (2 strains for each phenotype) were studied by using a killing curve method. Seven antibiotic concentrations were used from 0.25 to 16 mg/L by following a two-fold dilution. Viable counts were measured at T0, T3, and T6 h by using agar plates with inhibitors to prevent antibiotic carry-over. Tested antibiotics were moxifloxacin (MXF), levofloxacin (LEVO), amoxicillin (AMX), ceftriaxone (CRO), pyostacine (PYO). All strains were susceptible to MXF, LEVO and PYO.
Results: At T3 h a decrease of 2 log 10 cfu/mL was observed for 10 strains with MXF ≥ 2 mg/L, at T6 h, a decrease of 3 log 10 cfu/mL was observed with MXF ≥ 2 mg/L for 9 strains and a decrease of 2 log 10 cfu/mL for the three others strains. At T3 h, a decrease of 2 log 10 cfu/mL was observed for seven strains with LEVO ≥ 4 mg/L, at T6 h, a decrease of 3 log 10 cfu/mL was observed with LEVO ≥ 4 mg/L for nine strains and a decrease of 2 log 10 cfu/mL for the three others strains (the same strains as with MXF). At T3 h, a decrease of 2 log 10 cfu/mL was observed for one strain (*mefE* strain) with AMX 4 mg/L and for three strains for AMX 16 mg/L. At T6 h, a decrease of 2 log 10 cfu/mL was obtained for five strains and a decrease of 3 log 10 cfu/mL for three strains by AMX 4 mg/L. The bactericidal activity was not related to the penicillin and amoxicillin resistance phenotype. At T3 h, a decrease of >1 log 10 cfu/mL was observed with CRO 1 mg/L for only one strain (*mefE* strain) and at T6 h, with CRO 16 mg/L a decrease of 3 log 10 cfu/mL was observed with one strain and a decrease of 1 log 10 cfu/mL for nine strains. At T3 and T6 h, a decrease of 3 log 10 cfu/mL was observed with PYO ≥ 1 mg/L for four of 6 ERY-S strains and three of 6 ERY-R strains (one of four *ermB* strains).
Conclusions: The bactericidal activity of MXF and LEVO was observed from T3 h and was similar for both drugs but was obtained with 2 mg/L for MXF and with 4 mg/L for LEVO. PYO exhibited a rapid and intense bactericidal activity with strains without *ermB* gene. But, it had a weak bactericidal effect against ERY-R-*ermB* strains. The bactericidal activity of AMX was obtained at T6 h only. For CRO, the killing rate was very slow, even at high concentrations.

P1099 In vitro activity of rokitamycin against well-characterized macrolide-resistant *Streptococcus pneumoniae* strains

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Objectives: The level of susceptibility to macrolides (M), streptogramin B (SB) and lincosamides (L) in *S. pneumoniae* depends on the expression of different genetic determinants. For this reason certain molecules belonging to the above mentioned groups may be active against *S. pneumoniae* possessing either *ermB* or *mefA* genes. The aim of this study was to evaluate the in vitro activity of the 16-member macrolide rokitamycin towards well-characterized macrolide-resistant pneumococci.
Methods: A total of 40 erythromycin-resistant *S. pneumoniae* strains (20 showing a constitutive MLSB phenotype, and 20 belonging to the M-type) and 20 fully susceptible pneumococci were studied. *ermB* and *mefA* genes were amplified by PCR. Minimal inhibitory concentrations (MICs) of rokitamycin, erythromycin, azithromycin and clarithromycin were assessed by the microbroth dilution method (NCCLS, 2000).
Results: Rokitamycin and clarithromycin were the most potent drugs in terms of MIC_{90} (0.015 mg/L) against macrolide-susceptible pneumococci. For these two drugs MICs were generally 1 or 2 dilutions lower than those displayed by erythromycin and azithromycin (MIC_{90} 0.06 mg/L). Rokitamycin was also the most active drug against M-type strains (MIC_{90}: 0.06 mg/L), whit a potency in excess of 130-fold compared to erythromycin, clarithromycin and azithromycin (MIC_{90} values ranging from 8 to 16 mg/L). *S. pneumoniae* carrying the *ermB* gene were less susceptible to all drugs: MIC_{90} were 128 mg/L (rokitamycin and clarithromycin) and >256 mg/L (azithromycin and erythromycin). However, rokitamycin showed a bimodal distribution of MICs (1–4 and 32–128 mg/L).
Conclusion: In countries where *mefA* dictated resistance is dominant 16-membered macrolides may represent interesting therapeutic options.

P1100 Telithromycin is highly active against community-acquired respiratory tract infection (CARTI) pathogens collected from patients in Germany (PROTEKT 1999–2001)

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Objectives: PROTEKT is a global longitudinal surveillance study with good representation throughout Europe. The study is aimed at determination of resistance of key pathogens responsible for CARTI.
Methods: Within Germany, isolates were collected from eight centers during the winter season of 1999–2000 and 12 centers in 2000–2001 (preliminary data). MICs of 26 antibacterials were determined centrally by NCCLS broth microdilution methods and interpreted using available NCCLS breakpoints.
Results: Prevalence of resistance to penicillin G and erythromycin A (EryR; MIC $\geq$ 1 mg/L) among isolates of *S. pneumoniae* during 1999–2000 ($n = 325$) was as follows: penicillin G intermediate (PenI, MIC 0.12–1 mg/L), 20 (6.2%); penicillin G resistant (PenR, MIC $\geq$ 2 mg/L), 7 (2.2%); EryR, 51 (15.7%). Corresponding data for 2000–2001 ($n = 219$) were: PenI, 10 (4.6%); PenR, 7 (3.2%); EryR, 37 (16.9%). Mode MIC, MIC_{90} and susceptibility data for a selection of the antibacterials tested against *S. pneumoniae* are presented in Table 1.

Table 1 In vitro activity of 11 antibacterials against *S. pneumoniae* (1999–2000)

Antibacterial	Mode MIC (mg/L)	MIC_{90} (mg/L)	% Susceptible
Telithromycin	0.008	0.03	–[a]
Penicillin G	0.015	0.06	91.7
Cefuroxime	0.03	0.12	97.5
Erythromycin A	0.06	>64	84.3
Azithromycin	0.12	>64	84.9
Clarithromycin	0.03	>32	84.3
Co-trimoxazole	0.25	8	82.2
Levofloxacin	1	1	99.7
Linezolid	1	2	100
Teicoplanin	0.06	0.12	100
Quinupristin/dalfopristin	0.5	1	98.5

[a]NCCLS breakpoint not yet available.

Telithromycin was the most potent of the agents tested, having a mode MIC, MIC_{50} and MIC_{90} of 0.008, 0.008 and 0.03 mg/L, respectively, in both study years. Importantly, telithromycin retained potent activity against pneumococci resistant to other antibacterials. Telithromycin was also highly active against isolates of *S. pyogenes* (1999–2000: mode MIC, 0.015 mg/L; MIC_{90}, 0.03 mg/L; 2000–2001: mode MIC, 0.015 mg/L; MIC_{90}, 0.03 mg/L), *H. influenzae* (1999–2000: mode MIC, 1 mg/L; MIC_{90}, 2 mg/L; 2000/2001: mode MIC, 1 mg/L, MIC_{90}, 1 mg/L) and *M. catarrhalis* (2000 and 2001: mode MIC, 0.06 mg/L; MIC_{90}, 0.12 mg/L).
Conclusions: The PROTEKT surveillance study confirms the increasing prevalence of macrolide resistance among pneumococci in Germany. Telithromycin was highly active against isolates of the major bacterial pathogens implicated in CARTIs, including macrolide-resistant strains.

P1101 Telithromycin is highly active against blood isolates of *S. pneumoniae*, *S. pyogenes* and *H. influenzae* collected from patients with community-acquired respiratory tract infections (CARTIs; PROTEKT 1999–2000)

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Objectives: PROTEKT is a global longitudinal surveillance study (69 centers in 25 countries) initiated in 1999 to track the spread of antibacterial resistance among bacterial pathogens implicated in CARTIs. We present here the comparative activity of the new ketolide telithromycin against blood isolates collected during the first year of the study.
Methods: As part of PROTEKT, blood isolates of *S. pneumoniae* ($n = 213$), *S. pyogenes* ($n = 29$) and *H. influenzae* ($n = 20$) were collected from outpatients with CARTIs. MICs of 26 antibacterials were determined centrally by NCCLS broth microdilution methods and interpreted using available NCCLS breakpoints.
Results: Mode MIC, MIC_{90} and susceptibility data for a selection of the antibacterials tested against *S. pneumoniae* are presented in Table 1.

Table 1 In vitro activity of test antibacterials against *S. pneumoniae*

Antibacterial	Mode MIC (mg/L)	MIC_{90} (mg/L)	% Susceptibility
Telithromycin	0.008	0.12	–[a]
Penicillin G	0.015	2	78.4
Erythromycin A	0.06	8	81.2
Azithromycin	0.12	16	81.2
Clarithromycin	0.03	16	81.2
Co-trimoxazole	0.25	8	69.0
Levofloxacin	1	1	100
Linezolid	1	2	100
Quinupristin/dalfopristin	0.5	1	98.6

[a]NCCLS breakpoint not yet available.

On a weight basis, telithromycin was the most active of the agents tested, having mode MIC, MIC_{50} and MIC_{90} of 0.008, 0.008 and 0.12 mg/L, respectively. The prevalence of high-level penicillin G resistance (MIC $\geq$ 2 mg/L) among pneumococci was 12.2%, with a further 9.4% of isolates being penicillin G intermediate. Erythromycin A resistance was 18.8%. Importantly, telithromycin retained potent activity against these resistance phenotypes. Telithromycin was also highly active against invasive isolates of *S. pyogenes* (mode MIC, 0.015; MIC_{90}, 0.03 mg/L) and *H. influenzae* (mode MIC, 1 mg/L; MIC_{90}, 2 mg/L).
Conclusions: This subanalysis of the PROTEKT surveillance study confirms the potent activity of telithromycin against invasive isolates of bacterial pathogens implicated in CARTIs, irrespective of resistance phenotype. These data are consistent with the high clinical success rates and bacteriologic eradication rates that have been reported with telithromycin in patients with CAP-associated bacteremias.

P1102 Telithromycin is highly active against community-acquired respiratory tract infection pathogens collected from patients in Spain (PROTEKT 1999–2001)

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Objectives: PROTEKT, initiated in 1999, is a global longitudinal surveillance study of antibacterial resistance among bacterial CARTI pathogens. Data are now available for isolates collected in Spain during two consecutive winter seasons: 1999–2000 (2000) and 2000–2001 (2001; preliminary data).
Methods: Isolates were collected from two centers in 2000 and seven centers in 2001. MICs of 26 antibacterials were determined centrally by NCCLS broth microdilution methods and interpreted using available NCCLS breakpoints.
Results: Prevalence of resistance to penicillin G and erythromycin A among isolates of *S. pneumoniae* during 2000 ($n = 133$) was as follows: penicillin G intermediate (PenI, MIC 0.12–1 mg/L), 15 (11.3%); penicillin G resistant (PenR, MIC $\geq$ 2 mg/L), 56 (42.1%); erythromycin A resistant (EryR, MIC $\geq$ 1 mg/L), 38 (28.6%). Corresponding data for 2001 ($n = 199$) were as follows: PenI, 28 (14.1%); PenR, 66 (33.2%); EryR, 76 (38.2%). Mode MIC, MIC_{90} and susceptibility data for a selection of the antibacterials tested against *S. pneumoniae* are presented in Table 1. Telithromycin was the most potent of the agents tested, having a mode MIC, MIC_{50} and MIC_{90} of 0.008,

Table 1 In vitro activity of test antibacterials against *S. pneumoniae* (2000)

Antibacterial	Mode MIC (mg/L)	MIC_{90} (mg/L)	% Susceptibility
Telithromycin	0.008	0.03	–[a]
Penicillin G	0.015	2	46.6
Cefuroxime	0.03	8	50.4
Erythromycin A	0.06	>64	71.4
Azithromycin	0.12	>64	70.7
Clarithromycin	0.03	>32	71.4
Co-trimoxazole	0.25	16	45.1
Levofloxacin	0.5	1	100
Linezolid	1	1	100
Teicoplanin	0.06	0.12	100
Quinupristin/dalfopristin	0.5	1	99.2

[a]NCCLS breakpoint not yet available.

0.008 and 0.03 mg/L, respectively, in both study years. Importantly, telithromycin retained potent activity against pneumococci resistant to other antibacterials. Telithromycin was also highly active against isolates of *S. pyogenes* (2000: mode MIC, 0.015 mg/L; MIC_{90}, 0.25 mg/L; 2001: mode MIC, 0.008 mg/L; MIC_{90}, 0.5 mg/L), *H. influenzae* (2000 and 2001: mode MIC, 1 mg/L; MIC_{90}, 2 mg/L) and *M. catarrhalis* (2000: mode MIC, 0.06 mg/L; MIC_{90}, 0.06 mg/L; 2001: mode MIC, 0.06 mg/L; MIC_{90}, 0.12 mg/L).

Conclusions: The results of the PROTEKT surveillance study in Spain between 1999 and 2001 confirm the high prevalence of β-lactam, macrolide and cotrimoxazole resistance amongst pneumococci, and the potent activity of telithromycin against such isolates and other CARTI bacterial pathogens.

P1103 In vitro activity of telithromycin and macrolides against clinical isolates of *Streptococcus pneumoniae* from patients with community-acquired respiratory tract infections

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Objectives: The worldwide increase in antibacterial resistance among respiratory pathogens has prompted the need for new agents that retain activity against resistant pathogens and have a low potential to induce, or select for, resistant strains. Telithromycin is the first of a new class of antibiotics, the ketolides. Telithromycin displays potent in vitro activity against common and atypical/intracellular respiratory pathogens, including resistant strains. We report here an analysis of the in vitro activity of telithromycin versus macrolide comparators (clarithromycin [CLA] and azithromycin [AZI]), against clinical isolates of *Streptococcus pneumoniae* collected during Phase III clinical studies with telithromycin.

Methods: A total of 394 clinical isolates of *S. pneumoniae* were obtained from adult patients with community-acquired respiratory tract infections (pneumonia [$n = 250$], acute exacerbation of chronic bronchitis [$n = 28$] and acute maxillary sinusitis [$n = 116$]) who took part in one of 11 multicenter clinical trials. Tentative breakpoints used to determine in vitro susceptibility to telithromycin were: susceptible, ≤1.0 mg/L; intermediate, 2.0 mg/L; resistant, ≥4.0 mg/L. NCCLS breakpoints were used to determine susceptibility to CLA and AZI.

Results: All isolates ($n = 394$) showed intermediate or full susceptibility to telithromycin (mode MIC 0.015 mg/L), whereas 58 (14.7%) and 61 (15.5%) strains were resistant to CLA and AZI, respectively. All 47 pneumococcal strains that were resistant to penicillin G (MIC ≥ 2.0 mg/L) remained susceptible to telithromycin (mode MIC 0.03 mg/L). These isolates showed high levels of cross-resistance to both CLA (63.8%, $n = 30$) and AZI (68.1%, $n = 32$). A total of 58 strains (14.7%) were resistant to erythromycin A (MIC ≥ 1.0 mg/L). Nearly all of these strains were also resistant to both CLA and AZI (98.3%, $n = 57$), yet none were resistant to telithromycin (mode MIC 0.03 mg/L).

Conclusion: Compared with CLA and AZI, telithromycin provides excellent coverage against clinical isolates of *S. pneumoniae*, including penicillin G- and erythromycin A-resistant strains.

P1104 Telithromycin is highly active against isolates of *S. pneumoniae* from pediatric isolates collected in the PROTEKT Surveillance Study (1999–2000), irrespective of penicillin G or erythromycin A resistance

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Objectives: The rapid evolution of drug-resistant *S. pneumoniae* in the pediatric population is of particular concern. We present here the results of a subanalysis performed on the first year's data from the PROTEKT surveillance study to assess the prevalence of antibacterial resistance among pediatric isolates of *S. pneumoniae*, and the activity of the new ketolide, telithromycin, against such isolates.

Methods: *S. pneumoniae* isolates were collected during the 1999–2000 respiratory season from children (aged <12 years) with community-acquired respiratory tract infections (CARTIs). In total, 69 centers in 25 countries contributed data for 1999–2000. In vitro susceptibility of isolates to 26 antibacterials was determined by NCCLS broth microdilution at a central laboratory and interpreted using NCCLS breakpoints, where available. Susceptibility data were analyzed by culture source and age range.

Results: In total, 368 isolates of *S. pneumoniae* were collected from the 4 main culture sources (sputum, nasopharyngeal, ear and blood). Among these isolates, the overall prevalence of penicillin G resistance (PenR; MIC ≥ 2 mg/L) was 26%, and of erythromycin A resistance (EryR; MIC ≥ 1 mg/L) was 38%. Isolates resistant to erythromycin A were also cross-resistant to clarithromycin and azithromycin. The highest rates of resistance were recorded for children aged 1–2 years (PenR, 44%; EryR, 51%), and for isolates from the nasopharynx (PenR, 30%; EryR, 57%). Rates of EryR by age and culture source are summarized in Table 1.

Table 1 Prevalence of EryR among *S. pneumoniae* by age and culture source

	>Age (years)			
Culture source	≤1	>1–≤2	>2–<8	>8–<12
Sputum	19/56 (34%)	3/6 (50%)	14/40 (35%)	4/12 (33%)
Nasopharyngeal	27/49 (55%)	7/12 (58%)	24/39 (62%)	2/6 (33%)
Ear	16/54 (30%)	7/13 (54%)	6/24 (25%)	1/8 (13%)
Blood	1/27 (4%)	4/10 (40%)	3/12 (25%)	
Total	63/186 (51%)	21/41 (51%)	47/115 (41%)	7/26 (27%)

Overall, telithromycin had an $MIC_{50/90}$ of 0.015/0.12 mg/L. Against PenR and EryR *S. pneumoniae*, telithromycin had $MIC_{50/90}$ of 0.06/0.25 mg/L and 0.06/0.5 mg/L, respectively.

Conclusions: The high prevalence of EryR and PenR among pneumococci isolated from children with CARTIs is a cause for concern, and highlights the need for new antibacterials such as the ketolide telithromycin, which shows excellent activity against isolates of *S. pneumoniae* from pediatric patients, irrespective of penicillin, macrolide or multiple resistance.

P1105 Bactericidal activity of amoxicillin alone and in combination with clavulanic acid against *Streptococcus pneumoniae* isolates with reduced susceptibility to amoxicillin

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Objectives: Time-kill studies were performed to evaluate the antibacterial activity of amoxicillin alone and in combination with clavulanic acid against *Streptococcus pneumoniae* isolates with reduced susceptibility to amoxicillin. Included were two isolates with amoxicillin MICs of 2 mg/L, three isolates with MICs of 4 mg/L, three isolates with MICs of 8 mg/L and one isolate with a MIC of 16 mg/L.

Methods: Isolates were incubated in the presence of amoxicillin ± clavulanic acid at 0.25, 0.5, 1, 2 and 4 times the MIC. Viable counts were conducted at 2-h intervals for 8 h and again at 24 h.

Results: No notable differences in rate of kill were observed between amoxicillin and amoxicillin/clavulanic acid. A bactericidal effect (>2 log[10] decrease from the initial test inoculum) was observed at concentrations at or above the MIC, with some strains showing a reduced growth rate at 0.5 times MIC. At these concentrations, the inhibitory effect was shown to be time dependent through 8 h.

Conclusions: Overall, these results indicate that at or above the MIC, amoxicillin and amoxicillin/clavulanic acid produce a time-dependent bactericidal effect against *S. pneumoniae* isolates with reduced susceptibility to amoxicillin. The rate of kill seen with these isolates is similar to that reported for isolates that are considered highly susceptible to these agents.

P1106 Combination therapy with vancomycin and levofloxacin for severe bacterial pericarditis due to penicillin-resistant *Streptococcus pneumoniae* (PRSP): feasibility in a disease refractory to ceftriaxone, vancomycin, and quinupristin/dalfopristin (QP/DP)

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Objectives: Bacterial pericarditis is rare, and optimal therapy for life-threatening complication owing to PRSP is uncertain.
Results: A 29-year-old healthy black man presented with acute respiratory distress, fever (38.5 °C), and severe chest pain. Lingual consolidation on chest radiograph, and EKG indicated acute pericardial inflammation. Echocardiography showed moderate effusion and diastolic equalization of pressures, cardiac tamponade. The laboratory results are as follows: WBC 10.4 K/μL, ALT 546 U/L, AST 491 U/L, and ESR 110 mm/ h. An emergent pericardiocentesis catheter was placed and 360 cc of brownish, turbid effusion with several intragranulocytic Gram-positive diplococci was removed. *Streptococcus pneumoniae* was isolated from pericardial fluid (PF) and blood cultures, antimicrobial susceptibility (MIC) as follows: penicillin 4 μg/mL, ceftriaxone 3 μg/mL, erythromycin >21 mm, rifampin >19 mm, vancomycin >17 mm, levofloxacin >17 mm, linezolid 28 mm, and QP/DP 18 mm. Vancomycin (2 g daily) was added to ceftriaxone (4 g daily) 2 days later. Day 5, QP/DP (7.5 mg/kg every 8 h) was substituted for ceftriaxone as fever rose to 39 C, WBC 16.1 k/μL, and PF drainage increased to 120 cc/24 h. Day 9, he developed septic shock, WBC 31.8 k/μL, fever 40 C, and in pericardial fluid intragranulocytic diplococci persisted. Levofloxacin (750 mg daily, IV) was started and QP/DP was discontinued. The following day patient underwent surgical de-bridement and pericardectomy. Assisted ventilation was discontinued 96 h following surgery. He was discharged on day 23, and completed 4 weeks of antibiotic therapy.
Conclusions: Early surgical intervention is critical in patients with severe bacterial infection of the pericardium. High-dose levofloxacin in combination with vancomycin appears to be well tolerated and may provide an alternative in refractory infections owing to PRSP.

P1107 Study of antibiotic-resistant pneumococci isolated in Romania from January 1999 to December 2000

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Objectives: To study the antibiotic resistance in pneumococci isolated in Romania between January 1999 to December 2000.
Methods: Two hundred 42 strains of *Streptococcus pneumoniae* coming from sputum or tracheal aspirate ($N=107$), blood ($N=52$), CSF ($N=23$), pleural fluid ($N=4$), and others ($N=56$): ear fluid, sinus, pharynx, were collected between January 1999 and December 2000 at the National Reference Center for Streptococcus. The specimens from pharynx came from 47 patients with HIV. The isolates were serotyped and analyzed for susceptibility to the following antibiotics: penicillin (Pc), erythromycin (Em), clarithromycin (Cla), cefazolin (Kz), cefuroxim (Cef), cefotaxim (Ctx), cotrimoxazole (Sxt), chloramphenicol (Cm), ciprofloxacin (Cip), amoxicillin (Amx), amoxicillin/clavulanic acid (Amc), vancomycin (Va) by standard agar dilution MIC testing.
Results: Interpretative criteria were used according to NCCLS 1999. During the study period penicillin-resistant strains of *S. pneumoniae* were noted as follows: in sputum and tracheal aspirate 67% (MIC_{50}: 0.25 mg/L, MIC_{90}: 2 mg/L), in blood 42% (MIC_{50}: 0.12 mg/L, MIC_{90}: 1 mg/L), in CSF 8.2% (MIC_{50}: 0.06 mg/L, MIC_{90}: 2 mg/L) and in others 86% (MIC_{50}: 1 mg/L, MIC_{90}: 4 mg/L). The penicillin-resistant strains coming from sputum and tracheal aspirate revealed susceptibility to the following antibiotics: Amx (100%), Amc (100%), Ctx (90%), Cip (89%) and showed resistance to: Sxt (89%), Em (64%), Kz (34%), Cla (29%), Cef (27%), Cm (26%). No resistant strain to vancomycin was found. The following serotypes were resistant to penicillin: 6 (30%), 19 (22.3%), 7 (17.3%), 23 (14%), and 8 (14%).
Conclusions: The most efficient drugs against penicillin-resistant pneumococci were: Amx, Amc, Ctx and Cip. Clarithromycin was more active than erythromycin. There is an urgent need to implement a network in Romania for the surveillance of the antimicrobial usage and a stronger partnership between clinical medicine and public health.

P1108 Current antimicrobial susceptibilities of pneumococci and other *Streptococcus* spp. from France, Germany, Italy and the USA using TSN® data

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Objectives: Because β-lactams and macrolides are used to treat infections attributable to *S. pneumoniae* (SP), *S. pyogenes* (SPy), *S. agalactiae* (SAg) and viridans group streptococcus (VS), we present current susceptibilities (% S) of these species to these antimicrobial classes as reported to physicians following routine test procedures by clinical laboratories. We also review the impact of the new 2002 NCCLS (M100-S12) ceftriaxone (CRO), cefotaxime (CTX) and cefepime pneumococcal breakpoints for non-meningeal isolates.
Methods: We reviewed data (January 1999–September 2001) from The Surveillance Network® (TSN) Databases in France (Fr), Germany (Gy) Italy (It) and USA, to determine current susceptibilities from routine test results. We used 2001 NCCLS breakpoints, except for Fr data, for which CA-SFM (2001) criteria are used. To apply the revised 2002 NCCLS M100-S12 pneumococcal non-meningeal CRO/CTX breakpoints, we re-interpreted available quantitative MIC data.
Results: For SP, penicillin (PEN) and erythromycin (ERY) non-susceptibility (NS) was 72.6, 2.9, 8.4, and 56.4, and 54.4, 15.0, 34.4 and 37.7% for Fr, Gy, It and USA, respectively. Rates of CRO/CTX NS were 23.6/28.4% (Fr), 7.3/1.5% (It) and 23.1/29.2% (USA). For SPy or SAg, no isolates were reported as resistant to PEN. In contrast, 41.6 (Fr), 1.3 (It) and 25.3% (USA) of VS were PEN-resistant. ERY NS in SPy, SAg and VS, respectively, were 14.4/16.4/45.9% (Fr), 10.8/11.5/not tested percentage (Gy), 27.6/18.4/20.0% (It) and 12.6/24.5/40.0% (USA). Where TSN data was available for non-SP species, >99% of isolates remained susceptible to CRO except for 9.3% (CRO) and 10.4% (CTX) NS among VS from the USA. Interpreting CRO MICs for non-meningeal isolates of SP using 2002 NCCLS criteria showed the percentage S to be 97.8% (Fr), 100% (Gy), 98.1% (It) and 94.7% (USA).
Conclusions: Macrolide resistance (or non-susceptibility as discussed above) has emerged in all streptococcal species reported. PEN non-susceptibility for SP and VS varied notably by country. Among non-SP species, CRO or CTX were not routinely tested in European clinical laboratories, despite showing high activity when they were tested. Current levels of susceptibility to CRO or CTX among non-meningeal isolates of pneumococci will increase considerably when 2002 NCCLS M100-S12 breakpoints are introduced.

P1109 Antimicrobial susceptibilities of *H. influenzae* and *M. catarrhalis* causing lower respiratory tract infections: SENTRY Antimicrobial Surveillance Program, Europe 2000

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Objective: To study the occurrence of different β-lactam resistance phenotypes in *H. influenzae* (Hi) and *M. catarrhalis* (Mc) isolates and the resistance profile to other antimicrobials.

Methods: A total of 681 Hi and 285 Mc isolates were recovered in 16 laboratories from 12 European countries. Susceptibility was centralized (Univ. of Iowa) and performed as recommended by the NCCLS. Analysis of both organisms was made using NCCLS criteria for Hi (M100-S11).
Results and conclusions: The proportion of β-lactamase producers in Hi and Mc were 11.7 and 95.7%, respectively. The β-lactamase positive rate in Hi isolates from different laboratory sites ranged from 0 to 30%, verifying that these isolates were not recovered during the Sentry Antimicrobial Surveillance Program in all European geographic areas in 2000. Only 1% of Hi isolates were intermediate (2 isolates) or resistant (4 isolates) to ampicillin in the absence of β-lactamase production. With the exception of one of these 6 Hi isolates, all showed a reduced susceptibility to amoxicillin/clavulanate (4–8 μg/mL) and cefuroxime (8–>8 μg/mL) and displayed higher MICs to ceftriaxone (0.03 μg/mL) than that obtained in other Hi irrespective of β-lactamase production (<0.008–0.01 μg/mL). Within β-lactamase-producing Hi isolates, no amoxicillin/clavulanate or cefuroxime-resistant isolates were observed. All isolates of Mc and all but 3 of Hi were inhibited by 0.06 μg/mL of both ciprofloxacin and the new desfluoroquinolone BMS284756 compound. Clarithromycin resistance was uncommon in Hi (1.1%) and was not detected in Mc. Chloramphenicol, tetracycline and rifampicin resistance rates in Hi were 1.2, 0.7, and 0.7%, respectively; the corresponding values in Mc were 0, 3.9, and 0%, respectively. Cotrimoxazole resistance was only present in Hi (16.5%) and was randomly distributed among different sites.

P1110 In vitro activity of levofloxacin and other antibiotics against respiratory tract pathogens isolated in France during 2000–2001

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Objectives: The objective of this study was to evaluate the in vitro activity of levofloxacin (LVX) against recent respiratory tract pathogens (RTP) in France in comparison with other antibiotics.
Methods: A total of 1694 French clinical strains of adult respiratory tract infections were isolated from November 2000 to April 2001 in 30 participant centers. MICs of LVX, penicillin (PEN), amoxicillin (AMX), amoxiclav (AMC) and erythromycin (ERY) were determined by the agar dilution method in a central laboratory. Susceptibility rates were calculated according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie.
Results: MICs 50% (mg/L)/MICs 90% (mg/L)/percentages of susceptibility for each species are presented below: *Streptococcus pneumoniae* (SP): Overall SP strains ($n=675$): LVX: 1/2/99; PENI: 0.12/2/49; AMX: 0.06/2/72; ERY: 0.5/64/52.3; PEN susceptible strains (PSS) ($n=331$): LVX: 1/1/99.7; AMX: 0.015/0.03/100; ERY: 0.03/16/84; PEN intermediate strains (PIS) ($n=191$): LVX: 1/2/98.4; AMX: 0.5/1/74.3; ERY: 32/64/27.7; PEN-resistant strains (PRS) ($n=153$): LVX: 1/2/98; AMX: 2/4/8.5; ERY: 64/64/14.4. *Haemophilus influenzae* (HI) ($n=751$): LVX: 0.03/0.03/99.9; AMC: 0.5/2/98.8. *Moraxella catarrhalis* (MC) ($n=268$): LVX: 0.06/0.06/100; AMC: 0.03/0.12/100; ERY: 0.12/0.25/99.6.LVX shows a good activity against SP whatever the susceptibility to PEN. PEN non susceptible strains are divided in 55.5% of intermediate strains and 44.5% of resistant strains. Susceptibility of PRS to AMX is low and 17% of these strains shows a high level of resistance to AMX (MICs ≥ 4 mg/L). Resistance rates of SP to ERY are high, especially among PIS and PRS. About 33.2 and 92.1% of HI and MC isolates, respectively, are β-lactamase producers. Among the drugs tested, LVX has the best activity.
Conclusions: The results of this study show that LVX has a good activity against the most frequent RTP, and that resistance rates of SP to this antibiotic remain very low, whatever their susceptibility to penicillin. Resistance to penicillin and macrolides is high among SP strains. High level of AMX resistance becomes frequent among PRS of SP. Production of β-lactamase concern one-third of HI isolates and almost all MC strains. In conclusion, LVX appears to be a drug of choice for the treatment of respiratory tract infections.

P1111 National in vitro susceptibility of community-acquired respiratory tract pathogens (SEPRA)

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Objective: To evaluate Italian susceptibility data of antimicrobial agents against community-acquired respiratory tract pathogens.
Materials and methods: This multicenter, comparative antimicrobial in vitro susceptibility study included 10 Italian sites that have contributed to collection of clinical isolates from blood or sputum cultures. The broth microdilution method (Sensititre – Trek Diagnostic System) was used to determine the MIC of each of the study isolates, according to NCCLS guidelines (2001), and adequate analysis controls were utilized.
Results: Preliminary results on MIC_{90} (mg/L), MIC ranges and susceptibility rates (%) of the most common respiratory tract pathogens, collected during 2000 and 2001 are shown in Table 1.

Table 1

	S. pneumoniae ($n=348$)			*H. influenzae* ($n=270$)			*M. catarrhalis* ($n=81$)		
Antimicrobial agents	MIC_{90}	Range	S %	MIC_{90}	Range	S %	MIC_{90}	Range	S %
Penicillin	0.25	0.03–4	83.3	–	–	–	–	–	–
Ampicillin	–	–	–	2	0.12–32	90.4	–	–	–
Amoxicillin	1	0.12–32	98.6	–	–	–	–	–	–
Co-amoxiclav	0.5	0.12–16	96.5	1	0.03–32	98.5	1	0.12–32	98.8
Cefuroxime	1	0.12–8	89.9	2	0.12–32	98.9	4	0.12–32	96.3
Ceftriaxone	0.5	0.12–4	92.0	1	0.12–64	97.8	2	0.12–64	95.1
Clarithromycin	64	0.12–64	61.2	8	0.12–64	94.4	2	0.12–64	98.8
Co-trimoxazole	4	0.12–64	74.4	4	0.25–16	78.9	1	0.25–8	98.8
Levofloxacin	1	0.008–16	98.6	0.12	0.008–16	98.9	0.12	0.008–0.5	100
Moxifloxacin	0.25	0.002–4	98.6	0.06	0.002–8	98.9	0.06	0.002–0.12	–

Conclusions: These results demonstrate that the major community-acquired respiratory tract pathogens in Italy are susceptible to new fluoroquinolones, such as moxifloxacin, and confirm that these antimicrobials are an excellent option for empirical treatment.

P1112 Alexander Project 2000 Europe: comparative in vitro activity of 17 nonquinolone antimicrobials against 3380 adult community-acquired RTI (CARTI) isolates

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Objectives: The Alexander Project has collected adult CARTI isolates, including *Haemophilus influenzae* (HI), *Streptococcus pneumoniae* (SP), *Moraxella catarrhalis* (MC) and *Streptococcus pyogenes* (SPY), since 1992 in order to determine reliable surveillance data for use in guiding empirical therapy.
Methods: MICs and percentage susceptibilities of isolates collected in Europe in 2000 were determined against a range of nonquinolone antimicrobials according to strict NCCLS methodology and quality control procedures.

Results: Susceptibility patterns varied for individual countries. Data for Europe are summarized in the table.

	Mode MIC, MIC90 (mg/L) and %S										
	HI (1439)			SP (1707)			MC (145)[a]		SPY (89)		
Antimicrobial	Mode	MIC_{90}	%S	Mode	MIC_{90}	%S	Mode	MIC_{90}	Mode	MIC_{90}	%S
Penicillin	–	–	–	0.015	2	81.5	–	–	0.015	0.015	100
Ampicillin	0.25	8	88.5	–	–	–	4	16	–	–	100[b]
Amoxicillin	0.5	8	89.3	0.03	1	98.2	4	16	0.015	0.015	100[b]
Amox/clav	0.5	1	100	0.03	1	98.4	0.12	0.25	0.015	0.015	100[b]
Cefaclor	2	8	92.6	1	64	72.4	2	8	0.5	0.5	100[b]
Cefuroxime	0.5	2	99.1	0.03	4	87.3	1	2	0.015	0.015	100[b]
Cefixime	0.03	0.06	100	0.25	16	–	0.06	0.5	0.12	0.12	–
Ceftriaxone	0.004	0.008	100	0.03	1	89.2	0.015	0.5	0.015	0.03	100[b]
Cefprozil	2	8	94.3	0.12	8	88.4	2	8	0.12	0.12	100[b]
Cefdinir	0.25	0.5	99.4	0.06	4	87.1	0.25	0.25	0.015	0.015	100[b]
Erythromycin	4	8	–	0.06	>32	80.2	0.5	0.5	0.06	0.06	93.3
Clarithromycin	8	16	81.9	0.03	>32	80.3	0.5	0.5	0.03	0.06	–
Azithromycin	1	2	100	0.12	>32	80.3	0.12	0.12	0.12	0.25	93.3
Clindamycin	–	–	–	0.06	>2	84.7	–	–	0.06	0.06	97.8
Chloramphenicol	0.5	1	98.8	4	8	89.6	0.5	2	2	4	100
Doxycycline	0.5	0.5	99.8	0.12	8	79.6	0.12	0.25	0.12	4	89.9
Cotrimoxazole	0.06	4	85.3	0.25	8	71.4	0.25	0.25	0.25	0.25	–

%S, percentage susceptibility; amox/clav, amoxicillin/clavulanic acid.
[a]No NCCLS breakpoints defined for MC.
[b]Susceptibility based on penicillin.

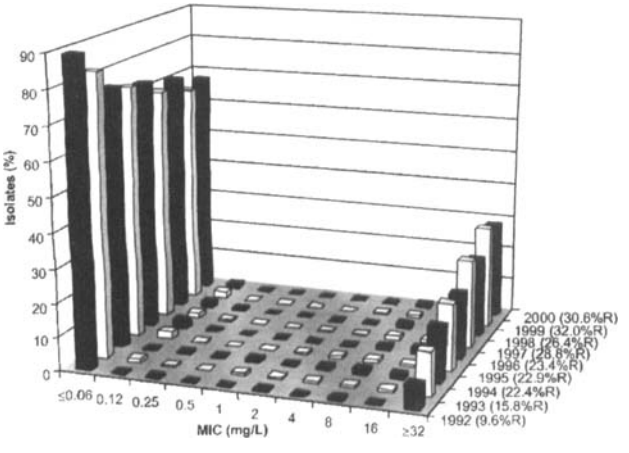

Figure 1

Conclusions: Amoxicillin/clavulanate was the only antimicrobial to which >90% of both SP and HI isolates were susceptible. Overall, the most potent antimicrobial (MIC[90] ≤ 2 mg/L) against the four CARTI pathogens was amoxicillin/clavulanate and the least potent were the macrolides and cefaclor.

P1113 The Alexander Project in Europe: decreased susceptibility of isolates of *Streptococcus pneumoniae* to macrolides 1992–2000

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Objectives: The Alexander Project has collected adult community-acquired respiratory tract infection (CARTI) isolates for 9 consecutive years in order to determine reliable surveillance data for use in guiding empirical therapy. Centers in five European countries have participated throughout the period 1992–2000: France, Germany, Italy, Spain and UK.

Methods: MICs were determined against erythromycin, clarithromycin and azithromycin according to strict NCCLS methodology and quality control procedures.

Results: Changes in the MIC of erythromycin for *Streptococcus pneumoniae* isolates collected between 1992 and 2000 in France, Germany, Italy, Spain and UK are shown in the Figure 1. Resistance to erythromycin (MIC ≥ 1 mg/L) has increased from 9.6% in 1992 to 30.6% in 2000. Resistance to clarithromycin (MIC ≥ 1 mg/L) and azithromycin (MIC ≥ 2 mg/L) increased from 9.0% (1992) to 30.6% (2000) and from 9.1% (1992) to 30.6% (2000), respectively. Changes in erythromycin resistance for individual countries were, from 1992 to 2000: France, 25.4–58.6%; Germany, 1.0–8.3%; Italy, 1.4–31.4%; Spain, 9.7–28.7% and UK, 2.4–11.9%.

Conclusions: Resistance to macrolides has increased significantly throughout the lifetime of the Alexander Project. Phenotypically, the increased MICs are indicative of MLS (B) resistance. This makes the continued use of these agents in the empirical treatment of CARTI doubtful.

P1114 In vitro activities of gemifloxacin and four other fluoroquinolones against contemporary respiratory tract pathogens recovered from outpatients in Germany

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Objectives: Gemifloxacin is an enhanced-affinity fluoroquinolone with potent activity against both Gram-negative and Gram-positive organisms that cause community-acquired respiratory tract infections (CARTI). We compared the in vitro activity of gemifloxacin with that of moxifloxacin, levofloxacin, gatifloxacin, ciprofloxacin and seven other licensed antimicrobials against *Streptococcus pneumoniae* (SP), *Haemophilus influenzae* (HI) and *Moraxella catarrhalis* (MC).

Methods: Organisms were recovered from outpatients in eight geographic areas in Germany between October 2000 and April 2001. A total of 158 SP, 135 HI and 86 MC isolates were recovered. Susceptibilities were determined by broth microdilution, according to NCCLS standards, in a central laboratory.

Results: All SP isolates were susceptible to penicillin. Macrolide resistance was detected in 4.4% of SP isolates. All HI isolates were susceptible to amoxicillin. Penicillin resistance was evident in 95.3% of MC isolates. $MIC_{50/90}$ values (mg/L) and percent susceptibilities (%S) are given in the table.

	SP (n=158)			HI (n=135)			MC[a] (n=86)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Gemifloxacin[b]	≤0.03	0.06	100	≤0.03	≤0.03	100	≤0.03	≤0.03	100
Moxifloxacin	0.125	0.25	100	≤0.03	0.06	100	0.06	0.06	NA
Levofloxacin	0.5	1	100	≤0.06	≤0.06	100	≤0.06	≤0.06	100
Gatifloxacin	0.25	0.5	100	≤0.06	≤0.06	100	≤0.06	≤0.06	100
Ciprofloxacin	1	2	NA	≤0.06	≤0.06	100	≤0.06	≤0.06	100
Penicillin	≤0.06	≤0.06	100	0.25	0.25	NA	4	8	4.7
Amoxicillin	≤0.06	≤0.06	100	0.25	0.5	100[c]	2	4	NA
Amox/clav	≤0.06	≤0.06	100	0.25	0.25	100	≤0.06	0.125	100
Cefuroxime	≤0.25	≤0.25	100	0.5	0.5	100	0.5	1	100
Cefpodoxime	≤0.06	≤0.06	100	≤0.06	≤0.06	100	0.25	0.5	100
Azithromycin	≤0.125	≤0.125	95.6	1	1	100	≤0.125	≤0.125	100
Clarithromycin	≤0.25	≤0.25	95.6	8	8	97	≤0.25	≤0.25	100

NA, no NCCLS breakpoint available, Amox/clav, amoxicillin/clavulanic acid.
[a]Breakpoint for *Staphylococcus aureus* were applied.

Among the fluoroquinolones tested, gemifloxacin was the most potent agent against *S. pneumoniae*. The rank order of potency (MIC_{90} [mg/L]) was gemifloxacin (0.06) > moxifloxacin (0.25) > gatifloxacin (0.5) > levofloxacin > ciprofloxacin (2). Gemifloxacin also showed excellent in vitro activity against HI and MC (MICs for all strains ≤0.03 mg/L).

Conclusion: The excellent in vitro potency of gemifloxacin suggests that it would be a beneficial therapeutic option for the treatment of CARTI.

P1115 In vitro emergence of spontaneous and multistep resistance in *S. pneumoniae, Moraxella catarrhalis* and *H. influenzae*

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Objectives: In vitro study to investigate the spontaneous emergence of resistance in *Streptococcus pneumoniae, Moraxella catarrhalis* and *H. influenzae* to faropenem (FAR), amoxycillin (AMX), amoxycillin/clavulanate (AMC), cefuroxime (CFX), and clarithromycin (CLA).

Methods:

- *Spontaneous emergence of resistance*: Resistant variants were elicited by spreading an inoculum of 10^7–10^8 cfu/mL over BHI agar plates supplemented with 5% bovine blood (*S. pneumoniae, M. catarrhalis*) or HTM agar plates (*H. influenzae*) and adding the test drugs: at 2 × MICs for each test organism [*H. influenzae* (n = 4, AMX and AMC-resistant strains included), *S. pneumoniae* (n = 6, penicillin and quinolone-resistant strains included), *M. catarrhalis* (n = 3, β-lactamase positive strains included)]. Following an overnight incubation at 37 °C the number of colonies growing on the drug-containing plates was counted. The frequency of resistant variants was calculated as the ratio of resistant variants arising after overnight incubation to the number of cfu originally inoculated.
- *Multistep emergence of resistance*: Bacteria were grown overnight in BHI or Fildes broth containing two-fold dilutions of the antibiotics. After an overnight incubation a second set of serial drug dilutions was performed using an inoculum from the test tube containing the highest drug concentration permitting visible growth. The transfer was repeated daily over a total period of 7 days.

Results: Following exposure of the indicator organisms, the following resistance mutation frequencies were observed.

Organism	FAR	AMX	AMC	CFX	CLA
S. pneumoniae	$<2.5\times10^{-7}$ to 1.2×10^{-4}	$<4.5\times10^{-8}$ to $<1.3\times10^{-6}$	$<6\times10^{-8}$ to 7×10^{-5}	$<1.6\times10^{-8}$ to 6×10^{-6}	$<7\times10^{-8}$ to 9.3×10^{-4}
H. influenzae	$<6\times10^{-7}$ to 3.4×10^{-3}	$<2.4\times10^{-8}$ to 1.5×10^{-5}	$<2\times10^{-8}$ to 5×10^{-6}	1.6×10^{-6} to 5.9×10^{-5}	n.t.
M. catarrhalis	$<7.5\times10^{-8}$ to 3.8×10^{-6}	$<3.5\times10^{-8}$ to 1.2×10^{-3}	$<1.5\times10^{-7}$ to 8×10^{-5}	$<7\times10^{-8}$ to 1.1×10^{-6}	3.4×10^{-7} to 4.4×10^{-5}

< = lower than the original inoculum; n.t. = not tested.

Faropenem did not induce multistep resistance in the test strains (except for one strain of AMC-resistant *H. influenzae*).

Conclusions: Overall, the spontaneous emergence of drug resistance to faropenem was similar to comparator drugs. However, for certain strains, comparator drug induced resistance mutations up to 1000-fold more frequently than with faropenem. In general Faropenem did not induce multistep resistance.

P1116 Telithromycin is highly active against isolates of *S. pneumoniae* and *H. influenzae* from pediatric isolates

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Objective: To investigate susceptibility of telithromycin (TEL) against *S. pneuminiae* including resistance to penicillin (PRSP) and erythromycin (ERSP) and *H. influenzae* isolated from pediatrics.

Introduction: The emergence of resistant strains of *S. pneumoniae* and *H. influenzae*, which are the primary pathogens of pediatric community-acquired respiratory tract infections (RTIs), to existing antibiotics has become an important treatment issue. In Japan, particularly, the rate of macrolide-resistant strains of *S. pneumoniae* has been growing rapidly. TEL, a novel ketolide with enhanced antibacterial activity, was developed by changing the structure of the macrolide clarithromycin (CAM). Owing to its unique structure, TEL demonstrates high in vitro susceptibility to PRSP and ERSP and also demonstrates good clinical efficacy against community-acquired RTIs caused by *S. pneumoniae* in adults.

Methods/results: The susceptibility of 60 isolated strains of *S. pneumoniae* and 56 isolated strains of *H. influenzae* to macrolide antibiotics and TEL, isolated from children in Japan, was investigated. TEL demonstrated high susceptibility to resistant *S. pneumoniae* including PRSP and ERSP. The $MIC_{50}/_{90}$ and MIC range for TEL against *S. pneumoniae* was 0.03/0.063 μg/mL and ≤0.01–0.25 μg/mL, respectively, versus >128/>128 μg/mL and ≤0.01–>128 μg/mL, respectively, for clarithromycin (CAM), >128/>128 μg/mL and ≤0.01–>128 μg/mL, respectively, for azithromycin (AZM) and 1/>128 μg/mL and 0.063–>128 μg/mL, respectively, for rokitamycin (RKM). TEL demonstrated good susceptibility to macrolide-resistant *H. influenzae* including BLNAR (β-lactamase-negative ampicillin-resistant *H. influenzae*). The $MIC_{50}/_{90}$ and MIC range of TEL against *H. influenzae* was 2/4 and 0.25–4 μg/mL, respectively, versus 8/16 and 2–16 μg/mL, respectively, to CAM, 1/2 and 0.125–4 μg/mL, respectively, to AZM and 16/32 and 2–32 μg/mL, respectively, to RKM.

Conclusion: TEL showed good susceptibility to Japanese pediatric isolates including PRSP, ERSP and macrolide-resistant *H. influenzae*. TEL can, therefore, be expected to demonstrate good clinical efficacy for the treatment of pediatric community-acquired RTIs in Japan.

P1117 Evaluation of in vitro activities of oral macrolides and β-lactam antibiotics against respiratory tract pathogens

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The 'in vitro' activities of azithromycin, clarithromycin, amoxicillin, amoxicillin/clavulanic acid, cefprozil, and cefaclor were evaluated against 597 strains isolated from upper and lower respiratory specimens from outpatients without age restriction from September 1999 to July 2000. The analyzed samples were as follows: 247 *H. influenzae*, 147 *S. aureus*, 114 *S. pneumoniae*, 51 *S. pyogenes*, and 38 *M. catarrhalis*. The Minimum Inhibitory Concentration (MIC) was determined by E-test methodology and interpreted following NCCLS criteria. Of 247 *H. influenzae*, 9.7% were positive for β-lactamase production. Among the β-lactamase producers, all (100%) were susceptible to amoxicillin/clavulanic acid and cefaclor, 98% to cefprozil, 96% to azithromycin, and 90% to clarithromycin. Of 147 *S. aureus* analyzed, 100% were susceptible to oxacillin, amoxicillin/clavulanic acid, cefprozil, and cefaclor, 71% to clarithromycin, and 68% to azithromycin. Among the *S. pneumoniae* (*n* = 114), full resistance to penicillin was not observed, whilst 14 (12.3%) presented intermediate resistance to penicillin. Overall, 100% were susceptible to amoxicillin and amoxicillin/clavulanic acid, 99% to cefprozil and cefaclor, 88% to azithromycin, and 86% to clarithromycin. Among the pneumococcal isolates with intermediate MIC to penicillin, 21.4% were resistant to azithromycin and 28.6% to clarithromycin. Among the 51 *S. pyogenes*, 8% presented resistance to azithromycin and clarithromycin, and all *M. catarrhalis* were found to be β-lactamase producers.

P1118 Antimicrobial susceptibility patterns of *Haemophilus influenzae*

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Objectives: To determine the susceptibility of 467 *Haemophilus influenzae* strains isolated from sputum/bronchial secretions during a period of 3 years (1999–2001) to 13 antimicrobial agents.

Methods: The susceptibilities to antibiotics were performed by microdilution method according to NCCLS guidelines. *H. influenzae* ATCC 49247

and *H. influenzae* ATCC 49766 were used as internal quality control strains.
Results: The MIC_{50}/MIC_{90} (mg/L) appeared, respectively: ampicillin 0.25/1, amoxicillin/clavulanic 0.25/1, cefaclor 2/8, cefuroxime 0.5/2, ceftriaxone <0.015/<0.015, ciprofloxacin 0.008/0.015, levofloxacin 0.015/0.03, gatifloxacin 0.008/0.015, azithromycin 2/2, clarithromycin 16/16, tetracycline 0.5/1, trimethoprim/sulfamethoxazole 0.12/8 (resistance 28%), Imipenem 0.12/0.25. A percentage of 7% of strains produced β-lactamase (range of MICs 2–>16).
Conclusions: (1) The production of β-lactamase was responsible for the 7% of resistant *H. influenzae* strains. (2) *H. influenzae* were highly sensitive to quinolones (MIC 0.008–0.03 mg/L) and imipenem (MIC 0.12–0.25 mg/L). (3) Azithromycin seems to be more effective than clarithromycin to the tested isolates. (4) The behavior of *H. influenzae* isolates to trimethoprim/sulfamethoxazole was very encouraging (sensitivity 72%).

P1119 Antibiotic resistance amongst ocular isolates of *Haemophilus influenzae* from outpatients in an eye hospital, Riyadh

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Objectives: *Haemophilus influenzae* is a renowned respiratory pathogen. International surveillance studies show the prevalence of antibiotic-resistant isolates is increasing, but very little is known about resistance in strains causing eye infections or isolated as commensals from normal eyes. The aim of this study was to investigate the prevalence of resistance to 10 antibiotics in ocular isolates of *H. influenzae*.
Methods: Two groups (pathogen and commensal) of ocular isolates of *H. influenzae* were collected during a 7-month period (November 1999 to May 2000) from patients presenting with eye infection and from healthy employees at the King Khaled Eye Specialist Hospital. The pathogen group ($n = 85$) yielded 100 discrete ocular isolates of *H. influenzae*. The commensal group ($n = 80$) yielded 18 isolates. Susceptibility testing was by agar dilution and the E-test (AB Biodisk). Nitrocefin was used to detect β-lactamases. Polymerase chain reaction was used to serotype strains and genotype β-lactamase positive isolates. Biotyping (pathogen group), was by API NH strips (BioMerieux).
Results: By NCCLS criteria all 118 isolates were susceptible to five antibiotics: ceftazidime, cefuroxime, ciprofloxacin, ofloxacin, and amoxicillin/clavulnic acid. E-test results were comparable to agar dilution. Resistance (% strains) was detected to the other five antibiotics: ampicillin (27%); tetracycline (19%), chloramphenicol (14%), clarithromycin (2%); and erythromycin (1%). All isolates were non-capsulated. A total of 31 isolates produced TEM-1 type β-lactamase; the majority of these, 20/31 (65%) were biotype II, 4/31 (13%) were biotype III, and 4/31 (13%) were biotype IV, and 3/31 (13%) were from the commensal group.
Conclusion: In marked contrast to respiratory infections, capsulated *H. influenzae* were not isolated from eye infections. β-lactamase positive strains were common and there was a significant level of resistance to tetracycline and chloramphenicol when compared to nonoccular reports. Further studies are recommended to characterize the subgroup of *H. influenzae* causing eye infections to determine optimum treatment as data from respiratory isolates may not be applicable.

P1120 Epidemiological studies and antibiotic susceptibility of invasive *Haemophilus influenzae* infection in Taiwan

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Objectives: To determine the epidemiological data and antibiotic susceptibility of invasive *H. influenzae* infection in Taiwan.
Methods: From 1996 to 2000, 148 patients' general data were analyzed and 114 *H. influenzae* strains isolated from pediatric patient's blood, CSF or sterile sites. The MICs of 10 antibiotics were determined by the broth microdilution test. The serotype was performed by the slide agglutination test.
Results: The incidence was very low, 1.6/100.000 population aged less than 15 years. The majority cases (95%) less than 5-year-old, and 7% cases less than 2-month-old. The characteristic of the diseases showed 63.9% was meningitis, 16.4% was pneumonia, and 12.3% was sepsis. The serotype revealed 82% was type b, 3% was non-type b and 15% was nontypeable. The β-lactamase production rate was 65.8%. The antibiotic sensitivity revealed the resistance rate for ampicillin, amoxicillin/clavulanate, clarithromycin, chloramphenicol and tetracycline were 65.8, 2.6, 15.8, 36.0 and 46.5%, respectively. All strains sensitive to the second and the third cephalosporins.
Conclusion: Extremely high ampicillin resistance of invasive *H. influenzae* were isolated from pediatric patients in Taiwan. The low incidence and low rate of type b strains were different from western countries.

P1121 Antimicrobial susceptibility of *Haemophilus influenzae* clinical isolates collected from four European countries, 2000–2001

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Objectives: *Haemophilus influenzae* (HI) is a common respiratory pathogen in adult and pediatric patients worldwide. Empiric treatment of patients with respiratory infections is often a β-lactam, macrolide, or fluoroquinolone, however, the prevalence of β-lactamase (BL) production in some countries has often eliminated β-lactams such as ampicillin and amoxicillin as potential therapies. We examined current levels of antimicrobial resistance among HI recently isolated in four European countries.
Methods: During 2000–2001, 840 isolates of HI were isolated from patient specimens collected in hospitals in France, Germany, Italy, and Spain and submitted to our central laboratory for antimicrobial susceptibility testing. The identification of each isolate was confirmed and each was tested for susceptibility to ampicillin (AMP), amoxicillin-clavulanate (AC), cefaclor (CFL), cefuroxime (FUR), azithromycin (AZI), clarithromycin (CLR), roxithromycin (ROX), ciprofloxacin (CIP) and levofloxacin (LEV) by broth microdilution (NCCLS, M7-A5). MICs were interpreted using the NCCLS 2000 published breakpoints, where available. BL production was tested using the chromogenic substrate nitrocefin.
Results: Overall, BL production was 18.8%. By country, BL production was 8.1% (range, 4.8–11.9% per site) in Germany, 8.6% (range, 2.4–19.0% per site) in Italy, 23.8% (range, 11.9–42.9% per site) in Spain, and 34.8% (range, 28.6–44.4% per site) in France. All isolates were susceptible (S) to AC, CIP, and LEV. Among the macrolides tested, AZI was the most active (MIC_{90}, 2 mg/L; 99.0–100% S), followed by CLR (MIC_{90}, 16 mg/L; 76.7–82.9% S) and ROX (MIC_{90}, 16 mg/L; no interpretive criteria). All of the macrolides tested were equally active against both BL-positive and BL-negative isolates. By age, BL production was highest among patients ≤14 years in France (41.6%), Spain (29.3%), and Germany (9.1%), and among patients ≥65 years in Italy (7.1%). BL production among isolates collected from respiratory sources ranged from 6.0% in Germany to 34.0% in France. The activity of the macrolides in all countries was consistent, regardless of age, specimen source, or BL production.
Discussion: BL production among HI varied significantly between countries. In all countries, HI remained highly susceptible (>99%) in vitro to AC, FUR, CIP, LEV, and AZI.

P1122 Azithromycin, clarithromycin and roxithromicin: in vitro efficacy and cross-resistance among *Haemophilus influenzae*-isolates in Finland

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Objectives: The consumption rates of azithromycin, clarithromycin and roxithromicin in Finland in the year 2000 (DDD per 1000 inhabitants/day)

were 0.76, 0.43 and 0.69, respectively (National Agency for Medicines and KELA. 2000: Finnish Statistics on Medicines) and have slightly increased in recent years. Seventy-seven percent of macrolides are prescribed for upper respiratory tract infections (otitis, sinusitis and acute bronchitis) (http://www.mikstra.fi), which is nonoptimal according to the Current Care guidelines (http://www.duodecim.fi/english, Current Care). According to Nissinen et al. (1995) the in vitro efficacy of erythromycin against *Haemophilus influenzae* isolates in Finland was marginal in 1988–90 (11.6% of the nontypeable strains from middle ear were susceptible). However, during the past few years the new macrolides (azithromycin, chlarithromycin and roxithromicin) have replaced erythromycin in clinical practice but the degree of resistance among these macrolides in Finland is not known. In the present study the in vitro efficacy of the macrolide group against *Haemophilus influenzae* was evaluated by analyzing their activity and cross-resistance among consecutive clinical isolates in Finland.

Methods: The strains ($n=376$) were collected from seven clinical microbiology laboratories in Finland. The MIC-values were determined using the E-test method (Biodisk, Sweden) and HTM-agar. The disc diffusion method and the breakpoints used for SIR-interpretations are based on the most recent NCCLS-guidelines or previously published data in case of roxithromycin (Erwin and Jones, 1993). The results were analyzed using the WHONET5.1-computer program.

Results and conclusions: According to the E-test results, 98% of the isolates were susceptible to azithromycin, 87% to clarithromycin and 97% to roxithromicin. On the basis of the MIC frequency distribution curves azithromycin was the most potent drug among the isolates included in this study. Clarithromycin and roxithromycin were less active. The E-test results also indicate that clarithromycin can be used as a representative of the macrolide group for *Haemophilus influenzae*, because no false susceptible results for azithromycin and roxithromicin are detected. When the disc diffusion method is used, chlarithromycin also predicts the susceptibility of azithromycine (no false-resistance results), whereas the use of azithromycin would not reveal 1% of chlarithromycin-resistant isolates.

P1123 Effect of moxifloxacin on bacterial virulence mechanisms in comparison with amoxicillin, clarithromycin and ceftriaxone

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Objectives: To compare moxifloxacin (MFX) activity with that of amoxicillin (AMX), clarithromycin (CLR) and ceftriaxone (CRO) against Gram-positive and Gram-negative bacteria, and effects of subMICs on bacterial virulence factors.

Methods: In vitro antimicrobial activities against *Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Haemophilus influenzae, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa* were evaluated by broth microdilution method. The effects of subMICs (1/4 and 1/8 × MIC) of antibiotics on bacterial adhesion to epithelial cells and fibroblasts were studied by incubating *S. pneumoniae, M. catarrhalis, S. aureus* and *H. influenzae*, previously exposed to antibiotics, with either human oral cells or MRC-5 monolayers. Adhesion was determined by counting bacteria adhered to 100 cells by scanning electron microscopy (SEM). Alterations in bacterial morphology induced on *S. aureus, H. influenzae* and *P. aeruginosa* by growth with subMICs (from 1/4 to 1/32 × MIC) of antibiotics were also evaluated by SEM.

Results: The MICs confirm that MFX has a marked activity against Gram-positives, while retaining potency against Gram-negative organisms. Although similar activities were observed for MFX, CRO and AMX against *S. pneumoniae* strains, none of the comparators were as effective as MFX against *S. aureus*. Among Gram-negative bacteria, MFX was mostly active against *Enterobacteriaceae* with the exception of *P. mirabilis*, showing marked potency against *H. influenzae* and *M. catarrhalis*. Only CRO exhibited a similar or slightly greater (*P. mirabilis*) or lower (*P. aeruginosa*) activity against *Enterobacteriaceae, H. influenzae* and *M. catarrhalis*. Pre-incubation with subMICs of the antibiotics significantly decreased the number of adhering bacteria in a generally dose-dependent manner. Buccal cells bearing low numbers of *M. catarrhalis* and *S. pneumoniae* were more frequently found in MFX-treated bacteria (1/8 × MIC) than in the other groups. Moreover, MFX strongly reduced the number of *S. pneumoniae* adhered to MRC-5 cells, when compared with the other drugs. MFX induced cellular elongation in 29% of *H. influenzae* at 1/4 × MIC and filamentation in 33, 27 and 13% of *P. aeruginosa* at 1/4, 1/8 and 1/16 × MIC, respectively.

Conclusions: MFX, by interfering with some bacterial pathogenic factors, displays a remarkable antimicrobial profile, representing a potentially advance in chemotherapy against Gram-positive and Gram-negative bacteria.

P1124 In vitro activity of ertapenem against community-acquired respiratory pathogens

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Objective: The in vitro efficacy of ertapenem, a new β-lactam drug, against a large number (over 500) of community-acquired respiratory pathogens was studied.

Methods: A total of 180 *S. pneumoniae* (including 50 penicillin-intermediate [Pen-I] and 30 penicillin-resistant [Pen-R]), 100 *S. pyogenes*, 70 *H. influenzae* (20 β-lactamase positive [bl+]), 70 *M. catarrhalis* (50 bL+), 100 methicillin-susceptible *S. aureus* (MSSA) and 30 *K. pneumoniae* were analyzed. With ertapenem, penicillin, ampicillin, amoxicillin, coclavulanate, cefuroxime, cefotaxime, ceftriaxone, clarithromycin, azithromycin, clindamycin, ciprofloxacin, levofloxacin, gentamicin, amikacin, vancomycin, tetracycline, chloramphenicol and rifampin were tested on the appropriate organisms. MICs were determined by a broth microdilution method (NCCLS, 2001). Bactericidal activity of ertapenem alone (4 × MIC) and in combination with gentamicin, vancomycin, clarithromycin, rifampin and levofloxacin (0.5 × MIC) were assessed against 15 *S. pneumoniae*.

Results: Against Pen-S *S. pneumoniae*, ertapenem displayed a potent activity (MIC_{90} 0.03 mg/L). Ertapenem showed MIC_{90} of 0.5 and 2 mg/L against Pen-I and Pen-R strains, respectively, with MIC_{90} values from two- to four-fold lower than those of other betalactams. *S. pyogenes*, irrespectively of their erythromycin-resistance phenotype, were inhibited by ertapenem at an MIC_{90} value of ≤0.015 mg/L and by the other drugs (range of MIC_{90} ≤ 0.015–>64 mg/L). Against β-lactamase-negative (bl–) and bl+ *H. influenzae* ertapenem showed MIC_{90} = 0.12 and 0.5 mg/L, respectively. MIC_{90} of the other drugs ranged from ≤0.0075 (ciprofloxacin) to >16 mg/L (ampicillin). Toward bl+ and bl– *M. catarrhalis* ertapenem demonstrated MIC_{90} of 0.03 and ≤0.0075 mg/L. The other antibiotics showed MIC_{90} values ranging from 0.015 mg/L (rifampin) to 8 mg/L (ampicillin). Against MSSA ertapenem was one of the most potent drugs (MIC_{90} 0.5 mg/L) together with rifampin, quinolones and tetracycline (MIC_{90} ranging from ≤0.03–0.5 mg/L). Time-kill curves showed a bactericidal activity of ertapenem against all pneumococci tested (>3 log in 24 h). The combination of ertapenem with the other drugs revealed prevalent synergistic effect and only three cases of indifference were noted. No case of antagonism was found.

Conclusion: Ertapenem, showing a potent in vitro broad-spectrum activity against the most common bacterial respiratory pathogens, may be useful in the treatment of community acquired respiratory infections.

In vitro evaluation of antiseptics

P1125 Evaluation of selected antiseptics effectiveness using a novel plate suspension test

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Objectives: To assess reduction factors (RF) of selected antiseptics using a novel plate suspension test.
Methods: Four antiseptics (D) 1% dioxydine (quinoxaline); (CHX) 0.05% chlorhexidine digluconate; (P) 1% poviargole (silver colloidal solution + PVP); and (K) 1% katapole (Ouats + PVP) were tested against *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 15442, and *C. albicans* ATCC 10231. In the test, metal cylinders (about 2.0 cm^3 capacity) were placed individually onto blood agar Petri plates and 0.9 mL of an antiseptic to test was carefully pipetted into the cylinder correspondingly, then an inoculum of 100 Ýl from bacterial suspension (10^8 cfu/mL) was added to antiseptic per cylinder. After each specified exposure period (5, 15, 30, 60 min and 3 h), a volume of 0.7 mL of reaction mixture was removed away, and the 200 Ýl of neutralizer was added to the rest. In 5 min, the cylinder was removed from blood agar and the neutralizer-reaction mixture poured was inoculated on the same agar plate by surface-plating technique. The cfu was counted after 18–24 h overnight incubation and the RF of each preparation was calculated.
Results: All the antiseptics listed were revealed to demonstrate a fair effectiveness. From investigated four antiseptics, K possessed relatively higher activity. Mean K RF was 5.7 (2.3 to >6), followed by D 4.9 (1.9 to >6), CHX 4.6 (1 to >6) and P 3.1 (1–5.2). It was established that all the antiseptics to be effective in the first 5–15 min, reducing microbial cell counts by 2.5–6 log and more. The investigated compounds showed various RF values towards Gram-positive and Gram-negative strains studied. The most effective antiseptics were: K (RF mean 6.2), CHX (5.5) against *S. aureus*; D (7.5), K (6.0), CHX (5.9) against *E. coli*; D (5.3), K (4.9), and CHX (4.8) against *P. aeruginosa*. P gave the relatively lower activity (2.7–4) against test bacteria used. The lower RF average values of 4.3, 2.7, 2.1, and 1.7 were demonstrated against *C. albicans* by K, P, D and CHX, respectively.
Conclusions: Our experience demonstrates the plate suspension method to be convenient for routine biocide testing. All the examined antiseptics were quite effective to decrease considerably microbial cell count within 5 min–3 h.

P1126 Efficacy of two different disinfectants against the spores and vegetative cells of *Bacillus subtilis*

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Objectives: Bacterial spores are highly resistant to inactivation by physical and chemical agents. Chemical sterilization agents are used in the healthcare setting to disinfect many medical instruments. Since *Bacillus* species has been noted to cause serious infections in hospitalized patients, the aim of the study is to investigate the effects of two traditional disinfectants Sirafan (quaternary ammonium compound) and sodium hypochlorite against the spores and vegetative cells of *Bacillus subtilis*.
Methods: The efficacy of these disinfectants was assessed by using a suspension test (DGHM), whereas the effects of these disinfectants on biofilm cells of the same strain on silicon disks were determined by conducting a carrier test method. Then the findings were compared. A 0.1, 0.5, 1 and 3% Sirafan, and 0.005, 0.01, 0.02, 0.03 and 0.04% sodium hypochlorite were applied. The eradication was evaluated in regards to the concentrations of disinfectants and time of exposure. The effect of disinfectants on the suspended spores and vegetative cells of *B. subtilis* was investigated between 1 and 30 min, whereas the sampling was done between 1 and 60 min for biofilms.
Results: The suspended vegetative cells of *B. subtilis* were eradicated by 0.5% Sirafan within 20 min and spores within 30 min. The biofilm of spores and cells of *B. subtilis* were eradicated by 0.1% Sirafan within 60 min. The suspended vegetative cells of *B. subtilis* were also killed by 0.01% sodium hypochlorite within 10 min and spores within 30 min by 0.02%. The biofilm of cells were eradicated by 0.01% sodium hypochlorite within 45 min, whereas the spores in biofilm form were eradicated within 60 min.
Conclusions: The results yielded from the suspension and carrier tests reveal that the eradication of biofilm-forming cells and spores demands for higher concentrations of disinfectants and longer time of exposure. These findings suggest the necessity to count on biofilm test results concomitant to suspension tests for reliable consideration.

P1127 In vitro antimicrobial activity of Greek *Thymus* bee-honeys

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Objectives: As a part of a systematic research on the chemical composition and on biological activities of the famous, through antiquity, Greek honeys, and as it has been observed an increasing interest in its use in traditional Mediterranean food; we report in this study the antimicrobial activities of six Greek *Thymus* bee-honeys samples produced in six different regions of Greece as well as of a pure isolated compound (a trihydroxy ketone) which was found to characterize the Greek thyme honey.
Methods: Our six thyme-honeys samples were studied microscopically (pollen analyzes) and predominant plants found were of *Thymus* species, ranging from 41.28 to 83.79%. From the five out of six examined samples, a trihydroxy ketone was isolated and identified by modern spectral means (1D, 2D NMR) as E-4-(1′,2′,4′-trihydroxy-2′,6′,6′-trimethylcyclohexyl)-but-3-en-2-one (1). The antimicrobial activities in all the studied samples and of pure isolated compound were determined, using the diffusion technique, by measuring the MIC of them against two Gram-positive bacteria: *S. aureus* and *S. epidermidis*, four Gram-negative bacteria: *E. coli*, *E. cloacae*, *K. pneumoniae* and *P. aeruginosa* and three pathogenic fungi *Candida albicans*, *C. tropicalis* and *C. glabrata*, all of them were strains of ATCC. Standard antibiotics were used in order to control the sensitivity of the tested organisms.
Results: Through the antimicrobial screening, the thyme honeys as well the characteristic triol (1) isolated, from five out of six studied samples, proved to be significantly active against all six tested bacteria as well as the tested fungi (MIC values: 0.04–2.1 mg/mL).
Conclusions: The results of our studies suggest that the activity of the thyme bee-honeys can be attributed, to a considerable degree, to the existence of the isolated trihydroxy ketone, which appeared to possess strong activities against all tested microbia. Besides, as this compound appears to occur only in thyme honeys, hence may be served as proof of floral source for these honeys which command considerable market premiums in Greece.

P1128 Activity of selected disinfectants against clinical strains of *Enterococcus* spp.

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Objectives: The ability of enterococci to prolonged survival on hands and environmental surfaces is a critical factor for transmission of these microorganisms from a person (patient or hospital staff) to the environment and then to another person. Thus, routine disinfection of environmental surfaces and handwashing with an adequate agent is mandatory. The aim of this study was to asses the activity of common antiseptic/disinfectant agents used against *Enterococcus* spp. strains isolated from hospitalized patients.
Methods: Aldewir (glutaric aldehyde: 4.0 g; ethanol: 26.0 g; isopropanol: 6.0 g), ftaloxid (magnesium monoperoxyphthalate; organic and inorganic acids; ammonium surfactants), terralin (alkyldimethylbenzylammomium chloride: 20.0 g; phenoxypropanol: 35.0 g), chlorhexidine gluconate, ethanol and isopropanol were tested against five strains of *E. faecalis* and five strains of *E. faecium*. The tests were carried out in accordance with European Standards (EN1040/97). The reduction factor (RF) of the bacteria counts exposed to the agents was determined.
Results: According to the European Standards, product shall demonstrate at least a 100 000 log (RF $\geq$ 5) reduction in viable counts of bacteria. It was found that aldewir, ftaloxid, terralin at the concentration and time recommended by the manufacturer as well as ethanol and isopropanol at the concentration of 70% (v/v) and time of 60 s reduced all enterococcal strain cell counts by over 100 000 (RF > 5). The activity of these agents did not decrease when they were tested at the lowered concentration of 20%. A 4% (v/v) aqueous solution of chlorhexidine gluconate produced a >5 log

cell reduction only for three of the 10 using strains. Additionally, the chlorhexidine gluconate was significantly less active at the lowered determination concentration of 20%; RF for all tested strains was <5.
Conclusion: Thus, it can be concluded that the disinfectant based on glutaric aldehyde, magnesium monoperoxyphthalate, quaternary ammonium compounds as well as ethanol and isopropanol show the required bactericidal activity against clinical strains of enterococci, whereas aqueous solution of chlorhexidine gluconate have the activity lower than required.

P1129 Old and new antiseptics against epidemic MRSA – are they effective?

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Objectives: Various antiseptic regimens are used to treat patients colonized with MRSA, however, treatment is not always successful. The objectives of this study were to determine whether treatment failure was due to reduced susceptibility to commonly used antiseptics and to investigate the effectiveness of two novel antiseptics.
Methods: The MIC for triclosan and chlorhexidine of a collection of 232 MRSA (72 EMRSA-15, 73 EMRSA-16 and 87 isolates of various other clones) isolated between 1997 and 2000 in 30 Scottish hospitals were tested using NCCLS criteria. The bactericidal activity of two commonly used antiseptic preparations, aquasept (2% triclosan) and corsodyl (1% chlorhexidine), was compared with that of two 'novel' antiseptics, Tea Tree Oil (Thursday Plantation) and Topro Aloe Vera (Alliance Products) against EMRSA-15, EMRSA-16 and six other Scottish MRSA (SMRSA) clones using a quantitative suspension test method based on the European Standard EN1040.
Results: The MIC_{90} of triclosan and chlorhexidine was 0.06 and 2 μg/mL, respectively, for EMRSA-15 and 0.03 and 2 μg/mL, respectively, for EMRSA-16. The majority of other Scottish MRSA clones were relatively sensitive, except for SMRSA99 (Iberian clone) and SMRSA117 which had triclosan MIC_{90} of 1 and 4 μg/mL, respectively, and chlorhexidine MIC_{90} of 4 mg/mL. In quantitative suspension tests, aquasept and corsodyl antiseptics gave complete kill of all eight clones at 30 s contact time. The bactericidal activity of Tea Tree Oil varied between different clones, however, complete kill (5 log reduction) was achieved for all clones after 30 min of contact time. Topro Aloe Vera gave a 2 log reduction at 30 s contact time, however, no further reduction was observed over a period of 6 h.
Conclusion: These results indicate that the antiseptics commonly used in hospitals have excellent activity against epidemic MRSA clones and that the 'novel' antiseptics, although not showing the same level of effectiveness, may be useful alternatives.

P1130 In vitro susceptibility to antimicrobial agents and disinfectants of group G streptococci

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Varese, I

Objectives: Group G streptococci (GGS) are isolated from different body sites, most commonly oro-pharyngeal, respiratory tract, and wound infections. Over the last year, GGS accounted for the 8.4% of strains isolated from skin lesions at the Center for Wound Infections of Varese, Italy. Due to the increase of severe infections caused by GGS, we evaluated the in vitro susceptibility of these microorganisms to several antimicrobial agents and disinfectants.
Methods: Over a 1-year period (November 2000–October 2001), we collected 20 GGS clinical isolates. Particularly, strains were recovered from wounds (10), oro-pharyngeal swabs (5), urinary tract (2), blood (2), and nasal swabs (1). Nine antibiotics, including ampicillin, vancomycin, ciprofloxacin, levofloxacin, chloramphenicol, erythromycin, clindamycin, cefotaxime and tetracyclines, were tested by means of both disk diffusion test and microdilution broth panels (Sceptor, Becton Dickinson, Sparks, MD). In addition, the strains isolated from wound infections were tested against five topic disinfectants, including chlorhexidine gluconate, merbromine, povidone iodine, silver nitrate and toluen-*p*-sulfochloramyde.
Results: All strains were susceptible to ampicillin, vancomycin, ciprofloxacin, levofloxacin, chloramphenicol, erythromycin, clindamycin and cefotaxime. On the contrary, only 8/20 GGS showed full susceptibility to tetracyclines. Among tested drugs, penicillin, ampicillin, cefotaxime, and clindamycin showed the lowest MIC_{90} with values of 0.06, 0.12, 0.25, and 0.25 mg/L, respectively. Tetracycline was the least-active drug, being characterized by a MIC_{90} of 8 mg/L. Susceptibility to disinfectants showed high variability, MBC90s of chlorexydine gluconate, silver nitrate and merbromine were 0.01, 0.01, and 0.02 g/L, respectively. MBC90s of povidone iodine and toluen-*p*-sulfochloramyde were 9.4 and 6.3 g/L, respectively.
Conclusions: Our study demonstrates that various antimicrobials and disinfectants are available for the treatment of tolerant organisms like group G streptococci. However, some patients resulted repeatedly positive for GGS regardless of an appropriate therapy. This finding suggests difficulties in eradicating infections, perhaps because these drugs fail to reach therapeutic levels at the site of infection. Finally, since few isolates were from blood cultures, an epidemiologic survey for GGS is very important in order to avoid spreading of drug resistances.

P1131 Antimicrobial activity of *Allium sativum* L.

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Iasi, RO

Introduction: Traditionally, garlic was used for centuries for dietary and medical properties. Organosulfur compounds and other secondary metabolites of garlic may by responsible for therapeutic effects.
Objectives: To investigate the antimicrobial activity of fresh garlic juice, aqueous and alcoholic extracts against different microbial species.
Methods: Antibacterial activity of garlic was tested by diffusion method in Mueller–Hinton agar. Different Gram-positive and Gram-negative strains, sensitive or multiresistant to antibiotics, were included in the study. Plates were inoculated with bacterial inocula according to the standard antibiogram procedure. Garlic samples were poured into the holes and the diameters of growth inhibition were measured after 24 h of incubation at 37 °C. The active substances were analyzed by gas chromatography and mass spectroscopy. The compounds were identified by comparing the mass spectra and retention indices with those in the literature.
Results: Garlic juice showed antimicrobial activity against all tested strains with the exception of *Pseudomonas aeruginosa*. The diameters of zones inhibition (mm) are followed: *Staphylococcus aureus* ATCC 25923: 40; *Sarcina lutea* ATCC 9341: 34; *Bacillus cereus*: 30; *Escherichia coli* ATCC 25922: 25; *E. coli* and *Salmonella* spp., extended-spectrum β-lactamase producers: 24 and 25, respectively; *Candida albicans*: 45. These values are significantly more higher that those obtained with both aqueous and alcoholic extracts.
Conclusions: Garlic juice exhibits a broad antimicrobial activity against Gram-positive and Gram-negative rods. Organisms that are multiresistant to antibiotics are susceptible to garlic. Among tested strains, only *P. aeruginosa* showed resistance to garlic.

P1132 A new procedure for the complete removal and the prevention of regrowth of biofilms in hemodialysis machines

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Introduction: Bacteria are known to attach to surfaces and create biofilms. Biofilms have been found in fluid pathways of hemodialysis systems, mostly inside silicone tubing of dialysis machines. These biofilms release bacterial pyrogens that can cross the dialysis membrane and induce chronic inflammatory disorders to patients in dialysis.
Objectives: Each of the pharmaceutical firms producing dialysis machines recommend a different cleaning procedure and the lack of literature about their action on biofilms led us to perform research studies to determine their efficacy on removing existing biofilms and on preventing further growth in order to give scientific arguments to dialysis area professionals.
Methods: The eight most commonly used cleaning procedures were tested on biofilms that were grown on silicone tubing in a reactor system that mimics dialysis machines. The first biofilm was grown with the addition of supplementary nutrients. The second biofilm was thinner and was grown without additional nutrients. The efficacy of the cleaning procedures was assessed on biofilm-fouled tubing samples in a close recirculating cleaning model. To study the preventive efficacy of those procedures, another model was set up in

which a contaminated media circulated for 4 h through the tubing and was then replaced by the cleaning chemicals every 4 h, until a mature biofilm develops on the control samples. Biofilm analyses were performed using staining methods and confocal microscopy, allowing the calculation of biofilm thickness and coverage. Then, the biofilm was removed using a mechanical scraper, the number of culturable bacteria was evaluated by plate counts on R2A medium and the endotoxin level was measured using the LAL assay.
Results: The most efficient cleaning procedure was a citric acid pretreatment followed by the autoclaving of the tubing. However, of the different procedures tested, none led to the complete removal of the biofilms. For this reason, we performed a screening of about 70 combinations of 13 other chemicals that led to the development of a very promising new cleaning procedure, allowing the complete removal of the biofilm and an excellent prevention of regrowth.
Conclusions: Further studies will improve and validate this new procedure in order to allow its marketing. That way, the microbiologic quality of dialysis liquids might be improved, increasing their biocompatibility and therefore the quality of life of dialyzed patients.

P1133 In vitro activity of topical disinfectants against bacteria isolated from wounds and diabetic ulcers

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Varese, I

Objectives: The choice of topic disinfectants to treat skin lesions is an important option of the infection control team. In order to help clinicians in choosing the most appropriate treatment, we wanted to assess: (i) the incidence of microorganisms isolated from wounds and diabetic ulcers; (ii) the activity of topic disinfectants against infecting bacteria; (iii) the relation between efficacy of disinfectants and resistance to antimicrobial drugs.
Methods: Bacterial identification and antimicrobial susceptibility testing were performed by the Sceptor System (Becton Dickinson, Sparks, MD). Susceptibility to topic disinfectants was assayed by homemade broth microdilution panels. Particularly, we studied the activity of five compounds as representative of different classes, including chlorhexidine gluconate (CG), povidone iodine (PI), toluen-*p*-sulfochloramyde (TS), merbromine (M), silver nitrate (SN). Minimum bactericidal concentration (MBC) was determined in 96-well plates, ranging from undiluted concentration (CG, 5 g/L; PI, 75 g/L; TS, 25 g/L; M, 20 g/L; SN, 3 g/L) to 12 two-fold dilutions.
Results: Over a 1-year period, 146 specimens gave a positive result; of them, 66 showed two or more infectious agents for a total of 220 isolates. The most prevalent species were *Staphylococcus aureus* ($n = 62$, 28%), *Pseudomonas aeruginosa* ($n = 46$, 21%), *Proteus mirabilis* ($n = 29$, 13%), and group G streptococci ($n = 18$, 8%). The resulting MBC90 (g/L) for the three major pathogens were, respectively: for *S. aureus* (CG, 0.16; PI, 9.4; TS, 6.3; SN, 0.2; M, 0.08); for *P. aeruginosa* (CG, 0.31; PI, 37.5; TS, 6.3; SN, 0.0015; M, 0.01); for *P. mirabilis* (CG, 2.5; PI, 19; TS, 3.1; SN, 0.02; M, 0.08). Resistance to oxacillin was detected in 47% of *S. aureus* isolates. Resistance rates of *P. Aeruginosa* were as follows: gentamicin, 27%; ceftazidime, 27%; imipenem, 10%; and amikacin, 10%. Finally, *P. mirabilis* strains showed resistance to gentamicin in 38% of the cases, but maintained full susceptibility to ceftazidime, imipenem, and amikacin. A few cases showed both a multidrug-resistant phenotype and resistance to three disinfectants.
Conclusions: Our study demonstrates that merbromine has the highest in vitro activity against all tested bacteria, but silver nitrate is a valuable option. Finally, high resistance rates to antibiotics suggest an appropriate antibiotic use in order to adequately treat skin lesions.

P1134 Antimicrobial activity of betadine disinfectant agent against multiresistant nosocomial pathogens

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Gdansk, PL

Objectives: Antimicrobial activity of the betadine disinfectant against the clinical multiresistant strains was examined. The 27 isolates, *Staphylococcus aureus* (three MRSA), *Enterococcus faecalis* (three HLAR), *E. faecium* (three VRE), *Pseudomonas aeruginosa* (three), *Acinetobacter baumannii* (three), *Enterobacter cloacae* (3), *Serratia marcescens* (three), *Klebsiella pneumoniae* (three ESBL+), *Escherichia coli* (three ESBL+), were tested for antimicrobial effectiveness in the standardized betadine solution.
Methods: Antimicrobial effectiveness was examined by a suspension method. The initial suspension containing 108 cfu/mL were prepared with tryptose soy broth and added to a disinfectant sample. In the assay, the disinfectant activity was arrested by neutralization after 1 min contact time. Disinfectant is considered to be bactericidal when a >5 log reduction is achieved.
Results: The initial suspensions of all strains and the reduction achieved by disinfectant were $(1.12–2.78) \times 10^7$ and $(0.275–1.92) \times 10^2$ cfu/mL. The reduction achieved a >5 log.
Conclusion: Betadine bactericidal effectiveness against multiresistant clinical bacterial strains is high and species independent.

Staphylococcus aureus

P1135 The clonal spread among Swedish children of a *Staphylococcus aureus* resistant to fusidic acid

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Objectives:
1 To investigate the epidemic spread of a *S. aureus* resistant to fusidic acid in superficial infections among children in Sweden.
2 To investigate the clonality of fusidic acid resistant *S. aureus*-isolates in superficial infections among Swedish children.
3 To investigate the use of fusidic acid in ointments in Sweden over time.

Methods:
1 A retrospective survey using data from laboratories in eight Swedish counties.
2 A prospective study using PFGE to fingerprint fusidic acid resistant *S. aureus*-isolates from the different counties.
3 A retrospective analysis of the sales of ointments containing fusidic acid in Sweden during the period 1993–2000.

Results: In all counties with available laboratory data, *S. aureus* resistant to fusidic acid were more common among strains isolated from patients in the age group 0–12 years than those from older patients (Table 1). The resistant *S. aureus* seemingly appeared in the county of Blekinge first, and subsequently (1998 or later) spread to other parts of Sweden. PFGE-patterns indicate the domination of a single clone. During the period 1993–2000, the yearly sales of ointments containing fusidic acid increased from 4 to 12 g per 1000 inhabitants in the age group 0–12 years and 3–6 g in the age group 13 years and older. The sales varied considerably among the counties.

	1997	1998	1999	2000
Skåne county	11 (3)	12 (4)	36 (7)	43 (11)
Blekinge county	38 (11)	42 (10)	51 (7)	48 (12)
Kronoberg contry	7 (9)	22 (8)	42 (9)	55 (13)
Kalmar county		7 (6)	46 (5)	34 (11)
Jönköping county	6 (2)	7 (3)	13 (3)	9 (4)
Västra Götaland county	10 (4)	13 (5)	34 (7)	38 (8)
Dalarna county				50 (12)
Norrbotten county			21 (11)	29 (13)

Proportions, in percentage, of *S. aureus* isolates from patients 0–12 years resistant to fusidic acid in eight Swedish counties. Numbers in brackets are the corresponding figures from the age group 13 years and older.

Conclusions: The apparent clonal spread of a *S. aureus* resistant to fusidic acid among children throughout Sweden is described. Irrespective of whether the increase in the use of fusidic acid ointments was caused by the epidemic per se or by the concomitant withdrawal from the market of a major alternative drug, the increase in the use of fusidic acid to which the clone was resistant facilitated the epidemic.

P1136 Typing of *Staphylococcus aureus* isolates from bovine mastitis by coagulase serotying and coagulase gene polymorphism (PCR-RFLP)

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Objectives: To type *S. aureus* isolates from bovine mastitis in Kuwait by coagulase serotyping. and PCR-RFLP of the coagulase gene.
Methods: One hundred and sixteen *S. aureus* isolates were collected from milk of mastitic cows in 12 dairy farms in Kuwait and studied for their relatedness using coagulase serotyping and coagulase gene polymorphisms. Coagulase serotyping was performed using coagulase antisera (Denka Seiken) according to the manufacturer's instructions. The coagulase (coa) gene polymorphisms was determined by the amplification of the 3′ end region of the coa gene by PCR using COAG2 and COAG3 primers followed by AluI restriction endonuclease digestion and analysis of the restriction fragment length polymorphism (RFLP) patterns of the amplified products by agarose gel electrophoresis.
Results: Sixty (51.7%) of the isolates were nontypeable by any of the coagulase antisera. However, 56 (48.3%) isolates were differentiated into seven coagulase serotypes. Coagulase serotype VI was the commonest serotype and was detected 30 (25.9%) isolates. Coagulase serotypes III and V were detected in six isolates (5.2%) each and coagulase serotype VII was detected in seven (6%) isolates. Coagulase serotypes I, II and IV were less frequent and none belonged to coagulase serotype VIII. PCR amplification of the coa gene gave PCR products of 714, 825, 861 and 987 bp in size. Five coa gene RFLP patterns. One RFLP pattern 1 was dominant and was detected in 101 (88%) isolates in all 12 farms. The rest belonged to four RFLP patterns.
Conclusion: Results of coa gene typing suggested that the majority of the *S. aureus* isolates from all the farms had a common origin. However, there was no correlation between results of coagulase serotyping and coa gene typing. The coagulase serotyping was not useful as a typing tool for studying *S. aureus* of animal origin. PCR-RFLP represents a valuable typing tool for assessing the relatedness of bacterial isolates.

P1137 Antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from 16 patients with a PFGE confirmed relapsed bacteremia

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Objectives: To identify antimicrobial susceptibility changes between initial and second episode strains isolated from *S. aureus* bacteremia with a relapse confirmed by pulse field gel electretrophoresis (PFGE).
Methods: From 1997 to 1999, we identified retrospectively 40 patients with a recurrent *S. aureus* bacteremia. Patient file records were analyzed and PFGE was performed on isolates from both episodes. PFGE banding patterns were identical in 16 patients, consistent with a relapsed infection. Antibiograms were performed by the diffusion method. Methicillin resistance was screened by the oxascreen test and confirmed by the latex MRSA or *mecA* PCR. Screening for hGISA and GISA (strains with reduced susceptibility to Vancomycin) was done by culture of strains on plates containing 6 mg/L of vancomycin and MIC E-test determination.
Results: Initial strains demonstrated the following antibiotic resistances (*N*, %): penicillin (16, 100%), oxacillin (7, 44%), gentamicin (1, 6.2%), erythromycin (7, 44%), fosfomicin (2, 12.5%), pefloxacin (6, 37.5%). No resistance to cotrimoxazole, rifampin, fusidic acid and glycopeptides was observed. The second strain was isolated 11–137 days after apparent resolution of the first episode. Acquired resistance was observed for seven patients:

- resistance to rifampicin for one patient and to fusidic acid for another patient;
- low level pefloxacin resistance (MICs 2–4 mg/L) for two patients which received only a 2-day fluoroquinolone treatment;
- change in glycopeptide susceptibility for three patients (1 MSSA/2 MRSA; MICs 6–8 mg/L) after initial glycopeptide treatment.

Most patients presented risk factors for early relapses such as indwelling foreign body (14, 87.5%), excessive time to initial antibiotic therapy (5, 31%) and hemodialysis dependence (5, 31%).
Conclusion: These results suggest that early relapse *S. aureus* bacteremia could be associated with an antimicrobial susceptibility changes without initial treatment failure. Particular attention should be focused on low level resistance to glycopeptides and fluoroquinolones.

P1138 Investigation of vancomycin resistance among *Staphylococcus aureus* isolates at a university hospital

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Objective: To evaluate vancomycin resistance among *Staphylococcus aureus* isolates obtained from nosocomial infections at a university hospital.
Methods: One hundred forty-five isolates collected over a period of 10 months in 2001 were examined. Methicillin sensitivity was detected with 1-μg-oxacillin disk. Of the isolates tested, 67 were methicillin-resistant. In-house-prepared brain heart infusion agar (BHIA) plates containing 6 μg of vancomycin per mL were used for screening. Plates inoculated with 10 μL of a suspension of each isolate equivalent in density to a 0.5 McFarland standard and incubated at 35 °C were examined for growth after 48 h. *Enterococcus faecalis* ATCC 51299 (vancomycin resistant) and *S. aureus* ATCC 29213 (vancomycin susceptible) were included as quality-control organisms.
Results: Thirty-four of the 145 *S. aureus* isolates were blood isolates, 26 were respiratory system isolates, 18 were continue ambulatory peritoneal dialysate isolates and 18 were urinary system isolates. No growth other than *E. faecalis* ATCC 51299 was detected on BHI agar plates containing 6 μg of vancomycin per mL.
Conclusion: Baskent University Hospital is a 300-bed, tertiary care hospital with most of its patients are on hemodialysis or continue ambulatory peritoneal dialysis (CAPD) program because of renal failure and at which approximately 80 kidney transplantations are performed annually. Annual vancomycin consumption at the hospital is 5000 g. Despite large amount of vancomycin use in high-risk patient group, no vancomycin resistant *S. aureus* isolate was detected. As these results demonstrate vancomycin resistance of *S. aureus* isolates is not yet an important problem for our hospital.

P1139 The biological cost of antibiotic resistance from the perspective of *Staphylococcus aureus*

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Introduction: Antibiotic resistance due to chromosomal mutations that cause structural modifications in the cellular target of the drug can be associated with a fitness burden.
Objectives: This study was aimed at analyzing (i) the biological cost of RNA polymerase (*rpoB*) mutations conferring rifampicin resistance on *S. aureus* (ii) the relationship between the cost of *rpoB* mutations and the level of resistance to rifampicin (iii) the distribution of *rpoB* mutations in vivo in view of the possible fitness burden associated with resistance.
Methods: A total of 54 *S. aureus* isolates was analyzed including 30 rifampicin resistant in vitro mutants derived from a single susceptible isogenic reference strain as well as 23 rifampicin resistant in vivo isolates obtained from six countries revealing 19 different PFGE genotypes. Mutations conferring rifampicin resistance were determined by partial DNA sequencing of the *rpoB* gene including resistance cluster I and II. The relative fitness of the rifampicin susceptible parent strain and the evolved rifampicin resistant in vitro mutants was determined from pair-wise competition experiments.
Results: With respect to in vitro mutations conferring rifampicin resistance 18 different *rpoB* genotypes could be identified. Only one genotype H481A showed no fitness burden whereas the other mutants were associated with a loss of fitness. A relationship between the cost of *rpoB* mutations and the level of resistance to rifampicin could not be demonstrated. Interestingly, 23 in vivo strains exhibit only seven different *rpoB* genotypes with mutation H481A, demonstrably not associated with a cost of fitness in vitro, being prevalent in 91% of isolates.

Conclusions: In vivo *S. aureus* adapt to the burden of resistance by the selection of mutations with little or no cost to their fitness. This phenomenon possibly is responsible for stabilizing the resistant bacteria in a population.

P1140 Nationwide laboratory surveillance for *Staphylcoccus aureus* with reduced susceptibility to vancomycin in South Korea

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Background and objectives: More than 70% of *S. aureus* isolated in tertiary hospitals in Korea were reported to be methicillin-resistant. And so glycopeptide antibiotics has been increasingly used for the treatment of methicillin-resistant *S. aureus* (MRSA) infections. To evaluate the current status of *S. aureus* with reduced susceptibility to vancomycin, we have done laboratory-based surveillance.
Methods: During the period from April 2001 to May 2001, 3746 MRSA isolates from 27 tertiary participant hospitals were screened on brain-heart infusion agar containing 4 μg/mL of vancomycin (BHI-4 agar). The minimum inhibitory concentrations (MICs) of vancomycin were determined by agar dilution, broth dilution and E-test methods. To detect the strains heterogeneously resistant to vancomycin (hetero-VRSA), population analysis and growth on BBL(R) MU 3 agar (Becton Dickinson, Japan) were done for the isolates grown on the BHI-4 agar. PFGE (Pulsed-field gel electreophoresis) was performed for molecular epidemiological analysis of isolates.
Results: Total 100 (2.7%) isolates grew confluent on the screening agar. None of them showed resistance to vancomycin, and vancomycin MICs for 15 isolates revealed 4 μg/mL by above mentioned methods. In these 15 isolates, only four contained resistant subpopulations similar to Mu3 strain, firstly identified hetero-VRSA. In 16 isolates, however, were suspected to be hetero-VRSA on BBL(R) MU 3 agar. Among 100 isolates, 40% differed from AMC 11094, first VRSA from Korea, by only one to three band on PFGE.
Conclusions: Despite high prevalence of MRSA in Korea, none of *S. aureus* isolates were resistant to vancomycin and less than 1% (4–16/3746) were heterogeneously resistant to vancomycin. But PFGE analysis suggested the possibility of re-emergence of VISA in Korea. This alerts us to the possibility emergence of VISA/VRSA and to need of necessary to periodic surveillance for VRSA.

P1141 Antimicrobial resistance of *Staphylococcus* aureus clinical isolates from a Greek hospital during a 3-year period

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Objectives: To study the antibiotic resistance of *Staphylococcus aureus* (SA) isolated from a Greek major hospital within a 3-year period.
Materials and methods: During a 3-year period (1998–2000) a total of 599 clinical isolates of *Staphylococcus aureus* were collected from patients treated in our hospital. The strains were isolated from blood cultures (22%), catheter tips (8.5%), bronchial cultures (15%) and from other sources (liquids, wounds, etc.) (54.5%). Of these isolates 75 (12.5%) were derived from the Intensive Care Unit (ICU) and 504 (87.5%) from the rest of the hospital. Isolates were identified as SA by a positive catalase test, a positive coagulase test and a positive nuclease test. Susceptibility to antibiotics was detected by the diffusion method according to NCCLS guidelines.
Results: Of the strains tested 252 (43.5%) were methicillin susceptible (MSSA) and 327 (56.5%) were methicillin resistant (MRSA). The percentages of resistance to antibiotics (except β-lactams and glycopeptides) for MRSA and MSSA were, respectively: Gentamicin (GM) 80–10%, Amikacin (AN) 73–17%, Ciprofloxacin (CIP) 80–6%, Erythromycin (E) 82–18%, Clindamycin CM 76–7%. Of SA from ICU 93% were MRSA while from the rest wards 51% were MRSA. The percentage of resistance for MRSA strains derived from ICU and from the other wards were, respectively: GM 99–75%, AN 99–67%, CIP 97–76%, E 96–79%, CM 96–70%.
Conclusions: It is worth noting that: MRSA isolated from ICU were much more resistant, with the exception of glycopeptides than MRSA from the rest of the hospital. The prevalence of Methicillin resistance and associated multiresistance in SA has increased significantly during the 3-year study period. All isolates were sensitive to vancomycin.

P1142 Antibiotic susceptibility of staphylococci (including MRS) isolated from hospitalized children

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Objectives: The aim of present study was the analysis of drug susceptibility of staphylococci (including MRS) isolated from children hospitalized in two pediatric clinics in 1998–2000.
Material and methods: Antibiotic ($n = 9$–10) susceptibility of 501 staphylococcal strains isolated from various clinical materials was determined by the disc diffusion method (NCCLS). Among tested strains there were 277 isolates (107 *S. aureus* and 170 CNS) from pediatric surgery (S) and 224 (43 *S. aureus* and 181 CNS) from pediatric nephrology (N) patients.
Results: All staphylococci were susceptible to vancomycin and teicoplanin (strains from urine also highly to nitrofurantoin). *S. aureus* strains were more susceptible than CNS to: erythromycin, clindamycin, gentamicin and cotrimoxazole. There were also differences in antibiotic susceptibility of the staph isolates from S and N, especially to erythromycin, clindamycin, doxycycline, gentamicin, cotrimoxazole and fluoroquinolones. Penicillin was practically ineffective. A total of 209 (42%) out 501 were MRS strains (62% from S and 38% from N). MRSA and MRCNS strains from S versus N constituted 23 and 62% versus 19 and 39%, respectively. MRS strains from S and N were susceptible to glycopeptides (100%), highly resistant to erythromycin (85 and 75%), clindamycin (a 74%), gentamicin (68% S), cotrimoxazole (60 and 61%) and less resistant to doxycycline (38 and 45%), gentamicin (38% N) fluoroquinolones (43 and 23%).
Conclusion: *S. aureus* in comparison to CNS strains were more susceptible to the majority of 10 antibiotics. MRS strains (constituting 42%) were isolated in prevalence from children in S. Only glycopeptides were active against 100% MRS strains, while against over 60% fluoroquinolones and gentamicin (strains from N) and doxycycline (strains from S).

P1143 Clinical implications of *Staphylococcus aureus* with reduced susceptibility to vancomycin

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Objectives: Rapid evolution of antibiotic resistance in *S. aureus*, including recent emergence of vancomycin-intermediate *S. aureus* (VISA), has been a serious concern. Infections of VISA and *S. aureus* with vancomycin MIC (minimum inhibitory concentration) of 4 μg/mL were reported to be the causes of treatment failures. To understand the clinical characteristics of infections with these strains, we reviewed the clinical characteristics of the patients infected with *S. aureus* with reduced susceptibility to vancomycin.
Methods: Clinical isolates of *S. aureus* were screened for reduced susceptibility to vancomycin. One hundred isolates of possible VISA, which grew confluently on brain-heart infusion agar containing 4 μg/mL of vancomycin, were referred to the National Institute of Health for confirmatory testing during the period from April 2001 to May 2001. Out of 100 isolates, 15 revealed vancomycin MIC of 4 μg/mL by agar dilution, broth dilution or E-test methods. Among them, four isolates were ascertained to be colonizers, not pathogens. The clinical characteristics of the 11 patients were analyzed retrospectively.
Results: The median age of the patients was 52 (11–74) years, and nine were male and two were female. Types of *S. aureus* infection included six chronic osteomyelitis, two postsurgical infections, one pneumonia, one intra-abdominal infection, and one catheter-related infection. While one case had no history of prior exposure to glycopetides, 10 were exposed for a prolonged period of time, ranging from 7 to 432 days. Despite adequate treatments, site of infections became culture negative in only one case. Two patients died of *S. aureus* infections.
Conclusions: Chronic osteomyelitis was the most common type of *S. aureus* infection with reduced susceptibility to vancomycin. Most infections due to *S. aureus* with vancomycin MIC of 4 μg/mL were not successfully treated.

Prolonged exposure to glycopeptides was associated with the emergence of *S. aureus* with reduced susceptibility to vancomycin.

P1144 In vitro antimicrobial susceptibility of *S. aureus* in suspensions, biofilms and resuspended biofilms

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Objectives: Device-related infections are difficult to treat and can become persistent infections because of the biofilm mode of bacterial growth. Once biofilms dislodge and enter the bloodstream, they can cause systemic infections. Diagnostic microbiology laboratories test and report susceptibilities of bacteria in suspension (planktonic bacteria). However, the susceptibilities of the biofilms are not done.
Methods: We tested the susceptibilities of the biofilms of 10 clinical isolates of *S. aureus* to oxacillin, synercid and vancomycin. The suspension bacteria were tested using broth microdilution technique (NCCLS, M7-A5). The biofilms were grown in 96-well microtiter plates by culturing 1×10^6 to 2×10^6 cfu/mL in 200 µL of TSB for 24 h. Plates where then washed twice with PBS and 100 µL of TSB was added. 100 µL of the antimicrobial agents were added at concentrations of 50, 500 and 1000 µg/mL and at the MIC_{90}. The microtiter plates were incubated for 24 h to allow interaction between the antimicrobial agents and the bacterial biofilms. Colony counts on serial dilutions were used to calculate percent viability. In parallel experiments, the biofilms were resuspended (disrupted) prior to the addition of the antimicrobial agents.
Results: The MIC_{90} of oxacillin, synercid and vancomycin in suspension were 0.25, 0.5 and 8 µg/mL, respectively. The mean percent viability of biofilms in the presence of oxacillin at 0.25, 50, 500, 1000 µg/mL were 25.8, 35.7, 43.0 and 23.5%, respectively, compared to 39.7, 7.04, 45.4 and 8.37%, respectively, for resuspended biofilms. The mean percent viability of biofilms in the presence of synercid at 0.5, 50, 500 and 1000 µg/mL were 17.6, 4.9, 22.6 and 25.1%, respectively, compared to 12.7, 19.2, 0.79 and 25.5%, respectively, for resuspended biofilms. The mean percent viability of biofilms in the presence of vancomycin at 8, 50, 500 and 1000 µg/mL were 100, 53.3, 15.3 and 7.43%, respectively, compared to 100, 15.2, 22.3 and 22.3%, respectively, for resuspended biofilms
Conclusion: The routine susceptibility testing using bacterial suspensions (planktonic mode of growth) does not predict susceptibilities of bacteria in the biofilms (sessile mode of growth).

P1145 Comparative activity of old and new quinolones against nosocomial *Staphylococcus aureus*

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Objectives: To compare in vitro activity of an old quinolone–ciprofloxacin (CIP), which is known to have a deficient activity against Gram-positive bacteria with new quinolones–levofloxacin (LEV) and moxifloxacin (MOX), which combine good Gram-negative activity with enhanced Gram-positive activity.
Methods: Overall, 140 *S. aureus* isolates obtained from hospitalized patients in Smolensk (Russia) were studied. MICs to CIP, LEV, MOX and Oxacillin (OXA) were determined by agar dilution method. Interpretation of results for OXA, CIP and LEV were performed according NCCLS recommendations (2001). Intermediately resistant and resistant strains were considered as non-susceptible. The *S. aureus* ATCC 29213 was used as a control strain.
Results: Among 140 isolates tested, 77 (55%) were methicillin-resistant (MRSA). MOX was the most active agent with $MIC_{90} = 0.125$ mg/L compare to 0.5 mg/L for CIP and LEV. The MIC_{50}, MIC_{90} and MICs ranges are shown in the table. The MICs of tested quinolones for methicillin-susceptible strains (MSSA) and MRSA were the same with the exception of MIC_{90} for MOX–0.125 mg/L for MSSA and 0.06 mg/L for MRSA. Against ciprofloxacin-susceptible MRSA the following MIC_{90} (MICs ranges) were observed: 0.5 (0.125–0.5) for CIP, 0.25 (0.125–0.5) for LEV and 0.06 (0.03–0.06) for MOX. Against nonsusceptible to CIP MRSA strains (10.4%) MIC_{90} (MICs ranges) were: 1 (0.5–4) for LEV and 0.25 (0.125–2) for MOX. Among all isolates only one strain was intermediately resistant to LEV (MIC = 4 mg/L), resistant to CIP (MIC = 16 mg/L) and had MIC = 2 mg/L for MOX.
Conclusions: According to the above data MOX is a potent agent against both methicillin-susceptible and -resistant *S. aureus*. MOX was more active than CIP and LEV. MIC_{90} to CIP and LEV were the same. However, MOX and LEV had a reduced activity against nonsusceptible to CIP MRSA isolates.

Antimicrobials	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)
MSSA ($N = 63$)			
CIP	0.5	0.5	0.25–1
LEV	0.25	0.5	0.125–0.5
MOX	0.06	0.125	0.03–0.25
MRSA ($N = 77$)			
CIP	0.5	0.5	0.25–16
LEV	0.25	0.5	0.125–4
MOX	0.06	0.06	0.03–2

P1146 Rapid detection of *S. aureus* from blood culture specimens

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Objectives: To evaluate a new rapid method based in real time PCR for the direct detection of *Staphylococcus aureus* in blood culture specimens.
Methods: One hundred *S. aureus* isolates and 90 diverse bacterial species were studied. DNA stock was prepared by phenol/chloroform extraction method. Seventy-five clinical blood samples were also used for direct detection of *S. aureus* extracted using a commercial method (Qiagen). PCR amplification was carried out using primers directed against a sequence of the *nucA* gene. Detection of the product was done by using a pair of probes that hybridize with an inner sequence of the amplicon (FRET assay).
Results: All the *S. aureus* strains tested positive in the FRET assays and none of the other bacterial species used, showing a sensitivity and specificity of 100%. The assay was not affected neither by blood inhibitors or exogenously added microorganisms. The detection limit was 20 cfu per assay. All the blood samples, that grew *S. aureus*, gave a positive PCR results but none of the samples, which grew other bacteria.
Conclusion: The *nucA* FRET-PCR assay described is extremely sensitive and specific for *S. aureus* direct detection in blood cultures specimens.

P1147 Long-term outcome of patients with *Staphylococcus aureus* bacteremia

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Objectives: *Staphylococcus aureus* bacteremia (SAB) is a leading cause of nosocomial infection and sepsis. While intravenous antimicrobial therapy for ≥14 days is thought to be crucial for prevention of secondary complications, little is known about long-term prognosis. We studied outcome and risk factors for death in a large cohort of patients with SAB including all departments of a tertiary medical center.
Methods: All patients with documented SAB from January 1997 to December 2000 at the university hospital of Cologne were included in this retrospective analysis. Clinical data were obtained by chart review and were available in 229 out of 284 identified cases (81%). For information on long-term outcome a questionnaire was sent to general practitioners and registration offices, and data were available in 202/229 patients (88%). Statistical analysis was performed using SPSS 6.1.
Results: A diagnosis of SAB was established in 153 male and 76 female patients (mean age 56 ± 18 years). Predisposing factors were present in 91% with surgery ($n = 77$) and cardiovascular diseases ($n = 70$) being the most prevalent conditions. Venous catheters were the most frequent source of SAB with 48 definite (21%) and 32 probable cases (14%), and in 95 persons (41.5%) the origin of SAB remained undefined. Most patients had nosocomial SAB (177, 77%). Sepsis developed in 168 (73%) and septic shock in 36 (16%) patients. Complicated SAB with organ infection was diagnosed in 45 persons (20%). In 13 cases (6%) MRSA was present. After 1-year, 123 persons (54%) were alive, 79 (345%) had died (46 within 30 days) and 27 (10.5%) were lost to follow-up. Risk factors for shorter survival were age >60 years ($P = 0.04$), a diagnosis of pneumonia ($P < 0.001$), malignant disease ($P < 0.001$), neutropenia ($P = 0007$), immunosuppressive therapy ($P < 0.001$), and source of bacter-

emia unknown ($P=0.001$). Mortality rates were highest in patients with neutropenia (77%), immunosuppressive therapy (62%) and malignant disease (58%). All deaths $n=11$, 65%) in patients with pneumonia ($n=17$) occurred within 30 days.
Conclusions: Patients with SAB have a substantial risk of short- and long-term mortality. The crude mortality rate after 1 year in our cohort was 39% (79/202). Prospective studies are needed to assess the specific mortality of SAB and to evaluate strategies for a more favorable outcome.

P1148 *Staphylococcus aureus* septicaemia in non-neutropenic adult patients: a retrospective clinical and microbiological analysis

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Introduction: *S. aureus* is one of the leading agents of nosocomial infection among adult patients.
Purpose: To determine predisposing factors and secondary complications of *S. aureus* septicemia (SAS) in non-neutropenic patients, as well as predictors of outcome in non-neutropenic patients with SAS.
Patients and methods: We performed a retrospective study of 56 patients who developed *S. aureus* sepsis (SAS) from January 1997 through June 2001 hospitalized in medical wards at Policlinico Umberto I-University of Rome 'La Sapienza'; we excluded from the study surgery patients and Intensive care unit's hospitalized patients.
Results: Median age was 61.9 years (24–89), male sex 29 (51%), infection was nosocomial acquired in 83.5%. More frequent underlying diseases were high blood pressure (37.5%), diabetes mellitus (26.8%), cirrhosis (26.8%) and malignancy (26.8%); SAS was intravascular device-related in 32%, mucocutaneous-infection in 16%, respiratory tract in 8.9% and unidentifiable focus-related in 43%. Metastatic infections were found in 12 patients (21.5%), with six (10.7%) developing infectious endocarditis; the rate of SAS relapse was 8.9%; Methicillin-resistance was found in 30.3%; most common empirical therapy was ceftriaxone (33.9%) and piperacillin ± tazobactam (19.6%) with addition of vancomycin or teicoplanin in 39.2% of cases. Overall mortality was 41% and attributable mortality 28.5%. Twenty-nine patients that developed metastatic infections or died for sepsis were compared with 27 patients that did not develop complications. Factors associated with an increased incidence of metastatic infections and SAS related mortality were: delay to adequate antibiotic therapy (2.46 gg vs. 1.15 gg, $P=0.02$), a 72-h surveillance blood culture positive (3.56 gg vs. 1.51 gg, $P=0.01$), high rate of septic shock (58.6% v 3.7%, $P=0.00001$), bacteremic pneumonia (17.2% vs. 0, $P=0.02$) as source of bacteremia and major severity of illness at the onset of SAS evaluated with ILLNESS SCORE (4.2 vs. 2.1 $P=0.002$).
Conclusion: SAS in non-neutropenic patients is associated with significant morbidity from high rate of metastatic infectious and with a considerable related mortality. Delay to adequate antibiotic therapy, 72-h surveillance cultures positive, septic shock, bacteremic pneumonia as source of bacteremia, major severity of illness at the onset of SAS were all predictive of a complicated course and a poor prognosis in term of morbidity and mortality.

P1149 Influence of nasal mupirocin on the incidence of *S. aureus* (SA) infections in children undergoing peritoneal dialysis (PD): a double-blind, placebo-controlled multicentre study

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Objectives: Nasal SA colonization is a major risk factor for catheter-related infections in continuous peritoneal dialysis (CPD) patients, including children. Patients or their caretakers may be the SA carriers and therefore the source for infection. The objective of our study was to investigate the eradication of SA by intranasal mupirocin of patients and caregivers and its efficacy on the reduction of SA site infections.
Methods: In a randomized, double-blind, placebo-cont-rolled multicentre-study, after screening (nasal and exit site) for SA carriers (children and/or caregivers) were randomized to mupirocin or placebo treatment for 5 days bid every 4 weeks.
Results: Overall, 122 patients and their caretakers from 18 centers were included. Median follow-up time: 7.8 months. In 58 families, both patient and caretakers remained SA negative. In 40 families, the patient but not the caretakers carried SA and in 24 families, at least one caretaker but not the patient was a carrier. All positive family members received nasal prophylaxis. SA infection within 12 months occurred in 20% of PD patients of families with at least one SA carrier but in only 5% of patients of noncarrier families. The number of infections was significantly reduced in the mupirocin arm during the first treatment year (1 vs. 5 infections; 11-month infection-free interval: 96% vs. 84%; $P<0.05$). However, two additional SA infections occurred during the second year, associated with SA recolonization of the caretaker in one case. Mupirocin significantly reduced carrier rates in children during the first treatment year ($P<0.002$) but not in caretakers, which might be related to selective noncompliance in caretakers.
Conclusions: Nasal mupirocin appears to be effective in reducing SA infections in children on CPD. However, it should be emphasized that decolonization of SA in children alone is not sufficient if caretakers are colonized.

Antifungal drugs

P1150 Early determination of in vitro resistance of filamentous fungi to antifungal drugs based on growth curves

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Objectives: Early and objective determination of the in vitro susceptibility of filamentous fungi may be critical for initiation of antifungal therapy. In this study, a new methodology for testing the resistance of filamentous fungi to three antifungal drugs was developed based on the first detectable growth by spectrophotometer.
Methods: A broth microdilution method (NCCLS guidelines, M-38P) was used in order to test the susceptibility to itraconazole (ICZ), amphotericin B (AB) and terbinafine (TB) of *Rhizopus oryzae* (RO), *Aspergillus fumigatus* (AF) and *Scedosporium prolificans* (SP) using two-fold serial dilutions. Microtiter plates were incubated at 37 °C for 24 h and growth curves obtained by measuring the optical density (OD) of each well by spectrophotometer (ht3, Anthos) every 10 min. The time to the first detectable growth (OD0) was determined for each drug-containing well and drug-free well. The OD0 was then correlated with the MIC scores (0, 1, 2, 3: the lowest drug concentration with absence of growth, slight growth, prominent and slight reduction of growth, respectively, or 4: the highest drug concentration showing no reduction of growth compared to growth control) at each concentration for each drug–organism combination.
Results: The OD0 of the growth control of RO, AF and SP was 5.4, 7.7 and 9.9 h of incubation, respectively. The OD0s increased concordantly with increasing drug concentrations. As an example, the OD0 at the MIC-2 occurred 4 h later then the growth control for ICZ (i.e. 9.3 h for RO and 11.7 h for AF) and for TB (i.e. 11.5 h for AF and 14.4 h for SP). Using the OD0, a distinction could be made between susceptible strains and resistant strains.
Conclusions: The use of a continuous monitoring system by a spectrophotometer permits discrimination of susceptible strains from resistant strains. This method may be useful for early determination of resistance in molds.

P1151 Desensitization to fluconazole in patients with human immunodeficiency virus infection: case report and review

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Introduction: HIV-infected patients suffering from meningitis by *Cryptococcus neoformans*, fluconazole is the preference treatment both after initial therapy

with amphotericin B as in secondary prophylaxis. Even though this drug is usually well tolerated, sometimes can give adverse effects. Procedures for desensitization have been developed. We present a case attended in our unit and a review of the literature. A 32-year-old man, old intravenous drug abuser, was suffering from HIV infection for 7 years. He had been treated with HAART, but he had left it 1 year ago. His last CD^{4+} lymphocyte count was 5 cells/mm^3. The viral load was 1 200 000 copies/mL. Four days before admission, he suffered from fever, headache and meningeal symptoms. CSF culture yielded *C. neoformans*. Amphotericin B was used as induced treatment, then the patient was switched to fluconazole (400 mg/day) to complete 8 weeks. After this time, the dose was decreased to 200 mg daily as secondary prophylaxis. By his own choice, the patient left the treatment. One month later, he came to our external-patients unit, and we encouraged him to take the prophylaxis with fluconazole. When it was restarted, he suffered fever and pruritic rashes. These symptoms resolved within a few days after discontinuing fluconazole and treating with antihistaminic. Five days later, the patient again suffered fever and severe headache. A new CSF analysis revealed relapse cryptococcal meningitis. A new induction with amphotericin B was indicated. At this moment, we elected to test dose and desensitize the patients using the procedure described by Craig beginning with 0.2 mg each day until 15 days, with excellent results. Now after 3 months, the patient remains free of symptoms.

Discussion: Though fluconazole is usually a well-tolerated drug, adverse effects are described. The skin lesions reported spread from a diffuse rash to a complete severe Stevens–Johnson syndrome. Our case suffered from serious skin lesions and general symptoms (fever) closely related with fluconazole. In our case, we contemplated the Craig's procedure because of the itraconazole failure and the major recidive possibility with weekly amphotericin B. During all this time, our patient remained without symptoms. In our opinion, this method is safe and can be functional in patients with fungal infection, especially cryptococcal, suffering from fluconazole hypersensitivity.

P1152 Effects of 5-hydroxytryptamine on several *Aspergillus* and *Candida* species in vitro

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Objectives: In humans, selective serotonin reuptake inhibitors (SSRIs) modify the concentration of 5-hydroxytryptamine (5-HT) and lead to an increase of 5-HT during therapy with SSRIs. Recently, we found that sertraline, a typical SSRI, has in vivo and in vitro antifungal activity. Therefore, we investigated the direct influence of 5-HT against clinical isolates of *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *C. albicans* (CBS) and *C. parapsilosis* (ATCC 22019). We examined the effects of 5-HT with regard to viability, morphology and physiology on the different species.

Methods: To determine the viability, a broth microdilution method was used. For morphological studies, examination by light microscopy and electron microscopy were performed. The red-fluorescent nucleic acid-binding dye Propidium-Iodid, which is impermeable to live cells, was applied to investigate the membrane integrity. In addition, the enzymatic activity of *C. albicans* for production of phospholipases was investigated by egg-yolk agar.

Results: 5-HT was fungicidal towards the tested fungi, at which *Candida* spp. seemed to be the most sensitive fungi. *A. terreus* represented the most resistant species with the MFC ranges of 25–50 mg/mL. The MFC ranges were for *C. albicans* 3.12–6.25 mg/mL and for *C. parapsilosis* 0.78–1.56 mg/mL. The application of light microscopy showed shrinkage of the cytoplasm and damage of the fungal organelles. By using the Propidium-Iodid staining method, a massive destruction of the plasma membrane was detected. A significant decrease ($P < 0.05$) on phospholipase activity of *C. albicans* at 5-HT concentrations of 0.19 mg/mL was observed.

Conclusion: In conclusion, we showed that 5-HT has antifungal activity. Further studies are required to evaluate the potential role of 5-HT in antifungal host defense.

P1153 Fungal colonization in neutropenic patients: a randomized, double-blind study comparing itraconazole solution and amphotericin B solution

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Objectives: The impact of prophylaxis with itraconazole solution and amphotericin B solution for fungal colonization from oropharynx and rectum was assessed in a randomized, double-blind study among 115 patients with hematological malignancies and neutropenia.

Methods: In the beginning of chemotherapy, patients were allocated randomly to receive itraconazole (5 mg/kg of body weight twice a day) or amphotericin B (1000 mg three times a day) until neutrophil recovery. Fungal surveillance cultures were obtained before, during and after neutropenia twice a week.

Results: A total of 59 patients received itraconazole, and 56 received amphotericin B. At the time of admission, *Candida* colonization in oropharynx and rectum occurred in 53 (46%) and 37 (32%) of the patients. *Candida albicans*, *C. glabrata* and *C. krusei* were the most common isolates. During neutropenia, fungal colonization in oropharynx occurred in 12 (19%) and 24 (42%) patients, in rectum occurred in 11 (18%) and 23 (41%) patients in the itraconazole and amphotericin B recipients ($P < 0.01$), respectively. Of these patients, 1.7% in the itraconazol group and 5.1% in the amphotericin B group had superficial fungal infections ($P < 0.01$). Invasive candidiasis developed in 3 (5.1%) patients, invasive aspergillosis occurred in one patient in the amphotericin B group ($P < 0.01$). The incidences of suspected fungal infection were not different between the groups.

Conclusions: Itraconazole solution reduced significantly *Candida* colonization and infection in neutropenic patients compared to amphotericin B solution.

P1154 In vitro and in vivo release of clotrimazole from ear drops formulation

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Objectives: Clotrimazole (CLT) has an antimicotic activity and it is also active against Gram positive bacteria. In regard to this data three formula eardrop containing 1.2% CLT was formulated. The products have been tested both physico-chemically and regarding the availability of CLT, using microbiological determination. We made also a clinical evaluation by treating some otitis and otomicosis cases.

Methods: We prepared three eardrops formulations:

- 1.2% CLT suspended in 1% tween 80 with solvents (20% propilenglycol, 2% benzylalcohol) and water;
- 1.2 CLT suspended in 2% PVP-water with 2% benzylalcohol and 20% propylenglycole;
- 1.2% CLT dissolved in 2% benzylalcohol and PEG300.

The products were characterized physico-chemically by measuring some parameters: pH, the electric conductivity and viscosity. The availability of CLT was assessed by microbiological tests, difusimetric methods. The bioavability was clinical evaluated on 63 patients with otomicosis and otitis.

Results: The products' pH varies between 5.06 and 6.40. The electrical conductivity is indirectly proportional to the values of pH. The viscosity varies between 2.93 and 7.45 mPas. The biggest viscosity has the third preparate with PEG300, respectively, 7.45 mPas. The microbiological tests, using *Sabouraud agar* medium, reveal that the CLT included in products is active against *Candida albicans*, *Sarcina lutea*, *Bacillus subtilis*, *Staphylococcus aureus*. The clinical data revealed the efficiency against external, medium otitis and otomicosis of second and third preparates, leading to their healing.

Conclusion: Although, the microbiological testes proved a higher activity for the third preparate (solution). We can note also a good efficiency for the second preparate (with a macromolecule that increases the contact time).

P1155 Promotion of mycelium formation by propofol

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Introduction: Fungal agents are becoming increasingly responsible for nosocomial infections, particularly in the setting of the ICU unit. Hypha and mycelium formation is assumed to be an important virulence factor by promoting adherence to host cells and for tissue invasion. Propofol, administered in a lipid emulsion, is a widely used anesthetic and sedative agent in critical care patients.

Objective: To evaluate the effect of propofol upon hypha and mycelium formation by *Candida* and *Aspergillus*.

Material and methods: Clinical isolates of *C. albicans* (3 strains), *A. fumigatus* (2 strains) and *A. flavus* (2 strains) were incubated with plain and with serial dilutions of propofol, in RPMI 1640 cell culture medium (Sigma). After incubation for 2 h at 37 °C, samples (200 cells) were hourly checked for budding and germ tube formation by blastoconidia or germination of spores in case of *Candida* and *Aspergillus*, respectively, up to 8 h.
Results: Propofol induced hypha formation both by *Aspergillus* and *Candida*, although much faster in the latter. After 2 h, an extensive rate of germ tube formation and of cell division (budding) by *C. albicans* was found, also in plain propofol. Similar results were found for *Aspergillus* regarding spore germination and hypha formation, although noticeable only after 5–6 h and particularly with *A. fumigatus*.
Conclusions: Propofol is potentially associated to a high risk of nosocomial primary fungemia, particularly by *Candida* spp., due to its high potential of contamination even when infused shortly after its use. Additionally, by promoting mycelium formation, seriously enhances the invasiveness and pathogenesis by *Candida* and *Aspergillus* organisms, promoting the establishment and progression of deep-seated fungal infections.

P1156 Evaluation of the E-test for susceptibility testing of clinical yeasts to amphotericin B, flucytosine, ketoconazole, fluconazole and itraconazole

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Introduction: The E-test is a diffusion method consisting of a strip with a gradient of drug concentrations that permits easy and rapid reading of MICs. Although this method had showed excellent results for numerous bacteria its yield for yeasts is still being studied.
Objective: To evaluate the E-test for susceptibility testing of clinical yeasts to amphotericin B, flucytosine, ketoconazole, fluconazole and itraconazole.
Methods: We compared the correlation of MICs of amphotericin B, flucytosine, ketoconazole, fluconazole and itraconazole by the E-test with those of the reference method (M-27 A, NCCLS, supplemented with 2% glucose). E-test was performed according to the manufacturer's recommendations. Three incubation times were evaluated for E-test: 24, 48 and 72 h. A total of 312 yeasts were tested: 56 *C. albicans*, 55 *C. parapsilosis*, 49 *C. glabrata*, 52 *C. tropicalis*, 49 *C. krusei*, and 51 *Cryptococcus neoformans*.
Results: Overall, the best performance for E-test was obtained after 48 h of incubation (89% correlation vs. 75% at 24 h and 84% at 72 h). At this condition, 53% of species-antifungal pairs had a correlation greater than 95%. The pairs with lower agreement were flucytosine-*C. krusei* (10%) and flucytosine-*C. neoformans* (60%). For the remaining species-antifungals pairs the range of correlation was of 79–93%. The agreement by species were 96 (*C. albicans*), 94 (*C. parapsilosis*), 92 (*C. tropicalis*), 92 (*C. glabrata*), 77 (*C. krusei*) and 83% (*C. neoformans*). The agreement by antifungals were 92 (amphotericin B), 78 (flucytosine), 91 (ketoconazole), 95 (fluconazole) and 90% (itraconazole).
Conclusions: In this study, optimal agreement with standard susceptibility test was obtained at 48 h of incubation. At this time, good results were obtained except for flucytosine when tested with *C. krusei* and *C. neoformans*. E-test is a ready-to-use method that represents an alternative for the antifungal susceptibility testing in the clinical laboratory routine.

P1157 Antifungal susceptibility testing of yeast bloodstream isolates

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Objectives: To determine in vitro activities of systemic antifungals against clinical yeast isolates causing nosocomial bloodstream infections.
Methods: A total of 90 yeast isolates, including 10 yeast species, were obtained from blood cultures of 69 patients. Most frequently, *Candida parapsilosis* was identified ($n = 45$), followed by *C. albicans* ($n = 19$) and *C. tropicalis* ($n = 10$). Standardized microdilution method according to M27-A document of NCCLS was used for the determination of the minimal inhibitory concentrations (MICs) of amphotericin B (AMB), flucytosine (FCZ), ketoconazole (KET), itraconazole (ITR), fluconazole (FLA) and voriconazole (VOR). Reading of results was done after 24 h of incubation at 37 °C with exception of *C. parapsilosis* strains, which were evaluated after 36 h.
Results: MIC ranges and most frequent MICs (in parentheses) in μg/mL were for C. Parapsilosis: AMB 0.125–1.0 (0.5), FCZ 0.062–0.5 (0.25), KET 0.031–0.5 (0.25), ITR 0.031–0.5 (0.25), FLA 0.25–4.0 (0.5) and VOR 0.031–0.125 (0.062), for *C. albicans*: AMB 0.125–0.5 (0.5), FCZ 0.062–0.125 (0.062), KET 0.031–0.25 (0.031), ITR 0.062–0.5 (0.25), FLA 0.062–2.0 (0.5) and VOR 0.015–0.125 (0.015) and for *C. tropicalis*: AMB 0.125–0.5 (0.25), FCZ 0.062–16.0 (0.062), KET 0.031–0.25 (0.031), ITR 0.031–0.5 (0.25), FLA 0.062–1.0 (0.5) and VOR 0.015–0.125 (0.015). MICs of all isolates were verified by repeated testing. According to NCCLS interpretive guidelines for AMB, FCZ, ITR and FLA, none of the isolates was evaluated as resistant. Significant changes in the MICs among the isolates of the same species obtained subsequently from individual patients were not observed.
Conclusions: The majority of our bloodstream isolates have low MIC values indicating susceptibility to systemic antifungals. Results of microdilution method used in our study were easily readable and, moreover, they showed very good reproducibility in repeated experiments.
Acknowledgments: This work was supported by grants NI6190-3 (IGA MH CR) and MSM 15110004 (ME CR).

P1158 PLD-118, a novel antifungal compound: tolerability, safety and pharmacokinetics after multiple oral dosing in healthy male volunteers

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Background: PLD-118 is a novel oral antifungal compound, primarily active against *Candida* spp., including azole-resistant strains.
Objective: Double-blind, randomized, placebo-controlled, sequentially ascending dose study was performed to assess tolerability, safety and pharmacokinetics of PLD-118 after multiple oral dosing in healthy male volunteers.
Methods: A total of 32 subjects, divided into four groups and randomized in a 3 : 1 ratio for active treatment and placebo, received multiple oral doses of 50 mg (tid), 100 mg (tid) or 150 mg (bid or tid) PLD-118. Each volunteer received a single dose on the first day, followed by a 72-h wash out period, multiple doses for 7 days, and a single dose on the last dosing day. Plasma samples were collected over 72 h at Day 1 and Day 11, as well as prior to and 1 h after the morning dose at Days 4–10. Urine samples were collected up to 72 h after the last dose.
Results: Mean pharmacokinetic data are summarized in the table below.

	Dose (mg)							
	50 mg (tid)		100 mg (tid)		150 mg (tid)		150 mg (bid)	
Study day	1	11	1	11	1	11	1	11
C_{max} (μg/mL)	1.6	2.4	3.2	5.6	4.5	5.9	4.7	6.0
AUC_{inf} (μg × h/mL)	8.7	14.4	19.6	39.8	27.4	46.9	24.5	38.6
t_{max} (h)	0.67	0.67	0.84	0.67	0.67	1.5	0.67	0.67
$t_{1/2}$ (h)	7.4	6.8	7.4	7.9	7.0	7.2	7.4	7.5
Ae_{72} (% of dose)	81	142	83	158	82	140	85	127

PLD-118 was well tolerated. The most frequent adverse event was dry mouth, occurring in five subjects. EEG abnormalities were detected in two subjects receiving PLD-118 and in two receiving placebo, suggesting normal biological scatter. There were no clinically significant drug-related changes of vital signs, ECG parameters or laboratory findings.
Conclusion: Presented results suggest good tolerability and favorable pharmacokinetic profile of PLD-118 in man, supporting further clinical development of the compound.

Neutropenia and related infections

P1159 *Staphylococcus haemolyticus* bacteremia in patients with hematological malignancies

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Objectives: We describe 22 cases of methicillin-resistant *S. haemolyticus* (MRSH) bacteremia occurred in a 7-month period in a hematological ward. From August 2000 to February 2001, 220 episodes of bacteremia have been observed in our pts with hematological malignancies. Out of these 123 (56%) were caused by coagulase-negative staphylococci and 22 (10%) by MRSH.
Methods: Predisposing factors, clinical characteristics, antimicrobial treatment of these 22 cases were compared with a control group of pts with *Staphylococcus aureus* (SA) bacteremia. There were no differences in sex, age, underlying HM and intensive antineoplastic chemotherapy in the two groups of pts. More pts with MRSH were neutropenic at the onset and during bacteremia ($P=0.0003$) and had a CVC in place ($P=0.04$) Prophylaxis with quinolones was given in 17 and 11 pts with MRSH and SA, respectively ($P=0.05$). Previous and/or ongoing antibiotics, including glycopeptides, were administered in a similar percentage in the two groups.
Results: All MRSH and SA isolates were inhibited by 4 mg/mL Vanco concentrations. On the other hand, Teico concentrations >8 mg/mL were required to inhibit 11 (50%) MRSH (up to >16 mg/mL to inhibit three MRSH isolates); all SA were inhibited by Teico concentrations <8 mg/mL. Crude mortality rate was 23% and 18% in MRSH and SA pts, respectively; attributable mortality was 4.5% in both groups. Duration of bacteremia was longer than 48 h in 15 MRSH pts vs. 6 SA pts ($P=0.003$). CVC was removed in five MRSH pts and three SA pts. Only 14 out of 22 MRSH bacteremias were treated with glycopeptides (10 Teico, 4 Vanco). No cases of endocarditis have been observed.
Conclusions: MRSH bacteremia is a frequent complication in pts with HM; as compared to SA bacteremia, is more frequently associated with neutropenia and CVC, despite poor susceptibility to antibiotics, associated morbidity and mortality appear negligible.

P1160 Septic shock in neutropenic patients

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Objective: To study the characteristics of septic syndrome in neutropenic patients.
Methods: We studied 94 septic episodes in 86 neutropenic patients (absolute number of polymorphonuclears <500/cm^3), owing to toxicity from chemotherapy. The majority of the patients (75.6%), suffered from hematological malignancies. The data analyzed included clinical characteristics, laboratory findings, duration of hospitalization and outcome.
Results: In one-third of the septic episodes, there were blood cultures found positive for either Gram-positive bacteria (45%, predominately *S. aureus* and Staph. coagulase-negative), or Gram-negative bacteria (48%, predominately *E. coli* and *Pseudomonas aeruginosa*). In 20% of the cases, there were more than one pathogens isolated from the blood cultures (polybacteremia). The most prevalent manifestation of sepsis in neutropenic patients was fever, present in 90% of the septic episodes. Evidence of severe sepsis (hypoxemia, oliguria, metabolic acidosis, DIC) was present in 18% of the septic episodes and 8.5% were in septic shock. Mortality was 14%. Prolonged neutropenia was associated with prolonged length of hospitalization and adverse outcome. The duration of hospitalization was shorter in patients with positive blood cultures (12.8 ± 7 days) than in patients with negative blood cultures (22.3 ± 5.4 days) ($P<0.05$).
Conclusions: Positive blood cultures in neutropenic septic patients lead to timely administration of appropriate antimicrobial therapy and therefore decrease the length of hospitalization. Fever is the main manifestation of sepsis in neutropenic patients. Prompt increase of the total number of polymorphonuclears in these patients, improves the outcome and shortens the length of hospital-stay.

P1161 Changing of empiric antibiotic regime to prevent *Enterococcus* infection in febrile neutropenic patients

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Objectives: To analyze the preventing effect of changed empiric antibiotic therapy on the occurrence of severe *Enterococcus* spp. infections in febrile neutropenic patients.
Methods: 197 febrile neutropenic periods of 131 patients were treated between 01 January of 2000–30 June of 2001. 68 patients suffered from non-Hodgkin lymphoma, 56 from acute leukemia, 7 from other hematological diseases such as aplastic anemia or drug-induced agranulocytosis. Thirty-seven cases were treated with high dose of cytostatic drugs. The absolute granulocyte (G) count was <0.1× G/L in 59 cases, 0.1–0.5× G/L in 70 cases and 0.5–1.0× G/L in 68 cases. Blood cultures and relevant microbiological samples were examined during febrile periods. Eighty infectious periods were clinically documented, 42 infectious periods were clinically and microbiologically documented and FUO were seen in 75 cases. Despite the fact that only one *Enterococcus faecium* sepsis could be detected between 1995 and 1999 in febrile neutropenic patients, in 2000 seven bacteremia ceased by multidrug-resistant *Enterococcus faecium* and three caused by *Enterococcus faecalis* was observed. In two cases, *Enterococcus faecium* caused urosepsis. The third-generation cephalosporin ± aminoglycosid empiric antibiotic therapy was changed either to piperacillin/tazobactame or in some cases to carbapenems from December of 2000 to prevent selection of enterococci.
Results: After changing empirical antibiotic regime in the hematological department only one *Enterococcus faecium* sepsis (January of 2001) and two *Enterococcus faecium* urinary tract infections (January of 2001 and April of 2001) were detected between January and November of 2001.
Conclusion: Changing empiric antibiotic therapy in the hematological department has stopped increasing of the multidrug-resistant *Enterococcus faecium* infections in the fever neutropenic patients can interrupt.

P1162 Betadine influence on colonization of nasopharynx of patients with leucopenia after chemiotherapy

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Introduction: Betadine solution is an iodide antiseptic with broad spectrum of antimicrobial activity (it has only local use). Application of this agent on skin or mucosa eliminates at least 85% microorganisms, which makes it one of the most effective antiseptics.
Objectives: Estimation of betadine activity on microorganisms colonizing nosopharynx of patients treated with chemiotherapeutics.
Methods: We examined 25 patients (pts) (22 with acute leukemia and 3 with lymphoma malignum) who demonstrated leucopenia after chemiotherapy. Those pts were treated in Hematological Ward. 12 pts had to gargled betadine 3 times a day and 13 pts were a control group (they used mixed solution with antibacterial, antifungal, etc. ingredients). All of them had bacteriological examinations (nosopharyngeal swab and saliva) before they started using betadine and on 7th, 14th, 21th day of examination. The main purpose of the test was to check the level of microorganisms elimination from the nosopharynx. Samples were identified by standard methods and disc – diffusion susceptibility testing was done according to NCCLS.
Results: The patients were examined from 30 March 2001–15 November 2001. 270 bacteriological tests were done. They included 86 nasal swabs, 84 pharyngeal swabs and 100 saliva samples. We noticed presence of pathogens taken before the treatment in 15 cases. There isolates were as followed: yeast (68 isolates; 49.3%), Gram-negative rods (52 isolates; 37.7%), Gram-positive coca (18 isolates; 13%). We cultured only common, multisusceptible strains. Results are presented as follows.

Number of microorganisms	Betadine (nr of patients)	Control group (nr of patients)
Decrease about 103 cfu/mL	3 (25%)	1 (7.6%)
Increase about 103 cfu/mL	2 (16.6%)	2 (15.4%)
No change	7 (58.3%)	10 (76.9%)

We did not observe correlation between gargling with antiseptic and the number of microorganisms in nasal samples.
Conclusions: We conclude that betadine is an effective disinfectant and in 83.4% examined patients prevented superinfections.

P1163 Infectious complications in acute leukemia patients in a Korean university hospital between 1998 and 2001

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Objectives: The purpose of this prospective study was to determine the spectra, frequency and their resistance pattern of pathogens that cause bacteremia and pneumonia in patients with acute leukemia.
Methods: The anatomical sites of infection was determined as a culture-positive site or a clinically evident site of infection, with the isolation of bacterial strain from either a sterile or a non-sterile body site, according to CDC definitions. Susceptibility of organisms was studied using a disc-diffusion technique according to NCCLS methods.
Results: Over the 3-year period, 360 febrile episodes, attributable to infection, were documented. The frequent sites of infection observed were: blood stream 122 (33.8%), lung 117 (32.5%), oral mucosa 114 (31.6%), gastrointestinal tract 97 (26.9%), perianal area 44 (12.2%), soft tissue 43 (11.9%), indwelling venous catheter 33 (9.1%), and paranasal sinus 23 (6.3%). A total of 162 organisms were isolated in the 122 bacteremic episodes. Overall, 86 Gram-positive bacteria (GPB) (53.0%), 73 Gram-negative bacteria (GNB) (45.0%) and 3 fungi (0.18%) were isolated. The most frequently isolated organisms were coagulase-negative staphylococci (23.4%), *E. coli* (16.6%), *K. pneumoniae* (11.1%), Enterococci (10.4%), Viridans group streptococci (9.8%), *E. cloacae* (8.6%) and *S. aureus* (7.4%). Seven (41.1%) of enterococci were VRE. In addition, 15 (20.5%) of GNB were the extended spectrum beta lactamase-producing organisms. Twenty percent of GPB were susceptible to cefotaxime, the extended spectrum beta lactams commonly used for empiric therapy, whereas 55.5% of GNB were susceptible to piperacillin, 56.1% to cefotaxime, 61.6% to ceftazidime and 75% to ciprofloxacin. Ninety-two organisms were isolated in the respiratory specimen cultures from 64 patients with microbiologically documented pneumonia; 57.6% were GNB, 30.4% GPB, 7.6% M. tuberculosis and 4.3% fungi. Antimicrobial susceptibility among the organisms that cause pneumonia was comparable to that of the same species for bacteremia. Overall, 50 patients (13.8%) died during or after 360 febrile episodes and 29 (8.0%) died of infectious complications.
Conclusions: Our results show that GPB are the major pathogens of bacteremia and the rates of resistance among bloodstream or respiratory pathogens are increasing. Greater consideration must be given to carbapenems and glycopeptides in empirical therapy for infections in patients with acute leukemia.

P1164 *Staphylococcus aureus* bacteremia in neutropenic and non-neutropenic patients: a retrospective case-control study

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Background: Bacteremia owing to *S. aureus* is an increasing problem for the widespread use of intravascular devices in clinical practice. Few data are presently available in the International Medical Literature about this infection in neutropenic patients (pts).
Aim of the study: To define the clinical significance of *S. aureus* bacteremia in neutropenic pts. with hematological malignancies.
Methods: Cases of *S. aureus* bacteremia were identified by review of microbiology laboratory records and defined according CDC criteria by review of pts. clinical charts. To determine the clinical impact of *S. aureus* bacteremia in neutropenic pts. a case control study was performed with cases of *S. aureus* bacteremia in non-neutropenic pts with similar age and with bacteremia occurring during the same period.
Results: Two groups of 39 neutropenic and non-neutropenic pts could be analyzed in the study, with similar median age (55.5 vs. 51.8 year, respectively), similar male to female ratio (25 : 14 vs. 21 : 18, respectively), and similar rate of methicillin-resistance (26% vs. 28%, respectively). Neutropenic pts. showed an higher frequency of mucositis ($P = 0.00001$), less days of fever ($P = 0.05$), less days of bacteremia ($P = 0.007$), less days to an adequate antibiotic treatment ($P = 0.002$), less days of antibiotic therapy ($P = 0.009$). In neutropenic pts. were observed no metastatic infection ($P = 0.002$) and a lower related mortality (5.1% vs. 28.5%, respectively, $P = 0.006$). Relapse was observed in 4 (10%) neutropenic patients vs. 2 (5%) non-neutropenic ones ($P = 0.3$).
Conclusion: Bacteremia owing to *S. aureus* in neutropenic pts. seems to be a low-inoculum bloodstream infection associated with low morbidity and mortality rates if adequate antistaphylococcal therapy is promptly administered.

P1165 Use of daptomycin in febrile, neutropenic patients with vancomycin-resistant *Enterococcus faecium* (VREF) bacteremia

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Objective: It is known that vancomycin-resistant enterococcal bacteremia in the neutropenic patient is associated with poor outcome. In a series of six prospectively followed bone marrow transplant recipients with VREF, mortality at 30 days was 100%. Daptomycin is an investigational lipopeptide with excellent bactericidal activity against VREF.
Methods: We examined the microbiologic and clinical outcome in patients with fever, neutropenia and bacteremia with VREF who were treated with daptomycin in an open label, emergency-use trial. We analyzed those patients who received more than one dose of daptomycin.
Results: There were 10 patients with 11 courses of fever and neutropenia with VREF bacteremia who received >1 dose of daptomycin, five were BMT patients, six had acute leukemia. Median age was 58 years. Eight patients were male, two females. Daptomycin dose was 6 mg/kg/day i.v. with doses adjusted for renal insufficiency. Median number of days of daptomycin treatment was 14 (range 3–18 days). Ten of 11 courses were complicated by persistent, profound neutropenia. Five of 11 courses (45%) had clinical and microbiologic cure, 3 of 11 (27%) relapsed, 1 had persistence and 2 could not be evaluated. Mortality 1 month postdaptomycin treatment use was 5 of 10 (50%).
Conclusions: Compared to the historical experience of bacteremia with VREF in fever and neutropenia in BMT, use of daptomycin in this population shows promise with microbiologic cure rates of 45% and survival of 50% ($P = 0.09$). However, VREF bacteremia in fever and neutropenia is still a very poor prognostic marker.

P1166 Randomized pilot phase trial of once-daily oral monotherapy of neutropenic fever

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Objectives: Intravenously administered antimicrobial agents have been the standard choice for the empirical management of fever in patients with cancer and neutropenia. If orally administered empirical monotherapy were not inferior to intravenous therapy, it could offer advantages such as improved quality of life and lower cost.
Methods: We perform a prospective randomized multicenter trial in the treatment of neutropenic fever, comparing efficacy and safety of i.v. piperacillin/tazobactam 4.5 g tid with oral levofloxacin 500 mg/day.
Core inclusion criteria are: oral temperature at least 38.5 °C once or 38 °C twice within 24 h, neutropenia <500 µL, expected to last shorter than

10 days. Patients are stratified for autologous stem-cell transplantation. Primary endpoint is defined as defervescence on day 4 followed by at least 7 afebrile days. Secondary endpoints are time to defervescence and toxicity.
Results: A total of 28 episodes of 23 patients ($m = 12, f = 11$; age 20–69 years; median 46 tears) have been included up to now. According to ISDA criteria fever of unknown origin accounted for 20 episodes (71%), microbiologically defined infection for 5 (18%), clinically defined infection for 3 (11%). Treatment was successful without the need of modification in 85% in the intravenous-therapy group treated with piperacillin/tazobactam and 73% in the oral monotherapy group treated with levofloxacin. The median time to defervescence was 3 days (range 1–4 days each). One patient in the oral-therapy group died with septic shock without isolation of a causative pathogen. There were no adverse effects like diarrhea, nausea or vomiting.
Conclusion: Oral levofloxacin 500 mg/day does not seem to be inferior to intravenous piperacillin/tazobactam tid in the empirical treatment of neutropenic fever in patients with low risk. Recruitment into the study continues in order to warrant a solid statistical analysis. This interim evaluation was intended according to the protocol.

P1167 Recurrent *Brevibacterium* bacteremia: role of long-term catheter

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Objectives: *Brevibacterium spp. is a rare cause of bacteremia in compromised* hosts with intravenous devices. The need for catheter removal in these cases has been suggested, although information on the efficacy of a conservative catheter antibiotic therapy is limited. We describe two cases of recurrent bacteremia owing to *Brevibacterium casei* associated with a long-term infected intravascular access.
Patients and methods: Two cases of *Brevibacterium* bacteremia were retrospectively studied over a two years period. Species identification was performed using API Coryne (Bio Merieux) and complementary biochemical tests. Susceptibility was performed by disc diffusion on Mueller–Hinton agar with 5% sheep blood. Culture isolates were typed using amplified fragment length polymorphism analysis of genomic DNA.
Results: The first patient, a 43-year-old woman, suffered from Crohn's disease with chronic entero-cutaneous fistulae. She had a totally implanted port for infusion of total parenteral feeding. A first episode of bacteremia owing to *B. casei* was treated with a 15-day course of vancomycin. Blood cultures were negative after treatment. Five months later, she became neutropenic and developed a second episode of bacteriemia with the same pathogen. Despite catheter removal and another course of vancomycin therapy, the patient developed sepsis and died. The second patient, a 31-year-old man, had a Hickman's catheter used for chronic hemodialysis after kidney transplantation failure. Following a first episode of bacteriemia with *B. casei*, the patient was also treated by a 15-day course of vancomycin. Control blood cultures were negative at the end of therapy. Two months later, the patient became septic again and the organism was re-isolated from blood. He received another 15-day course of vancomycin while the catheter was not removed. At two months follow-up, this patient's examination was unremarkable and control blood cultures remained negative. All isolated strains had the same antimicrobial-susceptibility profile.
Conclusions: These cases of catheter-related *B. casei* bacteriemia illustrate the relapsing nature of these infections after transient cure by antibiotic therapy with the catheter left in situ. Catheter removal may be the preferred therapeutic option for eradication of this device-associated infection.

P1168 Microorganisms from blood cultures in a hematology unit from 1999 to 2001

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Gdansk, P

Objectives: To analyze the results of blood cultures in patients suffering from malignant hematological disease.
Methods: We obtained blood cultures from 381 patients hospitalized in hematology ward from 01.01.1999 to 30.10.2001.
Two systems were used: Bact/Alert (Organon Teknika) and Vital (bioMerieux).
Results: We cultured 1170 microorganisms from our patients. Positive rate was 22.2%, 91 were polymicrobial cultures. The incidence of *Enterobacteriaceae* was 147 isolates (12.5%) with only 5 (0.4%) ESBL-producing isolates. Non-fermenting rods constituted 9.5% (112) of all microorganisms. The most prevalent were staphylococci, coagulase negative (CNS) – 535 isolates (45.7%), *Staphylococcus aureus* 47 isolates (4.1%) and MRSA 2 isolates. There were 79 (9.1%) enterococci with 30 isolates of them VRE. *Candida* spp. was isolated with the frequency of 5.9%.
Conclusion: Gram-positive coca are most prevalent microorganisms isolated from blood cultures with VRE being multiresistant. There was a significant increase in *Escherichia coli*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolation rate over 3 years, from 4 to 8%, 1.1 to 15% and 1.5 to 6%, respectively.

P1169 *Acinetobacter baumannii* strains isolated from oncologic patients

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Objectives: The aim of the study was to determine the clinical usefulness of biotyping and antibiotic resistance patterns in epidemiological investigation of *Acinetobacter* sp. infections.
Methods: We performed a retrospective study to evaluate the episodes of *A. baumannii* infections during the last 1.5 years. The isolates were identified by the API 20 NE system. Susceptibility to antimicrobial agents was determined according to NCCLS by the MIC and disc-diffusion methods.
Results: During the period from 01 January 2000 to 30 June 2001, 101 strains, from clinical material, were collected. The sources of isolates were as follows: respiratory specimens $n = 42$, wounds $n = 23$, urine $n = 19$, catheters $n = 14$ and other specimens $n = 3$. Repeat isolates from the same patient were excluded. Fifty-five isolates were collected from patients hospitalized at ICU, 35 isolates from surgery wards and 11 isolates from the other wards. By the API 20 NE three different biotypes were identified. The API profile 0001073 dominated (76%). Most of the *Acinetobacter baumannii* isolates were sensitive to imipenem (100%), meropenem (90%), ampicillin/sulbactam (60%) and gentamicin (37%).
Conclusions: In our study, *A. baumannii* mostly isolated at the intensive care unit and surgery wards and it predominately caused respiratory tract infections. In our experience imipenem remains the treatment of choice in *A. baumannii* infections

P1170 The clinical spectrum of infections in patients with primary humoral immunodeficiency: a clinical survey of patients from Iranian Primary ImmunoDeficiency Registry

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Background: Primary Humoral ImmunoDeficiencies (PHID) are currently increasingly recognized, thanks to novel advances in the immunology and its laboratory techniques. Patients with PHID frequently show infectious complications, which are often the first manifestation of disease and the major cause of their morbidity and mortality.
Objectives: To determine the spectrum of infectious complications in children with PHID.
Methods: We have reviewed the data from the clinical files of patients with PHID, diagnosed according to standard criteria, who were enrolled in Iranian Primary Immunodeficiency Registry.
Results: We analyzed 125 patients (84 males), with the diagnoses of primary antibody deficiency including common-variable immunodeficiency (64 pts), x-linked agammaglobulinemia (29 pts), IgA deficiency (20 pts), IgG-subclass deficiency (8 pts), and hyper-IgM syndrome (4 pts). The mean age of the patients at the time of study was 11 years. Acute and recurrent infections were found in all of our patients. A hundred and twelve patients out of 125 have had some form of respiratory system infections. Following the respiratory system was gastrointestinal tract involvement, seen in 77 patients; The rest of organs,

in descending order of frequency is musculoskeletal system in 21 patients, skin infections in 20, and central nervous system involvement in 18 patients. The majority of patients (92 pts, 73.6%) had pneumonia prior to diagnosis. The other infections were diarrhea (73 pts), otitis media (68 pts), sinusitis (61 pts), conjunctivitis (17 pts), bacterial meningitis (16 pts), oral candidiasis (14 pts), cutaneous abscesses (14 pts), septic arthritis (13 pts), urinary tract infections (13 pts), and osteomyelitis (5 pts). Also we had 24 patients with bronchiectasis, 12 with giardiosis, 6 with tuberculosis and 5 patients with *Pneumocystis carinii infection*. As the first presentation, 86 patients were presented with respiratory tract infections. Pneumonia was the most frequent presentation, with the frequency of 37.6% (47 pts). The rest of organs, involved as the first manifestation of primary immunodeficiency were gastrointestinal tract, skin and CNS. Sepsis was also seen in three patients as the primary manifestation.
Conclusions: Based on this study, we suggest that hypogammaglobulinemia should be considered in any patient with a history of recurrent infections in different organs, and such patients should have a full assessment of immune system.

P1171 The clinical spectrum of infections in patients with primary phagocytic disorders: a clinical survey of patients from Iranian Primary ImmunoDeficiency Registry

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Background: Primary phagocytic disorders, although not very common, comprise one of the challenging tasks of the infectious specialists. This group can be divided into those of deficient cell numbers and those with insufficient phagocytic function. Chronic granulomatous disease, being the most common disorder in this group, results from a defect in the oxidative burst of neutrophils.
Objective: To determine and compare the clinical spectrum of infections in patients with primary phagocytic disorders.
Methods: We had extracted data from the clinical files of patients with the diagnoses of primary phagocytic disorders, according to the standard criteria, who have registered in the Iranian primary immunodeficiency registry.
Results: We have reviewed 65 patients (42 males), with the mean age of 10.7 years at the time of study. They were all diagnosed as primary phagocytic deficiency including chronic granulomatous disease (38 patients), leukocyte adhesion defect (11 patients), hyper IgE syndrome (8 patients), Chediac–Higashi syndrome (4 patients), and Schwachman syndrome (4 patients). In 43 of our patients, the parents were first-degree relative. The leading infections were in the form of lymph node involvement (40 patients), abscess formation (37 patients), and pneumonia (35 patients). Abscesses have mostly occurred in the skin (31 patients), followed by liver abscesses (6 patients), pulmonary abscess (3 patients), and perianal (2 patients). The other sites of abscess formation were in the breasts, paravertebral, eyes, neck, and the teeth. The causative organism in most of the cases was *S. aureus*. The other infections were otitis media (21 patients), diarrhea (21 patients), oral candidiasis (16 patients), tuberculosis (12 patients), cellulites (10 patients), sinusitis (10 patients), septic arthritis (8 patients), and osteomyelitis (8 patients). We also had 6 cases with disseminated BCGosis and three patients with aspergillosis. Most of these infections were caused by catalase-positive organisms. On the whole, respiratory system was the leading site of involvement.
Conclusion: Phagocytic disorders should be considered in any patient with recurrent abscess formation in different organs of the body especially in the lymph nodes and the skin. Thus, any patient with a history of recurrent and/or unusual abscesses should have a full assessment of the immune system.

P1172 Dysregulation of myeloperoxidase level and life-threatening *Candida* infection in subjects receiving cancer chemiotherapy

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Background: Systemic fungal infections are a great problem in patients receiving cancer therapy. In those patients profound neutropenia following chemiotherapy is a serious risk factor for opportunistic fungal infections associated with high risk of mortality. Phagocytic cells represent the first line of defense in the control of *Candida* infection. Those cells are able to kill yeast's cells by activation of the respiratory burst and the dominant mechanism involves the myeloperoxidase-dependent pathway.
Objective: Evaluation the connection of MPO level with frequency of colonization and invasive *Candida* infections in patients undergoing chemiotherapy.
Methods: The clinical samples were obtained from 31 women with carcinoma ovariorum after the third course of treating with aggressive specimen Taxol and cisplatin, without symptoms of systemic fungal infection. They were examined for fungal colonization of mucosal membranes and presence of *Candida* antigens in bloodstream. For antigen detection we used *Platelia Candida*. The level of MPO was determined using ELISA method. The control group consisted of 21 healthy persons.
Results: Routine culture on Saburaud agar and biochemical identification of *Candida* species demonstrated that five patients were colonized on two or three sites. 4 of those persons were also positive for antigen presence in bloodstream. The mean level of MPO in persons colonized with *Candida* was 5.9 ng/mL (range 0–19.5). In patients with no *Candida* infection, the mean level of MPO was 33 ng/mL and in control group: 1691 ng/mL.
Conclusions: The results indicate that in the group of patients with drastic decreased level of MPO, there was frequently *Candida* invasion observed. Generally decrease of MPO level in oncologic patients is a predisposing risk factor for invasive *Candida* infection.

P1173 Continuous infusion of ceftazidime for patients with breast cancer and multiple myeloma receiving high-dose chemotherapy and peripheral blood stem-cell transplantation

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Objectives: This prospective study was performed to examine the safety and efficacy of a continuous infusion of ceftazidime in neutropenic febrile patients after high-dose chemotherapy (HDCT) and peripheral blood stem-cell transplantation (PBSCT) and to determine if the underlying disease would represent a risk factor for infectious complications.
Methods: From September 1995 to May 2000, 55 patients with breast cancer (BC, group I, 54 females, 1 male) and 32 patients with multiple myeloma (MM, group II, 10 females, 22 males) were included in this study. The febrile patients received 2 g intravenous bolus of ceftazidime, followed by a 4-g continuous infusion per 24 h using a portable infusion pump. If the fever persisted for 72 h, a glycopeptide antibiotic was added.
Results: The median age was 42 years (range 22–59) in group I and 52 years (range 35–63) in the group II. A total of 35 BC patients (64%) and 20 MM patients (63%) responded to the monotherapy of ceftazidime. After combination with a glycopeptide antibiotic, 11 patients vs. 10 patients became afebrile. The causes of fever in group I were: fever of unknown origin (FUO) in 49 patients, microbiologically documented infection (MDI) in 5 patients and clinically documented infection (CDI) in 1 patient. The causes of fever in group II: FUO in 22 patients, MDI in 8 patients and CDI in 3 patients. 41 fever episodes of the BC patients (75%) were successfully managed by outpatients treatment, vs. 22 episodes in the MM patients (69%). Significantly more episodes of MDI and CDI occurred in patients with MM ($P=0.05$).
Conclusion: Continuous infusion with ceftazidime is efficacious in both treatment groups. This study showed that patients after HDCT and PBSCT with BC and MM could be treated as outpatients. A close monitoring by a physician is essential to assure the safety. The outpatient treatment contributes a better use of health care resources.

P1174 Immunosuppression after experimental stroke causes severe lung infections

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Objective: Extensive epidemiological studies have demonstrated the high incidence of severe stroke related infections and their prominent role for morbidity and mortality in stroke patients.
Methods: We subjected mice to an experimental regional cerebral ischemia (stroke) by occlusion of the middle cerebral artery (MCAO) for

durations from 30 min to permanent occlusion. Fourteen hours, 2, 5, 14 or 42 days after experimental stroke the nimals were killed and blood, the thymus, and the spleen were harvested. We analyzed the tissues with FACS for the leukocyte subpopulations CD3+, CD4+, CD8+, CD45R+, DX5+, and CD11+0. We measured the proportion of apoptotic leukocytes by annexin-V-staining. We further performed functional testing of monocytes (TNF-α) and lymphocytes (IL-4/IFNα) with stimulation by ConA and LPS, respectively. The lung, thymus and intestines were examined histologically. Thymus histology included TUNEL-staining. Thymus-DNA was tested electrophoretically for laddering. Microbiological assessment was based on cultures of blood, bowel, and lung tissue of animals killed 3 days after stroke.

Results: The FACS-analysis demonstrated an early onset and long-standing reduction of circulating lymphocyte population, predominantly of NK- T- and B-cells. With annexin staining, we found a massive apoptosis rate of immature CD4+8+ thymocytes and of splenocytes. The apoptosis of thymocytes corresponded with a macroscopic atrophy of the harvested thymi. Beyond the demonstrated lymphopenia we observed a pronounced monocyte and lymphocyte dysfunction. The microbiological blood and lung tissue cultures of animals 3 days after stroke displayed a high bacterial load (mainly *E. coli*). This corresponded to the clinically observed septicemia and pneumonia.

Conclusion: Experimental stroke leads to an early onset and long-lasting lymphopenia as well as a significant dysfunction of the remaining lympho- and monocytes. The immunological organs thymus and spleen are not spared. This immuno-suppression syndrome provides the basis of a high vulnerability for mainly pulmonary Gram-negative infections. Our findings warrant the evaluation of specific immuno-modulatory and antimicrobial strategies in the adjuvant therapy of strokes.

P1175 The risk factors for candidemia in lung cancer patients after surgery

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Background: Invasive candidemia is a life-threatening complication especially in hospitalized cancer patients because of surgical operation. Early diagnosis and evaluation of the range of risk-factors leading to the better prognosis and prophylactic chemotherapy of those patients are still a major challenge. *Candida* colonization, dysfunction of humoral and cellular immune system and prolonged periods of hospital staying are considered as the high-risk factors of invasive candidemia development.

Objectives: Rate of *Candida* nasopharyngeal colonization at the first and last day of hospitalization, number of invasive *Candida* infection after surgical lung operation and relationship to immune factors (TNF-α, IL-12, C-reactive protein and myeloperoxidase concentration) and term of hospital staying were studied in 108 patients with lung cancer.

Results: Forty-three (39%) of hospital admitted patients were colonized with *Candida* in nasopharynx and additional 12 patients were colonized during hospital-stay. Pneumonia and wound infections were observed in 15 patients and 10 of them (66%) had been colonized with *Candida* at the first day of study. *Candida albicans* as the only pathogen was isolated from three patients. There was some relationships between colonization and infection by *Candida* and tested immune factors but significant relationships between candidemia and myeloperoxidase concentration (13.1 ng/mL vs. 170 ng/mL in healthy persons) were observed.

Conclusions: Our study confirmed that patients suffered from lung cancer are at high risk of infections with pathogenic *Candida* species that at high percentage colonized those patients in mucosal membranes of pharynx. Colonization is a progressive process during hospital staying and decreased immune factors might predisposed to infection incidence.

P1176 Frequency and clinical significance of *Escherichia coli* bacteremia caused by ESBL-producing strains in neutropenic patient

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Introduction: Over the last 15 years, numerous outbreaks of infection with organisms producing extended-spectrum beta-lactamases (ESBLs) have been observed world-wide. The advent of these pathogens has represented a great threat to the use of many classes of antibiotics, particularly cephalosporins, and an associated mortality ranging from 42 to 100%.

Aim of the study: Retrospective evaluation of the frequency and clinical significance of ESBL-producing *E. coli* bacteremia in neutropenic patients at Department of 'Biotecnologie Cellulari ed Ematologia' Policlinico Umberto I University of Rome 'La Sapienza'.

Methods: All *E. coli* blood isolates from January 2001 to October 2001 were identified as ESBL producers strains according to NCCLS criteria.

Results: During the study period, 4 (10%) out of 42 *E. coli* blood isolates were found ESBL producers. Review of clinical charts revealed the urinary tract as the source of infection in two cases and failure of ceftriaxone plus amikacin therapy in all septicemic patients with ESBL-producing strains. Piperacillin–azobactam was used as a second line therapy in all these patients and cure was obtained in three. The remaining patient eventually improved with meropenem therapy.

Conclusion: Our preliminary data confirm poor efficacy of third generation cephalosporins and suggest a potential role of piperacillin–azobactam against serious infection caused by ESBL-producing *E. coli*.

Tuberculosis and related infections

P1177 Epidemiologic and clinical features of tuberculosis: a review of 139 cases reported from 1996 to 2000 in a general hospital in Pisa, Italy

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Objectives: To describe epidemiological and clinical features of tuberculosis (tbc) cases identified recently in the hospital of Pisa (Tuscany, Italy).

Materials and methods: We performed a retrospective study of all cases of tbc notified to the Public Health Service in Pisa during January 1996–December 2000. The diagnosis of tbc was made following the criteria of the WHO.

Results: A total of 139 pts (83 males and 56 females) affected by tbc were identified. Diagnosis was microbiologically proven (positive culture for *M. tuberculosis*) in 81 pts. Mean age was 53.8 + 20.5 SD year (range 16–87). Thirty-five pts were extra-European community citizens (mostly from Africa). The incidence of tbc (*N*/100 000) was 8.4 in 1996 and 6.8 in 2000. Sixty-eight percent of pts had pulmonary tbc, 24% extrapulmonary and 7% mixed tbc. Extrapulmonary tbc was more frequent in extra-European community citizens than in Italian ones. Seven pts (5%) were presenting also advanced HIV infection. Microscopic examination for acid fast bacilli in sputum or bronchial secretion resulted negative in 17.4% of proven pulmonary tbc (positive culture for *M. tuberculosis*). The chest X-ray showed pleural effusion in 19 pts and cavity formation in 42 pts (more frequently recognized at CT imaging). Fever was not present in 42.4% of the pts at the moment of diagnosis. About 3.8% of the isolated strains of *M. tuberculosis* were in vitro multidrug-resistant.

Conclusions: The data presented showed an important rate of tbc in Pisa, similar to the incidence of tbc reported by other studies carried out on a national scale. We have yet to understand if the decreased rate observed in 2000 represents a new trend as reported in other north American countries. The isolation rate of multidrug-resistant strains of *M. tuberculosis* in Pisa seems to be similar to the rates reported in other areas of Europe.

P1178 Tuberculous meningitis in non-HIV children

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Objectives: To analyze clinical features, laboratory findings and prognosis of TBM in non-HIV children.
Methods: A total of 51 children with TBM admitted in our department in last 6 years were retrospectively studied.
Results: TBM in children represented 27.8% from all patients (pts) with TBM recorded in above-mentioned period of time. Age distribution was: <1 years: 5 cases; 1–3 years: 9 cases; 4–6 years: 13 cases; 7–10 years, 8 cases; and 14–16 years: 8 cases, with limits between 7 months and 16 year of age. A total of 24 pts (47%) were refereed from rural areas. Family exposure to TB infection was documented in 20 cases (39.2%) and 11 pts (21.5%) had personal history of TB. Twenty children (39.2%) had concomitant active pulmonary TB. Meningeal signs dominated clinical presentation in all cases; 13 pts (29.4%) had cranial nerves palsies, 7 pts (13.7%) – pyramidal signs, 6 pts (11.7%) had acute urinary retention, and seizures were noted in 7 children (13.7%). Coma was recorded in 14 cases (27.4%). Mean CSF cells count was 482 cells/mm^3, with significantly lower values in group under 7 years of age ($n=30$) versus children >7 year ($n=21$) (304/mm^3 vs. 586/mm^3, $P<0.05$); lymphocytic predominance >90% was found in 82.3% of cases. Protein level in CSF was greater than 1 g/L in 78.4% of pts. Direct CSF smears revealed AFB in 21 cases (41.1%) and CSF cultures were positive for *Mycobacterium tuberculosis* in 14 pts (27.4%); both CSF-sediment staining and CSF cultures were positive in three pts (5.9%). Five pts with coma eventually died. Neurological sequelae were noted in 12 pts (28.5%). Nine pts died (17.6%) and seven cases of them were <7 years.
Conclusions: TBM in children represents a high proportion of all TBM cases admitted in our clinic. Most of the cases are recorded in children less than 10 year of age. Most of the children had history of TB or exposure to TB. Mortality and neurological sequelae remain high.

P1179 Tuberculous meningitis in children – epidemiological, clinic and biologic aspects

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Objectives: To study the epidemiological, clinic and biologic aspects of tuberculous meningitis in children.
Methods: Retrospective analysis of 20 cases hospitalized in our clinic with the diagnosis of tuberculous meningitis between 1994 and 2001.
Results: The following results were obtained: the highest incidence was noticed in the preschool age group (50%), the disease affecting more the girls (60%) and the patients of urban origin (65%). Only, in 20% of cases there was evidence of contact with an infected patient. Seventy percent of cases had no other determination of the infection, 15% of cases were severe forms with encephalitic component. The extent in time of the debut varied from 1 week to 2 month, the hospitalization period being extended up to 60 days for 15 patients. The spinal fluid at the admission was clear in 70% of patients, in 14 cases the cell count results were under 300 cells/mm^3 and only in seven cases there were 100% lymphocytes. Most tuberculous meningitis (85%) had low level of glucose, elevated proteins up to 2 g/L and low chlorine levels in the spinal fluid. The diagnosis was established on positive acid-fast stain (75%) and/or cultures on Lowenstein–Jensen medium. Six patients developed hydrocephaly and three patients presented brain abscesses.
Conclusions: The recrudescence of tuberculosis noticed in the last decade is associated with the increase of the number of bacillary meningitis and meningoencephalitis. Nervous system tuberculosis is a severe form of infection with high risk of complications and not favorable evolution.

P1180 Concomitant occurrence of bronchopleural and pleurocutaneous fistula from *Mycobacterium tuberculosis* pulmonary infection

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Objectives: The presentation of a rare case of concomitant existence of both bronchopleural and pleurocutaneous fistula from *M. tuberculosis* infection in a patient with a history of past pulmonary tuberculosis.
Methods: The samples were examined for the presence of *Mycobacterium* spp. All samples were cultured on two Loewenstein–Jensen and smears were examined after Ziehl–Neelsen staining for acid-fast bacilli (AFB). Identification and susceptibility testing of isolate was carried out by the National Mycobacteria Reference Center. A 78-year-old woman was admitted to hospital owing to a growing right-sided subcutaneous tumor-like lesion on her anterolateral chest area, during the past 2 months. In her past history she reported having pulmonary tuberculosis (diagnosed 30 or more years ago) which was incompletely treated. The patient had been well until 2 months ago when the cutaneous lesion started to develop but no other symptoms were present. On examination, there was a rather soft bulging cutaneous mass, 3 cm in diameter with a fistula of 2.7 cm on its surface which was located over the right anterior axillary line at the fifth intercostal space. No signs of inflammation were detected over the lesion but a whitish pus-like fluid could be seen oozing from the fissure. Chest radiography revealed extensive calcified right fibrothorax, but no signs of parenchymal lesions. By the use of methylene-blue dye which was injected into the fissure of the cutaneous lesion, the existence of both bronchopleural and brochocutaneous fistula was established. Samples of sputum and cutaneous lesion fluid were collected for direct AFB smear and cultures while biopsy was obtained from the cutaneous lesion. *M. tuberculosis* was grown from cultures (sensitive to rifambicin, ethambutol, isoniazid and resistant to steptomycin) while histological findings in biopsy were typical of tuberculous granulomas. The patient was given a standard anti-TB regimen and surgical intervention followed for drainage of pleural empyma and management of fistula. The patient was discharged from hospital in a good condition with appropriate instructions.
Conclusions: Coexistence of bronchopleural and pleurocutaneous tuberculous fistulas is a rare but recognizable complication of reactivated past pulmonary tuberculosis. Radiological signs of extensive pleural involvement and history of incompletely treated past pulmonary TB infection can facilitate the diagnostic work-up in these cases.

P1181 Selection of a *Mycobacterium tuberculosis* strain after dissemination in patients co-infected with two different clones

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Objectives: Single episodes of tuberculosis (TB) can be caused by co-infection with more than one clone of *Mycobacterium tuberculosis* (MTB). Polyclonal infections can also be found in cases of disseminated TB, but it has not been established whether both strains are present in the different body sites or whether a clonal selection (compartmentalization) could occur in the different body locations.
Methods: Three patients with documented co-infection with different MTB strains in two independent body sites (respiratory and extrarespiratory) were studied. Isolates from each body location were plated onto solid medium to obtain isolated colonies. Ten colonies were picked from each sample and molecular fingerprints were obtained by spoligotyping and double-repetitive-elements-PCR (DRE-PCR).

Results: In two patients, the colonies analyzed were all identical for the same sample but different when comparing respiratory and extrarespiratory samples. In the third patient, two different typing patterns were obtained for the colonies selected from the respiratory sample but only one of these two strains was detected in the analysis of the extrarespiratory sample.
Conclusions: A clonal compartmentalization is observed in the dissemination of TB. The selection of a specific clone for dissemination in one of the patients suggests that this compartmentalization is mediated by selective events. These findings must be considered in the management of patients with disseminated tuberculosis.

P1182 Mycobacterial infections in a general hospital during a 2-year period (November 1999–November 2001)

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Objectives: To determine the incidence of mycobacterial infections in general hospital during 2-year period and the drug-resistance patterns of isolated *Mycobacteria*.
Methods: Over 2-year period, 2058 consecutive respiratory and extrapulmonary samples from 1144 patients (25–88 years old), were examined for the presence of *Mycobacterium* spp. Respiratory and urine specimens were processed according to the standard CDC NALC/NaOH method. All samples were cultured on two Loewenstein–Jensen solid media and smears were examined by microscopy after Ziehl–Neelsen staining. Cultures were incubated at 37 °C for 6 weeks. Identification and susceptibility testing of isolates were carried out by the National Mycobacteria Reference Center in Athens. The Amplified *Mycobacterium tuberculosis* Direct Test (AMTD II, Gen-Probe, bioMerieux) was applied to 64 respiratory and nonrespiratory specimens collected from 60 patients. The MTD-technique was performed in samples after decontamination.
Results: A total of 29 *Mycobacterium* spp. were isolated from the cultures of samples, obtained from 29 patients (2.5%). Of these patients 10.3% were immigrants. Of the isolates, two were from urine, two from gastric fluid, 21 from respiratory specimens (sputa, bronchial washings), one from peritoneal fluid, one from pleural fluid, one from pus of pleurocutaneous fistula and one from biopsy. Of these, 20 were new cases of tuberculosis and 9 chronic or relapses. Smear microscopy for acid-fast bacilli was positive in 18 samples (62.1%). *Mycobacterium tuberculosis* complex (MTBC) strains detected in samples from 21 patients. *Mycobacteria* other than *Mycobacterium tuberculosis* (MOTT) were detected in eight cases. Of 64 examined with MTD samples, 56/64 were true negative (negative-culture and one was *M. avium*) (87.5%) and 2/64 were false-negative (3.1%) (one gastric fluid, one biopsy). Of the *M. tuberculosis* isolates 9.5% were resistant to streptomycin, 4.8% to isoniazid, 0% to rifampicin and to ethambutol. Mortality rate in cases of mycobacterial infections was 10.3%.
Conclusions: The incidence of tuberculosis was 2.5%. Multiresistance was not recorded in *M. tuberculosis* isolates. Of the patients, 69% were new cases of tuberculosis and 31% chronic or relapses. Mortality rate in cases of mycobacterial infections was 10.3%.

P1183 Tuberculous meningitis: clinical features and outcome in 27 patients

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Objective: To study the clinical features and outcome in adults with tuberculous meningitis.
Patients and methods: Twenty-seven patients (24 male and 3 female; ages ranged 19–68 years old) of tuberculous meningitis were reviewed retrospectively. The cases had been diagnosed at Gulhane Medical Military Hospital (Ankara, Turkey) between 1997 and 2000. The clinical manifestations and outcome for each case were analyzed and included in the study.
Results: The clinical symptoms and signs on the admission were fever, neck stiffness, and headache in 27 (100%) patients; alteration in consciousness and focal neurological signs in 15 (55.5%), hemiplegia and seizures in one (3.7%). Fourteen patients were in the first stage of the disease (rational, no focal neurological signs or hydrocephalus), 12 in the second stage (confusion or focal neurological deficit), and one in the third stage (stuporous or dense paraplegia or hemiplegia). Abnormal chest X-ray were found in three (11.1%) patients (miliary pattern in two patients, pleural effusion in one). Cranial CT showed hydrocephalus in eight (29.6%) patients, tuberculomas in four (14.8). All the patients were treated with antituberculous drugs and steroids. The overall mortality for all of the patients was 14.8% (four cases). Permanent neurological sequelae were shown in nine patients (33.3%). Fourteen patients (51.8%) recovered completely.
Conclusion: Tuberculous meningitis continues to be an important problem in developing countries, including Turkey. The disease has significant mortality and morbidity rate. Therefore, it should be considered in the differential diagnosis in any patient presenting with fever, headache or neck stiffness; early diagnosis must be performed and specific therapy must be started without any delay.

P1184 *Mycobacterium kansasii* infections in patients with cancer

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Objective: The aim of this study is to know epidemiological features of *M. kansasii* infection in patients with cancer.
Patients and methods: We reviewed the medical records of all patients with cancer who were cared at our hospitals from which *M. kansasii* was isolated. First, we applied ATS diagnostic criteria for nontuberculous mycobacterial disease to the cases and then, patients were further classified according to modified criteria into: definitive disease (all ATS criteria), possible disease, probable-definitive disease, pulmonary/gastrointestinal colonization, indefinite and disseminated disease.
Results: *M. kansasii* was isolated from 16 patients with cancer which were cared for at Basurto and Santa Marina hospitals from 1998 to 2000. Nine patients had lung neoplasm, two of them with metastasis and another one with laryngeal neoplasm. Seven patients had the following localization of neoplasm: two laryngeal, one Kaposi's sarcoma, one testicle, one stomach, one prostate, and one non-Hodking lymphoma. Fifteen patients (93.8%) were male. Mean age was 65.93 years (range, 25–81 years). Four were HIV-positive and 12 HIV negative. The most common symptoms were weight loss 68.8%, cough 56.3%, sputum production 37.5%, fever 31.3%, dyspnea 31.3%, and chest pain 12.5%. The predisposing factors were COPD (62.5%), hepatopathy (25%), and gastrectomy (25%). Acid-fast bacillus smears were positive in 25% (4/16). No patient had disseminated disease. A total of 16 patients yielded pulmonary isolates, eight (50%) of them were classified as having definitive disease as met all ATS criteria, one (6.3%) as possible disease, five (31.2%) as colonization and two as indefinite. Four of the nine patients with pulmonary disease (definitive and possible) according to our criteria, did not receive antimycobacterial agents (two died before culture results were available). In the group classified as pulmonary colonization, two HIV-positive patients started treatment and none of the two HIV negative. The only patient with gastrointestinal colonization was HIV positive and was treated too. The antimycobacterial regimen included isoniazid, ethambutol, and rifampicin after *M. kansasii* was confirmed in culture.
Conclusions: *M. kansasii* causes disease and is a colonizing agent in cancer patients. In our area of Bilbao, *M. kansasii* disease has recently shown to be frequent in patients with cancer and should be suspected.

P1185 *Mycobacterium kansasii* disease in Bilbao, Spain

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Objective: The aim of this study is to know the clinical significance of the isolates and the epidemiology of the infection.
Patients and methods: We reviewed the clinical records of all patients with *M. kansasii* isolated from culture specimens at the Basurto Hospital from 1998 to 2000 and at the Santa Marina Hospital from 1998 to June of 1999. First, we applied ATS diagnostic criteria for nontuberculous mycobacterial disease to the cases and then, were further classified according to modified criteria into: definitive disease (all ATS criteria), possible disease, probable-definitive disease, pulmonary/gastrointestinal colonization, indefinite and disseminated disease.

Results: During the 3 years study period, *M. kansasii* was isolated from 96 patients: 30 HIV-positive and 66 HIV-negative patients, with male predominance (5/1) and a mean age 53.51 (range, 25–90 years). The mean age of HIV-negative patients was 20 years older than the HIV positive. There were two HIV-positive patients with disseminated disease. The geographic analysis was restricted to the area of Bilbao that contributed to 83% of patients and one was excluded because of homelessness. The peripheral districts of Bilbao showed higher incidence. The predisposing factors were COPD (33.3%), hepatopathy (22.9%), gastrectomy (13.5%) and neoplasia (19.4%). There was higher frequency of COPD in HIV negative ($P < 0.05$) and greater proportion of hepatopathy between HIV positive ($P < 0.05$) The respiratory symptoms were cough (61.6%), dyspnea (28.1%), hemoptysis (18.8%), and chest pain (17.7%). Fever was more frequent in HIV negative ($P < 0.05$). Chest X-rays showed infiltrates (38) or cavity lesions (35). HIV-positive and -negative patients did not differ significantly in chest X-rays. The predominant pulmonary presentation was unilateral and in the right upper lobe. Chest X-rays didn't show abnormalities in seven patients, four of them were HIV positive. Multidrug regimen containing isoniazid, rifampicin and ethambutol was initiated or previous therapy was changed when *M. kansasii* was recovered in 67.7% of patients, 24 HIV positive and 41 HIV negative. Only 57.28% patients met strict ATS criteria but according to our criteria 70% had *M. kansasii* related disease. Positive isolates also appeared in pulmonary colonization in (12.5%) and gastrointestinal colonization in (2.08%).
Conclusions: ATS criteria, with their requirement for multiple positive specimens may be excessively strict for clinical purposes.

P1186 Clinical and epidemiological characteristics of tuberculosis among Italian and foreign-born patients in Brescia, Italy

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Background: Tuberculosis (TB) is an emerging problem among foreign born persons who recently immigrated into Italy. The definition of clinical and epidemiological characteristics of TB in this population is important in order to define specific interventions for prevention and care.
Objectives: To describe and compare clinical and epidemiological characteristics of TB among Italian and foreign patients in a hospital setting.
Methods: We have systematically included in this analysis all TB cases observed at the Institute of Infectious and Tropical Diseases of the University of Brescia during the period 1990–1999. TB diagnosis has been based on TB culture isolation in all cases. We have retrospectively reviewed the clinical charts to collect information on age, gender, origin, skin reactivity to tuberculin, HIV serology, site of the disease, sputum results, drug-susceptibility pattern.
Results: This analysis include 187 cases. The annual incidence has shown an increasing trend during the study period: from 15 cases in 1990 to 39 cases in 1999, representing 0.94 and 3.54% of all hospital admission in the infectious disease unit in 1990 and 1999, respectively. The increasing trend was highest for foreigners and for HIV-negative patients. Male represented 77.5% of the cases, the mean age was 36.4 years. The site of the disease was pulmonary in 35.3%, extrapulmonary in 37.4%, and pulmonary and extrapulmonary in 27.3%. HIV co-infection was diagnosed in 43.9% of the cases, more commonly among Italian patients (70.5% vs. 20.5%, $P = 0.001$). Extrapulmonary disease was more frequent among foreigners (46.7% vs. 28.4%, $P = 0.035$); disseminated disease was more frequent among HIV co-infected persons (39% vs. 18.1%, $P = 0.001$). Sputum smear positivity was similar in Italian and foreign patients (60.3% vs. 63.4%) and among HIV-positive and HIV-negative cases (56.2% vs. 66.5%). More foreigners had a positive tuberculin skin test ((76.7% vs. 54.5%, $P = 0.028$) and HIV-negative persons (78.8% vs. 44.4%, $P = 0.001$). 21.7% of TB isolates were resistant to at least one drug, with similar rates among Italians and immigrants (23.7% vs. 20.3%) and HIV infected and non infected (20.4% vs. 22.7%). Resistance rates to rifampin and isoniazid were 3.1 and 12.4%, respectively.
Conclusions: We observe an increase in TB rates, in particular among foreign HIV-uninfected persons.

P1187 Risk factors for a bad outcome of patients with tuberculosis in a pneumological intensive care unit

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Objectives and methods: Until recently, just a few studies focussed on risk factors contributing to a bad outcome among patients with pulmonary tuberculosis in ICU-treatment. We reviewed the clinical records of 63 patients being admitted for pulmonary tuberculosis in our pneumological intensive care unit within the last 11 years.
Results: Among the patients were 58 with tuberculosis and 5 with atypical mycobacteriosis. Fifty patients were males and 13 females. Mean age was 48 years (15–84 years). The overall in-hospital mortality rate was 23.8%, none of the patients with atypical mycobacteriosis died. Six patients had a positive HIV-testing, but AIDS did not correlate with a bad outcome. Factors associated with a bad outcome ($P < 0.01$) were proven chronic pancreatitis in the patients history, as well as development of nosocomial pneumonia, acute renal failure, ARDS, bacterial septicemia and ventilator-associated pneumonia under therapy. The need for mechanical ventilation was also associated with a bad outcome.
Conclusions: We conclude, that preexisting chronic pancreatitis, the need for mechanical ventilation as well as complications under therapy like development of ARDS, acute renal failure, nosocomial pneumonia, ventilator-associated pneumonia and bacterial septicemia are strongly associated with a bad outcome among patients being treated for tuberculosis or atypical mycobacteriosis in an ICU.

P1188 Molecular epidemiology of tuberculosis in Equatorial Guinea

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Objective: To study the molecular epidemiology of tuberculosis (TB) in Equatorial Guinea.
Methods: Restriction fragment length polymorphism (RFLP) analysis associated with IS6110 was used to study *M. tuberculosis* isolates obtained from 1999 to 2000 from five districts of Equatorial Guinea. Patients whose isolates had identical patterns were grouped into clusters. Clinical, epidemiological and demographic data were reviewed to identify risk factors for TB transmission.
Results: Five hundred and six patients were diagnosed as having TB (47.8% with positive culture). A total of 185 (77.5%) of the 239 culture-positive pulmonary TB patients were available for RFLP. DNA fingerprinting identified 21 clusters: one cluster with 25 isolates, two with 15, one with 8, two with 6, three with 4, three with three, and finally, 9 with two isolates. Of all of the isolates of the positive cultures, 18.8% were resistant to one or more antituberculous drugs. Most of these resistant isolates were from among the largest cluster and were isoniazid resistant. Univariate analysis showed that resistance to antituberculous drugs was a factor observed significantly more frequently among patients infected with a clustered isolate.
Conclusions: The level of clustering (61.6%) observed in this study suggests a high degree of recent transmission or the scarce polymorphism of the isolates because of the high prevalence of TB in a country with 47.7% of the study population living on an island.

P1189 Clinico-microbiologic particularities of extrapulmonary tuberculosis (1997–2000)

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Objectives: The aim of this study is analysis of the cases of extrapulmonary tuberculosis and the particularities of *M. tuberculosis* resistance to antituberculous drugs between 1997 and 2000.

Methods: Retrospective study on 132 patients with extrapulmonary tuberculosis (meningitis, renal, bone, ganglion tuberculosis, tuberculosis pericarditis and generalized tuberculosis) at the hospitalized patients in the Clinic of Infectious Diseases of Iasi, Romania, between 1997 and 2000. They were analyzed from the clinic, microbiologic and therapeutic point of view. Isolation and identification of *M. tuberculosis* were realized by conventional method.
Results: The 132 patients with extrapulmonary tuberculosis presented 116 meningitis (11 with VIH infection); eight cases – generalized tuberculosis (three cases – polyserositis, two cases – granulia, two cases – meningitis with granulia), three cases presented ganglion tuberculosis, three cases – bone tuberculosis, one case – ovarian tuberculosis pericarditis. We observed the prevalence of the male sex with 62.12% cases, the age of patients varied between 5 months and 71 years old, with the prevalence of the young adult. The testing of antibiotic sensitivity was effectuated on 68 strains of *M. tuberculosis*, to isoniazid, streptomycin and rifampin; 60.20% strains being sensible to isoniazid, 20.8% to streptomycin and 14.7% to rifampin; the multiresistance being to 29.4% *M. tuberculosis* strains. The evolution under therapy with quadruple association of antituberculous drugs was favorable in 91.6%; the lethality being of 8.3%.
Conclusion: Multiresistance of *M. tuberculosis* represents a problem of tuberculosis therapy, in some cases of being necessary the association of fluoroquinolons, as the fifth anti-tuberculous drug.

P1190 Gammadelta T lymphocytes in the peripheral blood of tuberculosis (TB) patients with and without HIV co-infection: a prospective study

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Objective: To compare the profile of gammadelta T cell population in the peripheral blood of TB patients with and without HIV infection at the time TB diagnosis is established (time 0) and at the end of a 6-month anti-TB treatment (time 6).
Methods: From December 97 to October 01 consecutive TB patients from the Department of Infectious Diseases, Spedali Civili, Brescia were enrolled in the study. CD4+, CD8+ and Vdelta1 and Vdelta2 T cell counts were analyzed at time 0 and time 6. Lymphocyte surface membrane expression was evaluated with the FITC–TCRgammadelta, –Vdelta1, –Vdelta2 and PE–Vdelta1 monoclonal antibodies (mAbs).
Results: A total of 107 patients (73% male, 78% foreign born, 33% HIV +) were evaluated at time 0 and 41 reviewed at time 6. Median percentage (range) of total lymphocytes, CD4+ , CD8+ , total gammadelta T cells and delta1 and delta2 subsets among TB patients according to HIV serostatus at time 0 and time 6 are presented in Table 1.

Table 1 Media percentage (range) of total lymphocytes, CD4+, CD8+, gammadelta-T cells and delta1 and delta2 subsets (time 0 and time 6) among TB patients according to HIV serostatus

Cell pollution	TB HIV+ time 0 $n=33$	TB HIV+ time 6 $n=33$	*P*-value*	TB HIV+ time 0 $n=72$	TB HIV+ time 6 $n=38$	*P*-value*
Total lymphocytes	22.0 (4.6–77.0)	32.4 (8.3–52.6)	0.07	24.1 (5.4–65.0)	32.9 (20.0–59.5)	0.01
TCD4+	21.7 (1.2–52.1)	28.3 (1.7–53.5)	0.77	44.1 (3.4–74.0)	38.7 (11.1–56.6)	0.01
TCD8+	49.6 (8.1–72.9)	46.4 (17.9–71.7)	0.12	25.1 (6.1–82.2)	24.3 (14.0–69.0)	0.57
Total gammadelta T cells	3.9 (1.1–2.3)	3.7 (1.3–4.9)	0.17	3.1 (0.7–16.5)	3.6 (0.5–16.0)	0.46
Vdelta1	1.4 (0.1–31.3)	1.3 (0.5–6.3)	0.54	1.0 (0.1–7.7)	0.6 (0.1–4.6)	0.08
Vdelta2	1.2 (0.1–3.9)	1.2 (0.2–13.0)	0.48	1.3 (0.2–14.6)	1.8 (0.1–1.6)	0.12

*Wilcoxon's rank test.

Conclusions: Our results suggest a redistribution of lymphocyte population among HIV-seronegative TB patients after anti-TB therapy, with an increment of total lymphocytes percentage and Vdelta2 cells but with a reduction of TCD4 lymphocytes and Vdelta1 subset. Among HIV-seropositve TB patients we observed a tendency to total lymphocytes increasing without significant changes among the other subsets.

P1191 Altered expression of phagocyte Fcg receptors in active tuberculosis: effects of therapy

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Background: Macrophage and granulocyte receptors for IgG (FcgRs) are important in host defense against infection.
Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), monocytes cultured for 72 h (M/Mø), and granulocytes (G) in patients with active Tuberculosis (TB), during antituberculous therapy (anti-TB-Rx) and, after competition of anti-TB-Rx.
Methods: The surface expression of the three type of FcgRs, FcgRI, FcgRII and FcgRIII, on M, M/Mø and G were analyzed by flow cytometry in 37 HIV-negative patients with TB (33 men and 4 women), at diagnosis of TB, and monthly thereafter until competition of anti-TB-Rx. FcgRs expression was assessed on resting M, M/Mø and G, and on these cells after stimulation by culture in the presence of IFNγ.
Results: The expression of FcgRI and FcgRIII by M, M/Mø and G was significantly enhanced in patients with active TB by: $43 \pm 4\%$ and $22 \pm 2\%$ for M, respectively ($P < 0.001$), $58 \pm 6\%$ and $42 \pm 4\%$ for M/Mø, respectively ($P < 0.001$) and, $139 \pm 9\%$ and $37 \pm 3\%$ for G, respectively ($P < 0.001$). The expression of FcgRII by M, M/Mø and G was significantly decreased by $-36 \pm 1\%$ ($P = 0.02$), $-47 \pm 3\%$ ($P < 0.001$), and $-29 \pm 1\%$ ($P = 0.002$), respectively. These alterations of phagocyte FcgRs expression returned to normality after 8 weeks of effective anti-TB-Rx and, remained normal until the end of TB treatment. The expression of the three types of FcgRs, FcgRI, FcgRII and FcgRIII by M, M/Mø and G from patients with TB was significantly increased by culture in the presence of IFNγ ($P < 0.001$). Nevertheless, the expression of FcgRII by M, M/Mø and G from patients after effective anti-TB-Rx was not significantly increased by culture in the presence of IFNγ.
Conclusions: Macrophages and granulocytes from HIV-negative patients with active tuberculosis exhibit an increased expression of FcgRI and FcgRIII and, an impaired expression of FcgRII. These alterations of phagocyte FcgR expression during active tuberculosis disappear after effective antituberculous therapy.

P1192 Childhood tuberculosis in the indigenous Warao population in Venezuela

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Objectives: A definite diagnosis of childhood Tuberculosis is difficult in rural areas that do not have access to a large hospital. The Warao people of the Delta Amacuro State, Venezuela, have a very high prevalence of adult TB, and we suspected that the Warao children would also have a high prevalence, almost entirely undiagnosed.
Methods: We applied a simple methodology to select children suspicious for tuberculosis that is based on a rating system using clinical criteria, reactivity to tuberculin and intradomicilliary contact.
Results: Of the 502 children less than 15-year-old that were evaluated with this rating system, 27 were determined to be suspicious and further evaluated with a chest X-ray. Radiological confirmation of TB was found in 16 of the 27 suspicious children (60%). Of these 16, 13 were PPD positive and 7 patients had additional confirmation; 3 were positive by culture or smear examination and 5 were positive using a serologic TB diagnostic test. The prevalence of childhood tuberculosis in this population studied was 3.3%.
Conclusions: This methodology proved to be highly efficient in diagnosing childhood tuberculosis in this population, and should also be useful in other rural populations with a high prevalence of adult TB.

P1193 Immigrants are becoming the majority of TB cases in Milan metropolitan area

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Milan, I

Objective: To evaluate the incidence of immigrants among TB cases in Milan.
Methods: We retrospectively analyzed our TB cases survey from January 1990 to November 2001 (4611 pts). Patients ranged yearly between 346 and 420; in 2001 (January to 15 November) were 356.
Results: A total of 2877 subjects (62.4%) were Italians or from E.U. countries, while 1734 (37.6%) were immigrants from developing countries (IDC). In 1990, IDC represented 21% of the survey while in 1995 and 2000 were, respectively, 35 and 55%. In 2001, 215 IDC (60.4%) were diagnosed. Among the different ethnic groups, Philipinos and North African Arabs were, respectively, 45 and 30% in 1990, and decreased to 14 and 12% in 2001, while East Europeans and Peruvian/Ecuadorians increased in the same years from, respectively, 4 and 3% to 9 and 34%. Likewise, West Africans and immigrants from Indian Subcontinent increased from 5 to 13% and from 0 to 8%.
Conclusions: In the considered period, the number of TB cases seen per year at our Center remained substantially stable. However, Italians continued to decrease while IDC constantly increased, becoming the majority of cases since year 2000. This phenomenon has not yet been observed at national level, but is similar to what has already happened in other European Countries. Among IDC, while some groups remained stable in absolute numbers, others continued to increase in absolute and relative numbers, mostly because of a more recent immigration in our area.

P1194 Isoniazid preventive treatment (IPT) in children: a 443 case Italian survey

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Objective: To evaluate adherence, tolerability and effectiveness of IPT in children <14 years old.
Methods: A total of 443 children, 225 Italians (It) and 218 immigrants from developing countries (IDC) received the recommendation of IPT (November 1993–December 2000), as recommended by WHO/IUATLD. IPT consisted of isoniazid 10 mg/kg (max 300 mg daily), usually for 6 months; 227 children were close contacts of contagious TB cases (124 It, 103 IDC), of these 100 had an initial negative Mantoux test and IPT lasted 2 months until a second Mantoux was performed.
Results: A total of 367 subjects (190 It, 177 IDC) completed the treatment; 15 of them reported adverse reactions (AR) that did not cause IPT discontinuation (three increased AST/ALT, one vomiting, three gastritis, two headache, two unrest, one cramps, two itching). Fifty-five children did not complete IPT: five because of AR (two sight disturbances, two headache, one liver intolerance); 12 interrupted IPT spontaneously, 15 refused IPT before starting, 22 were lost to follow up. Twenty-one patients are still under treatment. So far, only one case of active TB occurred in a 'close contact' child.
Conclusions: IPT confirmed to be a well-tolerated treatment in children (4.5% overall side-effects incidence). IDC children completed IPT as often as the Italians, while in the adults it has been observed a lower adherence. In our experience, IPT proved so far to be effective in preventing the development of active TB disease from recent infection.

P1195 A systematic review of the adjunctive use of systemic corticosteroids for pulmonary tuberculosis

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Objective: To determine the safety and benefit of adjunctive systemic corticosteroids in the management of pulmonary tuberculosis.
Methods: A systematic review of 10 placebo-controlled clinical trials involving the use of prednisone, prednisolone, and/or adrenocorticotropin (ACTH) in conjunction with standard antituberculous chemotherapy. A total of 1769 steroid-treated patients were analyzed, most of whom had moderate-to-severe disease and cavitation. The mean daily prednisone or prednisolone dose was 32 mg (range 16–60 mg), and the mean daily ACTH dose was 40 units. The mean duration of steroid therapy was 87 days (range 30–180 days). Clinical, microbiologic, and radiographic outcome measures included time to defervescence, weight gain, normalization of serum albumin level and erythrocyte sedimentation rate, length of hospitalization, rate and rapidity of sputum conversion, and radiographic regression of pulmonary infiltrates and cavities.
Results: Corticosteroid therapy resulted in broad and significant clinical benefits in all studies reviewed, and more rapid radiographic resolution of pulmonary infiltrates and, to a lesser extent, closure of cavities (for the latter, especially in the first 4 months but extending up to 1 year after initiation of treatment). Steroids did not have any appreciable effect on the speed or rate of sputum conversion. Detrimental side-effects attributed to steroid therapy or a higher rate of bacteriologic relapse were not observed.
Discussion: The adjunctive use of systemic corticosteroid therapy can safely provide significant early and prolonged clinical and radiographic benefits to patients with advanced pulmonary tuberculosis.

P1196 Sporotrichoid *Mycobacterium fortuitum* infection in a healthy woman during pregnancy

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Introduction: Infections owing to the rapidly growing mycobacteria (RGM) have emerged as an important though infrequent cause of morbidity in patients with defects in cellular immune system. In noncompromised individuals, these infections may occasionally present as refractory prosthetic surgical device infections. Suppression of cellular immune responses during and immediately after pregnancy are understood, albeit, opportunistic infections owing to RGM are seldom seen in this setting.
Results: A painless red nodule developed on the right lower extremity in a 34-year-old HIV-seronegative primigravida during early third trimester of normal intrauterine pregnancy. After giving birth to a full-term healthy child, crops of lesions on the proximal lower leg spread caudally. Three distinct clusters of excoriated, nodular lesions with an erythematous, indurate base were present along the anterio-medial aspect of tibia. Otherwise, her physical examination was normal. Laboratory studies showed: WBC 6.6 k/μL, platelets 277 k/μL, AST 19 U/L, ALT 16 U/L, antinuclear antibodies <1 : 40, RF < 25 IU/mL, Angiotensin-1-Converting Enzyme 23 U/L, TSH 2.62 μIU/mL, and ESR 20 mm/h. Underlying facial compartments and bone were normal on three-phase 25.0 mCi technetium radionuclide bone and MRI scans. Biopsy of the right-leg lesion showed extensive necrotizing granulomas in the deep reticular dermis, and abundant superficial and deep dermal infiltration with neutrophils and lymphocytes. No microorganism was identified. In 2 weeks, RGM was isolated from skin biopsy specimens. Treatment with oral clarithromycin and ciprofloxacin (500 mg twice daily) was initiated. The DNA sequencing of RGM confirmed genetic relatedness to *Mycobacterium fortuitum* and results of antimicrobial susceptibility (MIC μg/mL) are as follows: amikacin 0.25, cefoxitin 8.0, ciprofloxacin 0.06, clarithromycin 2.0, doxycycline 0.12, erythromycin 0.25, and imipenem 0.25. Nine months following antibiotic therapy her lesions resolved completely.
Conclusions: Pregnancy is associated with suppression of T cell-mediated immunological responses and may rarely lead to opportunistic infections. De novo cutaneous and subcutaneous infections owing to RGM are extremely rare, and sporotrichoid lymphatic spread has been observed. In my patient, response to oral combination antibiotic therapy lead to an excellent clinical response.

P1197 Tuberculosis in dialysis patients

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Background: Immunocompromised patients with chronic renal failure have an increased risk of tuberculosis and extrapulmonary involvement is reported

to be predominant in this group of patients. The aim of this study was to determine the incidence and clinical presentation of TB in hemodialysis patients (HP).
Methods: We evaluated retrospectively the incidence, clinical characteristics, thoracic and extrathoracic involvement of TB in 510 HP during a 4-year period. (between October 1998 and October 2001)
Results: The overall incidence was 3.6%. The infection was limited to intrathoracic cavity in 38.4% of the patients, a single extrapulmonary side in 54.8%. It was disseminated in 6.8% of the cases. In patients with extrapulmonary TB, lymph node involvement was predominant with a ratio of 35.6% in all TB patients followed by peritoneum (6.8%), bone (5.5%), kidney (4.2%), muscles (2.7%). The most common symptom was fever, which was observed in 76.7% of the patients. 1% of the patient gave a history of previous exposure. PPD skin test was anergic in 63%, >10 mm in 26%, <10 mm in 11% of the cases. TB diagnosed by sputum smear (7.5%), and or culture or tissue biopsies which presented caseating granulomas and acid-fast staining bacilli. All of the patients were treated with four-drug therapy regiment. The success rate was 87.8%, 6.8% of the patients did not complete the therapy and 5.4% of the patients died during therapy because of disseminated TB.
Conclusions:
- The incidence of TB is higher in HP than general population;
- Extrapulmonary involvement was higher in these groups of patients;
- Because there was a high rate of energy, these patients require further clinical evaluation for TB.

P1198 Vitamin D deficiency is highly prevalent among immigrants with tuberculosis in London

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Objectives: Vitamin D is known to be important in the host defense against tuberculosis, both in vitro and in vivo. We previously showed, in a case–control study, there was significant Vitamin D deficiency in our Gujrati Indian patients, who are vegetarians. We, therefore, extended our observations to assess the overall prevalence of Vitamin D deficiency amongst all patients diagnosed with tuberculosis in our infectious diseases unit. We could then relate the Vitamin D levels to ethnic origin, religion, type of tuberculosis, sex, age, time in UK, and month of Vitamin D estimation. We analyzed all cases of tuberculosis, diagnosed between June 1998 and June 2001, who had routinely measured plasma 25-OH-vitamin D levels.
Results: Of the 210 patients, 76% of patients were Vitamin D deficient (25-OH Vitamin D less than 22 nM/L), with 56% having undetectable levels. About 68/79 Indian, 23/27 East African Indian, 29/34 Somalian, 7/12 Pakistani, 7/7 Afghani, 2/6 African and 15/21 Sri Lankan patients were deficient (with 56, 16, 23, 5, 4, 1 and 6 having undetectable levels, respectively). In contrast only 0/6 white Europeans and 1/8 South-east Asians had decreased 25-OH Vitamin D levels. Muslims, Hindus and Sikhs all had equivalent rates of Vitamin D deficiency. The greatest proportions of deficiency were found in patients with miliary, CNS, bone and pleural tuberculosis, and the lowest proportions among those with pulmonary TB. Among immigrants, Vitamin D deficiency was significantly correlated with the duration of residence in the UK.
Conclusions: Vitamin D deficiency is a major feature of patients with TB among all ethnic groups apart from white Europeans and South-east Asians. Religion (and therefore a vegetarian diet) appears to play only a minor role; possibly lack of exposure to sunlight is a more important determinant of Vitamin D deficiency. We consider it probable that Vitamin D deficiency contributes to the very high rate of reactivation of latent TB infection among immigrants to the UK.

P1199 A comparison between disk diffusion and microdilution for susceptibility testing of *Mycobacterium fortuitum* complex

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Objectives: We compared a disk diffusion method with the broth microdilution technique for susceptibility testing of *Mycobacterium fortuitum* complex organisms.
Methods: Ninety-four clinical isolates (*M. fortuitum* (48), *M. chelonae* (24), *M. mucogenicum* (4), *M. peregrinum* (12) and *M. abscessus* (6)) were tested together with the type strains of the five species. Broth microdilution was performed according to standard techniques. Disk diffusion technique was performed onto Muller–Hinton agar plates incubated at 30 °C during 3–4 days. Antibiotics tested were tetracycline, clarithromycin, azithromycin, erythromycin A, amikacin, levofloxacin and ciprofloxacin.
Results: All the strains grew on the Müller–Hinton agar plates. Sixty strains grew at 72 h and the other 34 strains were read at 96 h. Global results showed good correlation for all antimicrobials except for clarithromycin and azithromycin ($R > 0.7$, $P < 0.05$). However, when the results were analyzed by species, correlation was poor except for a few antimicrobials. These data can be explained by the polarization of MIC results for *M. chelonae* (quinolone-resistant and macrolide-susceptible) and *M. fortuitum* (quinolone-susceptible and macrolide-resistant) and the fact that 74.7% of the strains tested belonged to these species. The analysis of the results for resistant/susceptible results was good for all the antimicrobials tested except azithromycin and erythromycin.
Conclusions: The disk-diffusion technique could be useful as a screening technique for some antimicrobials, except for azithromycin and erythromycin, and perhaps all the bacteriostatic antibiotics, but the results must be confirmed by using an accepted reference technique.

Helicobacter pylori I

P1200 Evaluation of the Oxoid creaFAST(R) H-Pylori Combi-Kit for the identification of clarithromycin-resistant *Helicobacter pylori* in gastric biopsies

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Objectives: A recent application of in situ hybridization is a rapid screening method to identify bacteria in formalin-fixed paraffin embedded specimens. The method exploits the fact that there are multiple copies of rRNA within a bacterial cell, the sequences of which can confirm species identity, allowing development of simple and rapid in situ hybridization methods. One application of this method is the detection of antibiotic-resistant strains of *Helicobacter pylori* in gastric biopsies. Resistance to the macrolides and in particular, clarithromycin, an antibiotic used extensively to treat *H. pylori* infection, is conferred on bacteria by the presence of three defined mutations within 23S rRNA. An in situ hybridization method, the Oxoid creaFAST(R) H-Pylori Combi-Kit, is capable of detecting the macrolide-resistant strains of *H. pylori* in formalin-fixed paraffin embedded gastric biopsies. The method utilizes a mixture of fluorescent labeled oligonucleotide probes – fluorescein labeled probes specific for a 16S rRNA *H. pylori* specific sequence and Cy3-labeled probes specific for the 23S rRNA sequences that confer macrolide resistance. A large sample of archival biopsy specimens, from UK patients with known outcome for clarithromycin based proton pump inhibitor triple therapy, was investigated to evaluate the specificity and reliability of the method.
Methods: Eighty-five biopsies were selected from the archive from patients with clarithromycin-sensitive strains of *H. pylori* that had successful or unsuccessful eradication therapy and from patients with clarithromycin-resistant strains of *H. pylori*. The 4-μm sections were subjected to in situ hybridization as detailed in the kit with minor modification. Visualization of the hybrid products by fluorescence microscopy using a combined green/red

filter disclosed both the clarithromycin-sensitive and -resistant strains present in each biopsy.
Results: Identification of the clarithromycin-resistant strain of *H. pylori* by in situ hybridization showed close correlation with antimicrobial sensitivity testing by disc diffusion, defined as no inhibition around a 2-μg clarithromycin disc. The method was also capable of identifying the presence of coccoid forms of *H. pylori* in the biopsies.
Conclusions: The method illustrates the exceptional specificity of non-isotopic in situ hybridization for the identification of macrolide-resistant and -sensitive strains of *H. pylori*.

P1201 Identification of *H. pylori* directly from biopsy specimens by UreC PCR without culture and comparison with bacteriological methods

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Introduction: *Helicobacter pylori* is a microaerophilic Gram-negative fastidious bacterium. It has been associated with chronic gastritis, peptic ulcer and gastric carcinoma.
Methods and materials: In the present study by UreC PCR directly without culture 79 biopsies were studied and compared with bacteriological methods including Gram stain, rapid urease test and culture.
Results: Sixty-four cases were positive on PCR at sensitivity 91.4% and specifity 100%. Seventy-three cases were positive on rapid urease test at sensitivity 100% and specifity 66.7%. A total of 71 cases were positive on Gram stain at sensitivity 88.9% and specifity 100%. Forty cases were positive on culture at sensitivity 57.1% and specifity 100%.
Discussion: Based on the results obtained by UreC PCR we can identify *H. pylori* directly without culture on biopsy specimens. In comparison with bacteriological tests, UreC PCR enjoys a higher degree of sensitivity and specifity. In addition to identifying the bacterium, we can use methods such as RFLP with restriction endonuclease on products of PCR to determine genotyping, re-infection, infection with new strain, re-crudesence or drug resistance. Also, by using this method we can study in malignant cases embed tissues including MALT and the role of *H. pylori* in the diseases as retrospective study.

P1202 Lack of detection of *Helicobacter* spp. in the liver of patients with viral chronic hepatitis and hepatocellular carcinoma or metastatic liver carcinoma

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Objective: To test the hypothesis that *Helicobacter* species play a role in the enhancement of liver necro-inflammation and fibrosis and in the development of hepatocellular carcinoma (HCC), we searched DNA sequences of *Helicobacter* species in liver specimens from patients with viral-related chronic hepatitis and HCC or liver metastatic carcinoma.
Methods: We enrolled 28 consecutive patients with ultrasound evidence of hepatic nodule(s) on their first liver biopsy: 21 had a histological evidence of HCC (19 males and 2 females; median age 68 years, range 46–78; Group I) and 7 of metastatic liver carcinoma (4 males and 3 females; median age 69 years, range 48–81; Group II). In the same period we observed 27 consecutive patients with chronic hepatitis on their first liver biopsy (18 males and 7 females; median age 52 years, range 31–66) (Group III). *Helicobacter* sequences were searched by PCR using primers for the 16S rDNA of *Helicobacter* spp., designed to amplify a 400-bp fragment and detected by 2% agarose gel hybridization with a specific biotynilated probe. We used, as positive controls for the DNA extraction from liver tissue, hepatic-biopsy sections in which HBV infection was confirmed by the positivity for HBcAg and in which we amplified HBV-DNA by specific primers; positive controls for the amplification of *Helicobacter* spp. were obtained from gastric-biopsy sections in which *Helicobacter pylori* infection was confirmed by biochemical tests and by histological and histochemical examination.
Results: HBV-DNA was found in all five HBcAg positive liver biopsies of *Helicobacter* spp. The 16S rDNA was detected in all five biopsy specimens of gastric mucosal and in none of liver specimens from patients in all groups.
Conclusions: Our data suggest that *Helicobacter* species were not involved in the pathogenesis of virus-related HCC and chronic hepatitis or of liver metastatic carcinoma.

P1203 Detection of *Helicobacter pylori cagA* gene by polymerase chain reaction in gastric biopsies and fecal samples

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Objective: The polymerase chain reaction (PCR) has been used successfully to detect *Helicobacter pylori* from gastric biopsies and gastric juice. However, results using feces as biological samples for PCR are conflicting, largely owing to PCR inhibitor(s) usually found in stool. Aim of the study was to characterize *H. pylori* genotype in faecal sample using specific primers for the *cagA* gene. To overcome the problem of PCR inhibitors new DNA extraction kit was used.
Methods: One hundred antral biopsies and 40 fecal samples were obtained from symptomatic patient undergoing upper endoscopy. Biopsies were cultured using standard *H. pylori* method. PCR was used to detect *cagA*-positive *H. pylori* from both biopsies and stool.
Results: The *cagA* gene was detected in fecal and biopsy sample of 10/40 (25%) and 61/100 (61%), respectively. Duodenal ulcer and/or antral erosions were observed in 9 of 12 *cagA*-positive patients (75%) and in 3 of 12 (25%) *cagA*-negative patients.
Conclusions: Our results indicate that using DNA extraction techniques to remove PCR inhibitor will make faecal sample a more suitable for the detection of *cagA* status in *H. pylori*. Moreover, they confirm the existence of a significant relationship between *cagA* status and duodenal ulcer.

P1204 The evaluation of nested PCR, PCR, culture, staining and histopathological results for the detection of *H. pylori* in gastric biopsy specimens of symptomatic adults

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Objectives: Infection from *Helicobacter pylori* plays a role in several gastroduodenal diseases. Different tests are available for diagnosing *H. pylori* infection. Polymerase chain reaction (PCR) is a rapid, sensitive and accurate method for the specific detection of *H. pylori* from gastric biopsy specimens. The aims of this study were to compare nested PCR, PCR, culture, Giemsa staining, and histological findings and to assess the diagnostic value of these tests.
Methods: In our study, 104 gastric tissue specimens from symptomatic adult patients were examined by nested PCR, PCR, culture, Giemsa staining, and histological methods for detection of *H. pylori*. The infection state was considered as positive or negative when the results of at least two of the five tests agreed. In this respect, sensitivity, specificity, positive and negative predictive values of all groups were determined, and statistical comparisons between the results were made with kappa test by using SPSS for Windows 10.0.
Results: According to our results, positivity was achieved in 24% (25/104) with Giemsa staining, 34% (36/104) with histopathology, 36% (38/104) with PCR and 41% (43/104) with nested PCR, respectively, whereas *H. pylori* was isolated in only 33% (35/104) of the cultures on the biopsy specimens. Both the sensitivity and the positive predictive value of the nested PCR method were 100%, and both the specificity and negative predictive value were 98%. Only the results of the nested PCR method were in agreement with our definition of *H. pylori* infection state ($n = 44$, $P > 0.05$).
Conclusions: As a conclusion, our results suggest the nested PCR as a highly valuable method in the detection of *H. pylori* with a reasonably high sensitivity and specificity.

P1205 Detection of anti-Caga antibodies in patients with *H. pylori* gastric infection

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Objective: To evaluate the role of detection of anti-Caga antibodies in patients with *H. pylori* (H.P.) gastric infection with diagnosed ulcer of the duodenum and chronic superficial gastritis.
Material and methods: Our study consisted of 80 adult patients with H.P. gastric infection. A total of 68 of the patients were diagnosed with duodenal ulcer and 12 with chronic superficial gastritis. The diagnosis of H.P. infection was based on gastroscopy (two samples of the gastric mucus). The presence of H.P. in biopsies was detected microbiologically (microaerophil conditions). For the detection and classification of IgA and IgG anti-Caga antibodies were used *Helicobacter* p120 (Caga) ELISA test (VIVA Diagnostica).
Results: In all the patients with duodenal ulcer IgG antibodies anti-Caga were found (average optical density 1.12 ± 0.62), when only in 36 patients were positive IgA antibodies anti-Caga were found (average optical density 0.90 ± 0.52). Six of the twelve patients with chronic superficial gastritis were positive in anti-Caga antibodies (IgG and IgA). Five patients had only IgA anti-Caga and the last patient was negative for anti-Caga antibodies.
Conclusion: The presence of IgG anti-Caga antibodies is strongly correlated with duodenal ulcer in the course of H.P. infection. This fact is a strong indication that Caga antigen plays an important role in the pathogenesis of duodenal ulcer.

P1206 Determination of the antigen in stool for diagnosis of *H. pylori* infection and value of this method in the post-treatment follow-up of *H. pylori* eradication

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Aim: To compare the determination of antigen in stool for diagnosis of *H. pylori* with other invasive methods in cases diagnosed as gastritis and duodenal ulcer in terms of etiopathology and to evaluate the effect of this method in *H. pylori* eradication in the post-treatment follow-up.
Methods and materials: Eighty patients who had been admitted to gastoenterohepatology policlinics of two different centers with dyspepsia were prospectively included in this study. Endoscopy was performed and they were diagnosed as chronic gastritis or gastritis and duodenal ulcer. Four tissue samples were obtained during endoscopy for culture, urea determination, pathological investigation and PCR studies. In addition to these, HPSA investigation of all patients were performed with HPSA E A (Meridian Diagnostic, USA) test kit. *H. pylori* was considered as positive when only culture was positive or two out of three invasive test methods were positive. HP(+) cases were treated with three antibiotics (omeprazol + amoxicilin + claritromicin) for 15 days. Forty-five patients which could be followed 1 month after treatment were evaluated again.
Results: Out of 50 HP(+) cases according to the criteria of this study, in 46 (92%) HPSA were detected positive (sensitivity 92%). Out of 30 HP(–) cases, in 27 HPSA were found negative (specificity 90%). Out of 45 HP(+) cases which had follow-up after treatment, in 37 (86.1%) infection was eradicated. Thirty-five (94.6%) of these 37 cases had HPSA results negative whereas in seven cases out of eight having infection HPSA was detected positive (HPSA sensitivity after therapy 82.5%, HPSA specificity after therapy 94%).
Conclusion: This study emphasizes that HPSA test in stool samples is an accurate and a simple method for identification of *H. pylori* infection. In addition to this it is also indicated that in the post-treatment follow-up of bacterial eradication, HPSA test has more advantages in comparison to invasive methods.

P1207 A semiquantitative measurement of *Helicobacter pylori* antigen in stools

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Objective: To correlate a semiquantitative measurement of *Helicobacter pylori* antigen amount in stools with delta value of 13C-urea breath test (UBT). This value has been used to predict the severity of gastric damage and outcome of *H. pylori* treatment.
Methods: Fifteen dyspeptic patients with *H. pylori* infection confirmed by positivity to histology, rapid urease test, UBT and stool antigen (Hpsa) detection were studied. In a first stage we tested Hpsa with known amounts of *H. pylori* (from 0.2 to 2 millions cfu/mL) both alone and mixed with stools. In this way we have obtained a curve of saturation in both cases, even if the presence of feces (owing to protein content) increased bacterial concentration required to obtain test positivity and saturation point. For the Hpsa measurement we applied the principle of 'standard points' to the numerical value expressed by the absorbency units.
Results: Our measurement of the Hpsa correlated with Delta UBT for each patient using Pearson *r*-test ($r = 0.77$; $P < 0.01$). In addition, by Student's *t*-test a significant correlation with the CagA states was observed for either Delta UBT and Hpsa higher values ($P < 0.01$).
Conclusions: Our data suggest that an estimation of *H. pylori* antigen concentration in stools is feasible and similarly to that reported for Delta UBT may represent a semiquantitative determination of *H. pylori* load.

P1208 Comparison among different laboratory methods for the determination of *Helicobacter pylori* infection

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Objective: The aim of this study was to assay two different commercial methods, for the direct search of H.p. in stools, in comparison with the culture, Hurease test, direct identification of the bacterium, in dyspeptic patients. Serological tests for identification H.p. antigens and CagA cytotoxin, were performed on all specimens.
Materials and methods: Each of 36 specimens was tested by the following methods:

- Culture, Hurease test, Bacterial direct identification, Search for the antigen in stools with (Meridian H.p. SA, Helory AST Eurospital).
- All samples with positive culture and/or bacterial direct identification were considered positive;
- All samples with negative culture and bacterial direct identification were considered negative.

Results: By using the above mentioned criteria we were able to identify 18 negative and 18 positive specimens.

	Meridian H.p. SA	Helory AST Eurospital
Negative 18		
Negative specimens	13	14
Positive specimens	5	4
Specificity (%)	72.2	77.8
Positive 18		
Positive specimens	18	18
Negative specimens	0	0
Sensibility (%)	100	100

Conclusions: The two commercial methods for the direct search of H.p. in stools showed 100% sensibility, the Eurospital test showed higher specificity, however, all false-positive samples obtained with both methods were low positive.

P1209 Immunodiagnosis of *Helicobacter pylori* infection by femtolab in dyspectic patients

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Introduction: *Helicobacter pylori* (Hp) is a common human pathogen implicated in chronic inflammatory process, which may ultimately lead to the development of peptic ulcer disease or gastric carcinoma. Hp antigens can be measured in human stools with an enzyme immunoassay (EIA) and this could turn into a valuable noninvasive diagnostic tool.
Objective: To evaluate the usefulness of a new monoclonal EIA for detecting Hp antigens in dyspeptic patients' feces (FemtoLab *H. pylori* Cnx, Martinsried, Germany).
Methods: Gastric biopsies were taken from 76 dyspeptic patients undergoing endoscopy (50 men, 26 women, age range 17–84, mean 52 ± 16.5) for histology (H) (haematoxylin and eosin, Giemsa staining) and rapid urease test (RUT). The presence of Hp in stool specimens was determined by HpSA (Platinum Premier HpSA™ Meridian Diagnostic Inc., Cincinnati, USA) and Stick H. pyl (OPERON S.A, Zaragoza, Spain). FemtoLab also investigated Hp antigens in feces at three different times, which uses a monoclonal anti-*H. pylori* capture antibody absorbed in microwells. Hp status was established by a minimum concordance of three reference tests (H, RUT, HpSA or Stick pyl). Sensitivity, specificity, positive- and negative-predictive values (PPV and PNV, respectively) of each determination were calculated. Concordance between determinations was estimated by Kappa statistics.
Results: According to the reference methods the sensitivity and specificity of FemtoLab immunoassay ranged from 98 to 100% and 84–90%, respectively (PPV: 84–90%, PNV: 94–100%). Correlation coefficients ranged from 0.85 to 0.92.
Conclusions: FemtoLab stool test is a highly sensitive and specific test. At the same time, the new stool assay was a reliable and easy-to-perform tool for diagnosis of *Helicobacter pylori* infection.

P1210 Non-invasive post-treatment assessment of *H. pylori*: a comparison between monoclonal and polyclonal stool tests

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Introduction: Stool antigen test and urea-broth test are the most accurate and widely used techniques for noninvasive assessment of *H. pylori* (HP) infection. A new second-generation stool antigen test based on monoclonal antibodies anti-HP has been proposed.
Aim: The comparison of two different stool antigen tests for post-treatment assessment of HP.
Patients and methods: Ninety-seven patients (53 F, 44 M, age 17–73 years) have been included. Each patient was treated with standard triple-therapy regimen for 1 week. Post-treatment evaluation was performed between 4 and 10 weeks after therapy by means of urea breath-test (UBT) or endoscopy, depending on clinical situation of patient. HP status was assessed by means of rapid urease test (RUT), histology (H) and culture (C). HP infection was confirmed if UBT, C or both RUT and H were positive. HP was considered absent if UBT was negative or, in case of gastroscopy, all the three invasive tests (RUT,C,H) were negative. Stool specimen were collected, stored, frozen (−20 °C) and then tested with two different stool tests (Premier Platinum HpSA Meridian; Femtolab Connex) according to the manifactures protocols.
Results: Shown in the following table:

Tests	TP	FN	FP	TN	Sensibility	Specificity	PPV	NPV
Femtolab	42	4	4	55	91%	93%	91%	93%
HpSA	35	10	2	62	78%	97%	95%	86%

Conclusions: Both stool tests are easy to perform and results clearly evaluable. Owing to the high sensibility and specificity they can be recommended for noninvasive evaluation of HP infection after therapy.

P1211 Detection and identification of *Helicobacter pylori* in gastric biopsy and resection specimens

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Objectives: To compare the sensitivity of detecting *H. pylori* in gastric biopsy and resection specimens using traditional detection methods (haematoxylin and eosin (HE) stain, modified Giemsa stain) with immunohistochemistry using a commercially available anti-*H. pylori* antibody (Dako, Denmark).
Methods: Gastric antral biopsy specimens showing chronic gastritis (28 cases) together with tissue blocks from gastrectomy specimens for duodenal ulcer were histology reviewed. The paraffin sections were stained with classical histological HE and modified Giemsa and immunoenzymatic by alcaline phosphatase antialkaline phosphatase (APAAP) method for the identification of *H. pylori*.
Results: The HE, modified Giemsa and immunoenzymatic treated sections were carefully examined for the presence of *H. pylori*. HE-stained *H. pylori* appeared as slightly basophilic, spiral-shaped organisms attached to the apical surface of the surface mucus cells. However, curved bacteria were only detected when found in great numbers. Using a modified Giemsa stain, the spiral-shaped bacteria of *H. pylori* stained blue, were attached to the brush border of the gastric foveolar epithelial cells and inside gastric pits. In some cases masked bacteria hidden within mucus were obvious only in immunostained preparations (red deposits). *H. pylori* was identified in 36.6% sections stained with HE, but it could be identified with greater frequency in sections stained with modified Giemsa (78.3%). It could be detected at a still greater frequency in staining with APAAP (90%). Immunohistochemistry was positive in all cases in which *H. pylori* was detected by other methods.
Conclusion: Immunoenzymatic staining of tissue sections by the APAAP procedure is a highly sensitive and easy to use method for detecting *H. pylori* in gastric biopsy and resection specimens.

Atherosclerosis: *Chlamydia* and others

P1212 IL-12 and IFN-γ serum concentrations in abdominal aortic aneurysm (AAA) patients with persistent *Chlamydia pneumoniae* infection

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Objectives: The aim of our study was to evaluate the frequency of persistent *C. pneumoniae* infection and its activity in AAA patients by measuring *C. pneumoniae* specific IgG, IgM and IgA levels and the concentrations of IL-12 and IFN-γ in patients' serum. A total of 28 patients operated for AAA (5 F, 23 M, mean age 68.5 years) and 20 control subjects matched for age and sex (4 F, 16 M) without clinical signs and symptoms of cardiovascular and pulmonary disease took part in our study.
Methods: Microimmunofluorescence method was applied to evaluate the level of anti-*C. pneumoniae* IgG, IgA and IgM (*C. pneumoniae* MIF test, Labsystem). The concentrations of cytokines were evaluated using ELISA method (optEIA set, Pharmingen). The results were evaluated by means of Student's *t*-test and considered statistically significant when $P \leq 0.05$.
Results: Serologic markers of persistent *C. pneumoniae* infection have been detected in 25/28 (89.3%) patients and in 6/20 (30%) healthy controls. In 40% (10/25) of patients with serologic markers of persistent *C. pneumoniae* infection high titers of specific IgG (over 1:1024) and IgA (over 1:64) indicated active infection—reinfection or exacerbation of chronic infection. Mean concentrations of IL-12 (3.86 pg/mL) and IFN-γ (6.86 pg/mL) in this group,

lower than in the rest of patients (IL-12 = 24.94 pg/mL, IFN-γ = 36.9 pg/mL) and in healthy controls (IL-12 = 17.55 pg/mL, IFN-γ = 41.82 pg/mL), can indicate lack of protection against intracellular pathogens.
Conclusion: Since all patients with active *C. pneumoniae* infection were diagnosed as having symptomatic AAA, we suggest that active infection can exacerbate inflammation in the AAA wall. Patients with small AAA and active infection may be candidates to antimicrobial treatment, which can slow down the progression of the disease.

P1213 *Chlamydia pneumoniae*-specific IgG and IgA antibodies in patients suffering from ischemic ulcerations of the legs

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Objectives: Serum samples taken from 49 patients (ranging in age from 30 to 70 years, mean 52.1 years) suffering from ischemic ulcers of the legs were examined to determine the seroprevalence of IgG and IgA specific antibodies against *C. pneumoniae*. Twenty-three healthy blood donors in similar age ($P = 0.43$) constituted the control group.
Methods: Specific IgG and IgA antibodies were determined in sera by the enzyme immunoassays (Labsystems, Finland, Helsinki). Sera containing anti-*C. pneumoniae* IgG antibodies with titers of 45 EIU or higher and IgA with titers of 12 EIU or higher, were considered positive.
Results: The results are presented in table.The seropositivity rate of IgG and IgA antibodies in sera of examined patients was significantly higher ($P < 0.00001$) than in the control group.

	IgG and IgA+		Only IgG+		Only IgA+		IgG and IgA−	
Antibodies	*n*	%	*n*	%	*n*	%	*n*	%
Patients ($n = 49$)	29	59.1	8	16.3	5	10.2	7	14.3
Control ($n = 23$)	9	39.1	5	21.7	2	8.7	7	30.4

The coexistence of specific IgA and IgG antibodies was more frequently detected in the patients with ischemic ulcers than in the healthy donors ($P < 0.00001$)
Conclusions: The higher seroprevalence of anti-*C. pneumoniae*-specific IgG or IgA antibodies may indicate that persistent infection caused by *C. pneumoniae* occurs more often among patients suffering from ischemic ulcers of the legs than among healthy people.

P1214 Infection of *Chlamydia pneumoniae* increased of serum level of interleukin-1β, but not interleukin-8 during atherosclerosis

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Objectives: Atherosclerosis is a major cause of stroke, coronary heart disease, peripheral vascular disease, and aortic aneurysm. Because of the prevalence and importance of these diseases, atherosclerotic lesions within arteries have been extensively studied. While many risk factors have been identified, the mechanism by which the lesions are formed remains unknown. The most popular concept is that the endothelium lining the lumen of the artery becomes damaged. This damage alters the properties of the endothelium and leads to a cascade of events culminating in fibrosis, necrosis, lipid accumulation, and eventually calcification. There have been several candidates forwarded as putative initiators of endothelial injury including microorganisms. Recent studies have shown an association between an obligate intracellular bacterium, *Chlamydia pneumoniae*, and atherosclerosis.
Object and methods: A total of 80 patients with stable angina pectoris, FC III, aged from 44 to 77 years and 30 persons of control group were examined. Immunoglobulin G (IgG) antibodies to *C. pneumoniae* were measured using the ELISA method. Levels of interleukin-1β (IL-1β) and interleukin-8 (IL-8) in serum were measured by ELISA.
Results: IgG antibodies to *C. pneumoniae* were found in 48.75% patients with stable angina pectoris versus 15.38% persons from control group. Level of IL-1β in serum of seropositive to *C. pneumoniae* patients was 56.03 ± 2.25 pg/mL versus 39.55 ± 3.89 pg/mL in seronegative patients ($P < 0.01$). We revealed a strong correlation between titres of anti-*C. pneumoniae* IgG and IL-1β serum level ($r = 0.96$; $P < 0.05$). Level of IL-8 in serum of seropositive patients was 0.842 ± 0.052 ng/mL and in serum of seronegative patients was 0.845 ± 0.042 ($P > 0.05$).
Conclusion: The results of this study showed that chronic *C. pneumoniae* infection is an independent risk factor for the development of coronary heart disease. This infection increases the level IL-1β but not IL-8 in serum. Infection of *C. pneumoniae* increases of level IL-1β but not IL-8 in serum of patients with stable angina pectoris. Final verification of a pathogenetic role of chronic chlamydial infection in the development of coronary heart disease is still lacking.

P1215 Specific antibodies against *Chlamydia* pneumoniae in patients with advanced atherosclerosis

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Introduction: Number of studies pointed the association between chronic *Chlamydia pneumoniae* infection and atherosclerosis. The presence of specific antibodies in serum is one of the important indicator of the infection.
Objective: To determine IgA, IgG and IgM specific antibodies against *C. pneumoniae* in patients undergoing carotid endarterectomy due to advanced atherosclerosis.
Materials and methods: The study group consisted of 32 patients aged 45–88 years (mean age 66 years). All patients were undergoing carotid endarterectomy due to advanced carotid atherosclerotic lesions. The control group consisted of 28 children aged 1–3 years. Sera were stored at −20 °C until serological tests were performed. All sera were analyzed by the EIA test (*C. pneumoniae* IgM EIA, *C. pneumoniae* IgG EIA, *C. pneumoniae* IgA EIA, Labsystems, Finland).
Results: All tested sera from the study group were negative for antibodies against *C. pneumoniae* in IgM, but positive in IgG of 75% and in IgA of 68.7% cases. In control group, 100% of cases were negative for antibodies against *C. pneumoniae* in IgM and IgG, and 86% in IgA. Differences between study and control groups were statistically significant (Table 1).

Table 1 The prevalence of serological markers *C. pneumoniae* infection in study group in comparison with control group

	Study group		Control group		
	n	%	*n*	%	*P* (Fisher test)
IgM	0/32	0	0/28	0	NS*
IgG	24/32	75	0/28	0	<0.00001*
IgA	22/32	68.7	4/28	14.3	0.0001*

*NS: not significant.

Conclusion: The results indicated significantly high frequency of raised levels of *C. pneumoniae* IgG and IgA antibodies in sera of patients with advanced atherosclerosis.

P1216 Is there any link between the presence of *Chlamydia pneumoniae* (CP) antigen in pharynx, anti-CP antibodies in sera and inflammatory markers in patients with coronary heart disease?

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Objectives: In 1988 Saikku et al. presented serological evidence of an association of *Chlamydia pneumoniae* with chronic coronary heart disease

and acute myocardial infarction but still there are controversies in causative relation and the pathogenic pathways. The aim of our work was to examine the presence of CP antigen in pharynx, anti-CP antibodies in sera and some inflammatory markers in patients with coronary heart disease.

Material and methods: Our study compromised 47 patients (32 men and 15 women, aged 40–79) hospitalized in 2000–2001 in Clinic of Cardiology because of angina pectoris ($n = 35$) or acute myocardial infarction ($n = 12$). We measured level of C-reactive protein (CRP), fibrinogen, IgG and IgA antibodies against CP in blood samples and examined pharyngeal swab specimens for CP antigen. Serologic testing for CP was performed by SeroCP—IgG and IgA ELISA test (Savyon Diagnostic Ltd., Israel) while test *C. pneumoniae*—Antigen IFT (Medac, Germany) was used for detection CP-elementary bodies (EB) in pharyngeal swab specimens. As a control group, we examined 15 age and gender-matched subjects without cardiovascular disorders.

Results: Antichlamydial IgG and IgA response (>1.1) was found in 39/47 (82.9%) and 24/47 (51%), respectively, in the control group in 9/15 and 6/15. CP-EB in pharyngeal swab specimens were found in 6/47 (12.7%) of the examined patients and 2/15 in the control group. CRP levels were positively correlated to plasma fibrinogen ($r = 0.37$, $P < 0.05$) but were not correlated to CP serology and the presence of CP antigen in pharynx.

Conclusions: Higher prevalence rate of IgG- and IgA-specific anti-CP antibodies in patients with coronary heart disease than in control group may suggest the specific anti-CP immunization in this group of patients. In both groups there was no strict association between high seropositivity for CP and the presence CP-EB in pharyngeal swab specimens.

P1217 Serological study of cytomegalovirus (CMV) and *Chlamydia pneumoniae* (C.pn.) infections in patients with coronary artery disease

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Objective: To evaluate the possible relation between coronary artery disease and serological evidence for *Chlamydia pneumoniae* and CMV infections.

Methods: A total of 272 sera samples were collected and investigated for the presence of IgG, IgA and IgM antibodies against *C. pneumoniae* (C.pn.) as well as IgG and IgM antibodies against Cytomegalovirus (CMV). A total of 172 sera samples derived from hospitalized patients (of mean age 63 ± 12 years) affected by coronary artery disease (CAD), angiographically documented and 100 sera samples from healthy subjects (control group) with the mean age 57 ± 10 years, not affected by CAD. Antibodies against CMV and C.pn. were performed by ELISA methods, levels of C-reactive protein (CRP) by nephelometry and levels of total cholesterol, HDL-cholesterol and triglycerides by an automated biochemical analyzer (enzymatic methods).

Results: In the CAD group, the presence of specific IgG antibodies against C.pn. was 79.07%, IgA 74.22% and IgM 4.65% while in the control group was 30, 7 and 0%, respectively. Specific IgG antibodies against CMV were detected in the 97.68% of the CAD group and IgM antibodies in the 4.65% of them. The percentages in the control group were 8.5 and 0%, respectively. High CRP values were detected in the 61.63% of patients with CAD while in the control group, were found normal values.

Conclusions: Higher levels of CRP, IgG, IgM and IgA antibodies against C.pn. as well as IgG and IgM antibodies against CMV were found in CAD group of patients than in control group. According to the results above, it appears that there is a possible relation between CAD and chronic infection by C.pn. and CMV.

P1218 Characterization of the cytokine response to *Chlamydia pneumoniae* and *Chlamydia trachomatis*

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Objectives: Infections with *Chlamydia* attract increasing interest, since there is emerging evidence that *Chlamydia pneumoniae* (Cp), a common respiratory tract pathogen, and *C. trachomatis* (Ct), a sexually transmitted urinary tract pathogen, are associated with chronic diseases like atherosclerosis, multiple sclerosis and arthritis. All these *Chlamydia*-associated manifestations involve inflammatory reactions, however, not much is known about recognition and activation of the immune system by these pathogens. The aim of this study was to characterize the cytokine profile released from leukocytes after stimulation with Cp or Ct.

Methods: Human whole blood or murine bone marrow macrophages were incubated for 24 h in the presence of 106/mL viable Cp, Ct or 100 pg/mL Lipopolysaccharide (LPS, *Salmonella abortus* equi (S.a.e.)) and cytokine release was determined by ELISA.

Results: Both, Cp and Ct induced a distinct cytokine profile, different from the one induced by LPS. Ct induced similar amounts of the pro-inflammatory cytokines TNF-α, IL1-β and IL-8, as well as twice as much IFN-γ, compared to stimulation with LPS, but less anti-inflammatory IL-10. In contrast, Cp induced significant less of the pro-inflammatory cytokines and failed to induce any IFN-γ, but led to a stronger induction of the anti-inflammatory IL-10. The same results were obtained with UV- and heat-inactivated Cp and Ct. In the presence of a blocking anti-CD14 antibody, the Cp- and Ct-induced cytokine release was not reduced, while the action of LPS was blunted, suggesting that leukocyte activation is independent of CD14. Incubation of bone marrow macrophages obtained from C3H/HeJ and TLR2−/− mice revealed that both TLR4 and TLR2 are important for the release of murine IL-6 and TNF-α in response to *Chlamydia*.

Conclusions: Taken together, stimulation of inflammatory responses by *Chlamydia* appear to be only partially dependent on their LPS. Although carrying the same LPS, Cp compared to Ct or LPS (S.a.e.) induced a predominantly anti-inflammatory cytokine pattern, what might be due to the involvement of different receptor combinations.

P1219 Are fungi involved in the atherosclerosis process?

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Objectives: There is growing evidence of a relationship between some infective agents (*Chlamydia pneumoniae* and cytomegalovirus) and atherosclerotic process. We sought to determine a similar implication between fungi and atherosclerosis.

Methods: We studied atherosclerotic arterial fragments from 30 patients (24 M) undergoing peripheral vascular surgery (aorta, carotid, femoral and iliac arteries) and 30 fragments of nonatherosclerotic internal mammary arteries from patients undergoing coronary artery bypass surgery (27 M). Each fragment was separated to two pieces: one for mycological analysis and the other for direct microscopy examination and culture in specific mediums. All fragments were handled with strict cautions in order to prevent any external contamination.

Results: Ten (16.6%) fragment cultures showed fungal growth: 9 fragments originating from atherosclerotic lesions (9/30) and only one from an internal mammary artery (1/30, $P < 0.05$). Three fungal species were identified: *Aspergillus*, *Cladosporium* and *Trichoderma*. No correlation was found between the presence of fungi and atherosclerosis risk factors.

Conclusion: This study shows a significantly high affinity of some fungi species to atherosclerotic arterial intima. Further investigations are needed to clarify fungal involvement in atherosclerotic process.

Gynecological infections

P1220 Study of group B *Streptococcus* (GBS) colonization in pregnant women with gestational age over 20 weeks in an Iranian hospital

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Objectives: To determine the rate of GBS colonization in the pregnant women during 20 weeks and more gestational age in one referral Gyneco-logic/Obstetrics center in Hamadan, west of Iran.
Methods: A total of 544 pregnant women selected randomly and two vaginal cotton swabs were taken, one for Gram's staining and the other for culture. The second sample streaked on blood agar medium and incubated at 37 °C. After overnight, the colonies were observed for β-hemolysis, then *Bacitracin* test was done on β-hemolysis colonies. The Na-hypporate hydrolysis test was done on *Bacitracin* resistant colonies. The positive hydrolysis for Na-hypporate was considered as GBS positive.
Results: A total of 145 women (26.7%) were colonized by GBS. There was not correlation and women's age, gestational age, parity, sexual activities, and previous contraception method.
Conclusion: We concluded that GBS colonization in this population is higher than normal rates mentioned in the text references. Because of remarkable rate of GBS colonization in this area, we recommend doing prophylaxis by penicillin G in GBS-colonized women that they have a risk factor for pregnancy-related infections.

P1221 A study of risk factors of vaginal colonization with group B streptococci, in pregnant women, Trujillo, Peru

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Objective: Colonization with group B streptococci (GBS) at any time during pregnancy is an important risk factor for neonatal sepsis. We study 442 pregnant women to determine the principal risk factors to colonize with GBS and we associated with higher or lower colonization rates.
Methods: We evaluated an instrument made specially for this purpose; previously we made a pilot study to determine the validity of this instrument. It consisted in questions related to the principal factors in accordance with the literature revised, such as social characteristics investigated (age, profession, residence, marital status, education, and others).
Results: We determined the influence of a previous pregnancy with pre presence of GBS. 27.8% were primiparas; 28.9% smokers; 2.7% with a history of alcohol use; 0.9% with a history of sexually transmitted disease; 33.5% unmarried. No statistically significant differences were found in regard to age, weight, marital status, history of drug use.
Conclusion: The influence of the number of previous pregnancies proved to be significant. Pregnant women with two or more previous pregnancies tended to be more frequently colonized than primigravidae or secundigravidae women. These findings are discussed in relation to the possible reservoir of the microorganisms and the route of infection.

P1222 Recurrent neonatal invasive group B streptococcal disease in Denmark 1984–1999

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Introduction: Group B *Streptococcus* (GBS), is one of the most frequent causes of mortality and morbidity in neonates. In Denmark, the vast majority of GBS strains isolated from patients with invasive infection is sent to Statens Serum Institut for surveillance of type distribution.
Objective: Our aim was to estimate the frequency of recurrent GBS infections in neonates (0–90 days of age) in Denmark from 1984 to 1999 and to describe the clinical features of these infections. We wanted to put the recurrent GBS infections in the perspective of the total GBS infections in neonates received in the same period in the Streptococcus Unit.
Methods: Serotyping of GBS isolates was performed by the Lancefield capillary-precipitation method.
Results: In the period 1984–1999, 402 cases of invasive GBS-disease in neonates (0–90 days old) was registered at the Streptococcus Unit. A total of 221 (55%) of these cases were among boys and 171 (43%) were in girls. In 10 (2%) the sex was not stated. The incidence was 0.4/1000 live births and increased from 0.1/1000 live births in 1984 to 0.8/1000 live birth in 1995. The Streptococcus Unit received 432 GBS isolates from blood or CSF from the 402 neonates (0–90 days of age) in the study period. A total 311 (72%) were from blood and 91 (21%) were from CSF. The most frequent serotypes were serotype III (59% (41–83%), Ia 16% (0–32%), Ib 8% (0–20%), NT 7% (0–33%) and II 6% (0–15%). Recurrence of neonatal GBS infection occurred in four (1%) of the 402 cases in Denmark in the period 1984–1999. Three out of the four were boys. The mean ages of the children at the first and second episode of the invasive GBS infections were, respectively, 5.5 days (0–13 days) and 27.8 days (26–31 days). The interval with no antibiotic therapy between the two episodes varied between 2 and 7 days. None of the patient died. Isolates from three of the patients were serotype III both at first and second episode. The isolates from the remaining patients two episodes were serotype Ia.
Conclusions: Invasive neonatal GBS infection is still a frequent problem in Denmark. The incidence was 0.4/1000 live births in the period from 1984 to 1999. Recurrent GBS infection occurred only in 4/402 cases (1%). The interval with no antibiotic therapy between the two episodes varied from 2 to 7 days. In all four cases, the serotype identified at both the first and the recurrent episode were identical. Serotype III is the dominating serotype both in invasive neonatal GBS infection in Denmark 1984–1999 and in the recurrent episodes.

P1223 Direct latex agglutination testing of selective broth for the detection of Group B streptococci (GBS) in pregnant women

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Objectives: To determine the value of the direct latex agglutination testing of selective broth for the rapid detection of Group B streptococcal colonization in pregnant women.
Material and methods: Vaginal and rectal specimens from pregnant women were inoculated onto Columbia with colistin and nalidixic acid agar (CNA) and a selective broth (Todd Hewitt supplemented with gentamicin, nalidixic acid and 5% blood). After 24 h of incubation in 5% CO_2 all negative CNA cultures were incubated for an additional 24 h and all selective broth were subcultured onto CNA. In addition, latex testing for GBS was performing according to the manufacturer's protocol on the selective broth after 24 h of incubation. Antimicrobial susceptibility by disk diffusion testing was performed directly from selective broths positive by the latex method.
Results: A total of 114 specimens from 67 pregnant women with GBS colonization and 120 specimens from 66 pregnant women with no colonization were evaluated. Of the 114 positive specimens, the direct latex method detected 91 (80.7%). In 23 cases, the direct latex method was negative. Of those, there were 11 specimens in which GBS was isolated from initial CNA but subcultures of the selective broths were negative. Of the 120 specimens negative for GBS in only one case the latex method was positive. In this case, presence of GBS in the broth was confirmed by inoculation of the broth onto a different selective solid medium (Granada agar). Antimicrobial susceptibility testing was performed in 96 samples, of those in 68 (70.8%) results were available.
Conclusions: The direct latex test of selective broth is a rapid method for detecting GBS colonization but it cannot replace the traditional subculture method.

P1224 A 4-year seroepidemiological screening for CMV, rubella and *Toxoplasma* infections among women of child-bearing age

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Objectives: The aim of the study was to determine the seroprevalence of *Rubella* virus, *Cytomegalovirus* (CMV) and *Toxoplasma gondii* antibodies in a population of pregnant women in Greece. Findings of the survey will provide empirical data on immunization coverage and will help to prevent congenital infections.
Material and methods: During a 4-year period (1997–2000) (A) 1872 Greek women (67%) and (B) 922 immigrants form East Europe (32.9%), were screened for IgG and IgM antibodies to rubella virus, CMV and *Toxoplasma gondii*. IgG antibody titers to rubella were determined by hemagglutination inhibition (Rubeokit, Bio-Rad, Pasteur, France), to CMV and *Toxoplasma* by indirect immunofluorescence (Merifluor CMV IFA, Gull-Meridian And Toxo Spot IF, Biomerieux, France, respectively). Enzyme Immunoassay method has been used for the detection of IgM antibodies (IMX System Abbott lab. Illinois, USA).
Results: The findings of this study showed that approximately 21.4% of screened Greek women and 20.4% of immigrants were seronegative to rubella. Values between 50 and 200 IU/mL of IgG antibodies were detected in 65.2 and 62.9% of both groups A and B, respectively. Increased levels of rubella IgG antibodies (>200 IU/mL) were found in 15.8 and 13.3% among the women of child-bearing age. Seroprevalence of *Toxoplasma* IgG antibodies (>200 IU/mL) was determined in 2.4 and 3.3% of population A and B. One thousand four hundred twenty-two (76%) women and 697 (75.5%) were susceptible – IgG antibodies <25 IU/mL) – to *Toxoplasma*. CMV IgG antibody titers (20–80) were found in 1191 and 636 of the 2794 women, while 626 (33.4%) and 246 (26.6%) were seronegative. Finally. high CMV IgG titers (^{3}80) were determined in 0.96 and 1.2% of the screened population. Seropositive rate of rubella IgM antibodies 0.9%, *Toxoplasma* 0.9% and CMV 2.3% was found in women tested.
Conclusions: (1) A substantial number of women that were susceptible to a primary infection with CMV (about 26–33%), rubella virus (21%) and *Toxoplasma gondii* (76%) was found. (2) The results did not show a statistically significant difference between the two groups of populations examined. (3) rubella immunization strategy should be reconsidered for women of child-bearing age.

P1225 The persistence of antirubella antibodies in woman of child-bearing age

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Objectives: Introduction of monovalent and conjugated vaccines against measles, mumps and rubella helped to decrease significantly the morbidity. Therefore, World Health Organisation and USA Childhood Immunisation Initiative introduced the program of measles and rubella eradication. *Rubella* is a mild disease, usually without consequences and complications. Clinically serious is congenital rubella, when nonimmunized pregnant woman is infected with rubella virus during first month of pregnancy. The main aim of vaccination is associated with elimination of congenital rubella. In Poland, the vaccination of 13 old girls was introduced in 1989. The current data on serologic status of rubella antibodies in women in reproductive age are lacking. The aim of the study was an evaluation of antirubella antibody levels in women 15–30 years old. It was thought that such data might be of value in view of planned introduction to the National Vaccination Program of additional vaccination against rubella of woman immediately after delivery.
Methods: To establish the level of antibody the test kit ETI-RUBEK-G Plus Kit Diasorin was used. The method for quantitative determination of specific IgG to rubella virus is an immunoenzymatic assay, based on ELISA technique.
Results: The total of 865 serum samples has been tested. In 767 sera (88.7%) the protective titers of antirubella antibodies were detected. The decrease of antirubella antibodies titer together with increasing age of tested women was observed. The geometric mean titer of rubella antibody was 158.7 IU/mL among women at age of 15 years, and 66.8 IU/mL in the age group of 30 years. Among investigated women 11.3% had no protective antirubella antibodies.
Conclusion: The decrease of antirubella antibody titer together with increasing age of tested women was observed. It is necessary to take into consideration possibility of introducing additional dose of vaccine for women in reproductive age.

P1226 In vitro and in vivo antibacterial activities of telithromycin (TEL) against gynecological pathogens

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Objective: To evaluate in vitro and in vitro antibacterial activities of TEL against gynecological pathogens.
Methods and results: In vitro: the antibacterial activity of TEL against recent clinical isolates (isolated in 2000) of *S. agalactiae* (33), *E. faecalis* (22), *N. gonorrhoeae* (30), *P. anaerobius* (20), *F. magna* (20), *B. fragilis* (25) and *P. bivia* (30) was compared with those of erythromycin A, clarithromycin, azithromycin, ampicillin and levofloxacin. TEL inhibited more than 50% of clinical isolates of *S. agalactiae, E. faecalis, N. gonorrhoeae, P. anaerobius, F. magna, B. fragilis* and *P. bivia* at the concentration of 0.016, 0.063, 0.063, 0.032, 0.032, 0.5 and 0.25 μg/mL, respectively. TEL inhibited more than 90% of clinical isolates of *S. agalactiae, E. faecalis, N. gonorrhoeae, P. anaerobius, F. magna, B. fragilis* and *P. bivia* at the concentration of 0.016, 4, 0.125, 0.063, 0.063, 4 and 1 μg/mL, respectively. In vivo: The efficacy of TEL was evaluated using experimental intra-abdominal abscess in mice caused by *B. fragilis* (MIC of TEL: 0.016 μg/mL). TEL inhibited the abscess formation and significantly decreased the viable cell counts in abscess in comparison with the untreated group.
Conclusions: These results suggested TEL would be also useful for the treatment of anaerobic infections concerning in vitro and in vivo antibacterial activities.

P1227 Prevalence and antifungal susceptibility patterns of yeasts from women with vulvovaginitis

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Introduction: The number of life-threatening fungal infections observed worldwide has increased significantly. The major agents are *Candida* species and *C. albicans* is the predominant pathogen. The therapeutic choice is restricted to a few agents; among these azoles have been more extensively used, but resistance to such drug agents is emerging.
Objectives: To evaluate the incidence of *Candida* spp. and other yeasts and their resistance to antifungal agents among patients with vulvovaginitis. The relatedness of the isolates from patients with recurrent vaginitis was also investigated.
Methods: The population of this study included patients with symptomatic vaginitis attending the Department of Obstetrics Gynecology, University Hospital of Pavia, Italy. Vaginal and vulvar swabs were collected and cultured on Cromo Albicans Agar (Biolife). The fungal isolates were identified by use of the YBC card (Vitek system BioMérieux) and confirmed by API-*Candida* (BioMérieux). Antifungal susceptibility testing was carried out by a broth microdilution test YeastOne (Sensititre) and the E-test (AB Biodisk) using RPMI 1640 glucose agar plate. Quality control strains included were according to the manifactureer. Genotypic characterization was performed by the pulsed field gel electrophoresis of genomic DNA using the reagent kit four (Bio-Rad).
Results: A total of 39 specimens from 36 patients were cultured. The identification of isolates revealed a prevalence of *C. albicans* (52.9%), followed by *C. krusei* (17.6%) *C. glabrata* (11.8%), *S. cerevisiae* (11.8%), and *C. parapsilosis* (5.9%). The concordance of results obtained with the two identification methods used was high (99.8%). *C. albicans* resulted always sensible to amphotericin B, fluconazole, 5-flucytosine and rarely resistant to itraconazole and ketoconazole (2%). *C. krusei* isolates were sensible to amphotericin B, itraconazole and 5-flucytosine, resistant to ketoconazole and showed an intermediate level of resistance to fluconazole. *C. glabrata, S. cerevisiae*, and

C. parapsilosis resulted sensible to the tested drugs. In 26.5% of the cases, the results of the two antifungal susceptibility tests were incongruous.
Conclusions: It seems there may be an increasing trend of non*albicans* species in vulvo vaginitis. Further studies are needed to make a surveillance of the reifections by the above species because in three patients with recurrent vaginitis the latter isolates developed resistance to itraconazole and ketoconazole.

P1228 Antifungal activity of the 'paracymeno' on *Candida* species

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Introduction: Vulvovaginal candidosis (VVC) is one of the most common gynecological infections. The reduced number of available antifungals and the increasing resistance urged the search of alternative therapy. Previous research of our team demonstrated the antifungal activity of some essential oils (1). Paracymeno is a component of the essential oils *Thymus vulgaris* and Thymus zygi.
Objectives: To determine the: (1) antifungal activity of paracymeno, estimated as the minimal inhibitory concentration (MIC) on commonly pathogenic *Candida* species; (2) effect upon germ tube formation by *C. albicans*; (3) the chemical composition of the essential oil; (4) mechanism(s) of activity by flow cytometry (FCM) using two fluorocrom FUN-1(2) and propidium iodide (3).
Materials and methods: Ten clinical strains (five *C. albicans* and five non-albicans) and two ATCC strains (*C. albicans* 10231 and *C. krusei*), either sensitive or resistant to classic antifungals. MIC was determined according the NCCLS protocol M 27-A. The ability to form germ tubes was assessed in a germ tube formation assay. The composition of the essential oils was investigated by gas-chromatography and gas-chromatography/mass spectrometry. The mechanisms of activity were elucidated by flow cytometry using propidium iodide (PI) to evaluate lesion of the membrane and FUN-1 to study metabolic disturbance with different concentrations of the oil, after serial times of incubation.
Results: MIC was 1/32 for all tested strains, except for *C. krusei* which was 1/16. Dilutions well above MIC concentrations totally inhibited germ tube formation. The concentration of paracymeno was 11.7 and 21.2% of essential oil of *Thymus vulgaris* and *Thymus zygis*, respectively. The dilutions associated to an extensive rate of killing induced a permeation to PI, related to a direct damage of the cytoplasmic membrane. In a kinetic study assaying the MIC concentration, 15 min were sufficient to produce killing of 57.4% of the cells in the suspension. Lower concentrations, unable to produce lesion of membrane, induced metabolic disturbance as shown by FUN-1 staining.
Conclusions: Paracymeno demonstrated a potent antifungal activity, due mainly to direct damage to the cytoplasmic membrane of *Candida* species. The essential oil shows however, greater antifungal activity than its component paracymeno. Our results support the use of paracymeno in the treatment of mucocutaneous candidosis, namely VVC, in case of being devoided of toxicity and side-effects.

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P1229 *Gardnerella vaginalis* and other microorganisms from women in reproductive age with colpitis

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Objective: *Gardnerella vaginalis* (GV) is an etiological factor of colpitis, a sexually transmitted disease (STD). The most common symptoms are: vaginal itching and discharges with foul smell and comfortless copulation.
Methods: We tested 420 samples from womens' genital tracts. They had symptoms of colpitis. They were heterosexual age 24–50. From all of them cervical and vaginal swabs were taken. Samples were cultured on different plates to evaluate aerobes, anaerobes and fungi.
Results: We obtained 538 bacterial isolates from genitourinary tract in 2000 years. The most frequent were aerobic: *Escherichia coli* (22.9%), *Enterococcus faecalis* (20.4%) and anaerobic: *Gardnerella vaginalis* (17.1%), *Peptostreptococcus* sp. (4.1%), *Prevotella* sp. (3.9%). GV was susceptible to penicillin, clindamycin, tetracycline, doxycycline, metronidazole (MET). In spite of susceptibility in vitro to MET we observed some cases clinically resistant to this antibiotic. Successful outcome was obtained after clindamycin treatment.
Conclusions: GV is the most common pathogen isolated from colpitis-like infections (17.1%). We observed that infections caused by GV had more troublesome symptoms and were more difficult to treat than colpitis caused by others pathogens.

P1230 The association between bacterial vaginosis, cervicovaginal IL-6 level and preterm delivery

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Objectives: Bacterial vaginosis (BV) is the most prevalent cause of vaginal symptoms among women of child-bearing age. Several studies have been published during the last few years that suggest that women with BV tend to have a higher incidence of preterm labor and premature rupture of membranes than women with normal flora. An increased level of cytokines has also been found in the vaginal fluid of women with abnormal vaginal flora. The aim of our study was to determine the possible association between BV, cervicovaginal IL-6 levels and preterm delivery.
Methods and results: Vaginal samples for BV diagnosis and IL-6 analysis were taken from 56 unselected pregnant women. By using Nugent criteria, BV was diagnosed in 35.7%, intermediate flora in 7.1% and normal vaginal flora in 57.1% of examined pregnant women. The IL-6 levels in cervicovaginal fluid of pregnant women with BV were higher (median 128.15 pg/mL) than those in pregnant women with normal vaginal flora (median 118.05 pg/mL) ($P < 0.01$). Median value of cervicovaginal IL-6 was statistically significantly higher among pregnant women with BV who delivered preterm compared with pregnant women with BV who delivered at term ($P < 0.01$).
Conclusion: Our results show that pregnant women with BV and high levels of cervicovaginal IL-6 deliver more often preterm compared with pregnant women with BV and low levels of cervicovaginal IL-6.

P1231 Molecular epidemiological study of vertical transmission of vaginal *Lactobacillus* species from mothers to newborn infants

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Objectives: Lactobacilli may act as probiotics providing beneficial effects to the host by improving the balance of colonic bacteria. Meanwhile, there is little information available regarding how and when infants became colonized with lactobacilli in their gut. We investigated mother-to-newborn infant transmission of *Lactobacillus* species in Japanese.
Methods: Vaginal swabs were obtained from the anterior and posterior vaginal fornices of pregnant women and the spontaneously defecated stool was collected from their newborn infants. All infants were born by uncomplicated vaginal delivery and basically fed breast milk. All specimens were cultured anaerobically on LBS agar. Lactobacilli were identified by a two-step multiplex PCR to the species level. *Lactobacillus* strains were fingerprinted by arbitrarily primed polymerase chain reaction (AP-PCR) using ERIC1R and ERIC2 primers.
Results: Of 86 pregnant women tested, 71 (82.6%) were positive for vaginal lactobacilli. At 5 days after birth, 24 (33.8%) of the 71 infants whose mothers were lactobacilli positive had fecal lactobacilli while only one (6.7%) of the 15 infants delivered from the vaginal lactobacilli-negative mothers was lactobacilli positive ($P < 0.01$). Lactobacillus crispatus was the most prevalent species as vaginal lactobacilli in mothers and fecal lactobacilli in infants at 5 days of age whereas *Lactobacillus gasseri* was the most common in infants at 1 month of age, followed by *L. crispatus*. Identification to the species level followed by AP-PCR typing demonstrated that 23.3% of the 86 infants were likely to be colonized in the intestine by vaginal lactobacilli of their mothers and that only two infants of them retained the same vaginal lactobacilli until 1 month of age.

Conclusions: These results suggest that approximately one-fourth of infants acquire vaginal lactobacilli of mothers at birth and that the acquired lactobacilli do not last in the intestine of the infant for long-term but rather are replaced by ones from milk or unknown sources after birth. AP-PCR with primers ERIC1R and ERIC2 is suggested to be a potential tool for typing of lactobacilli.

P1232 The possible influence of H_2O_2-producing lactobacilli on asymptomatic cervical shedding of *Herpes simplex* virus

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Objectives: Antimicrobial activity of H_2O_2-producing lactobacilli in female genital tract represents an important nonspecific mechanism of local host defense in the prevention of STDs. The aim of this study was to examine the prevalence of H_2O_2-producing lactobacilli and asymptomatic cervical shedding of *Herpes simplex* virus type 1 and type 2.
Methods: The study population consisted of 67 asymptomatic women of reproductive age. Vaginal lactobacilli were isolated and identified to the genus level by colony morphology, characteristic Gram's stain and catalase-negative test. The ability of lactobacilli to produce H_2O_2 was tested by tetramethylbenzidine (TMB) plate method. *Herpes simplex* virus types 1 and 2 were detected in endocervical smears by the test of direct immunofluorescence with type specific monoclonal FITC labeled antibodies.
Results: Lactobacilli were isolated and identified from 54 of 67 (80.6%) women. Among the isolated strains of lactobacilli the percentage of H_2O_2-producing strains was 66.67%. Asymptomatic cervical shedding of *Herpes simplex* virus types 1 and 2 was detected among 4 of 36 (11.12%) women with H_2O_2-producing lactobacilli and among 4 of 13 (30.77%) women without isolated lactobacilli.
Conclusion: The results of this study suggests the lack of correlation between the presence of asymptomatic cervical shedding of *Herpes simplex* viruses and the presence of H_2O_2-producing lactobacilli.

P1233 Hybrid capture based human papillomavirus typing in cervical specimens compared to cytology (Pap test) and colposcopic biopsy

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Introduction: Human Papillomavirus (HPV) infections are related to several cutaneous and mucosal dysplasias, including both benign and malignant lesions. recent developments in HPV DNA detection techniques have shown that HPV infections in female genital tract are common but the progression of the disease may require several factors such as the persistence of the infection, the viral load, co-infection with the presence of immunodeficiencies and probably the presence of other cofactors.
Objectives: The utility of a molecular assay for the detection of HPV in cervical smears was evaluated.
Methods: A total of 114 women aged between 21 and 69 years (36 median) with normal cytology, with atypical squamous cells of undetermined significance (ASCUS), with low (LSIL) and high (HSIL) grade squamous intraepithelial lesions in cervical cytology, were studied. In all women with ASCUS, LSIL and HSIL, a colposcopically directed biopsies were performed. hybrid capture (HC II) testing for 14 human Papillomavirus types (digene hybrid capture II system) were tested on all 114 women.
Results: Based on cytology results, 40 patients showed normal cytology, three ASCUS, 53 LSIL and 18 HSIL. With the molecular assay, HPV D was detected in 65/114 (58%) patients. the high-risk HPV types was present in 46/65 (70%) patients, the low-risk HPV types in 9/65 (14%) patients; in nine (16%) women was present high and low-risk HPV types. The sensitivity of hybrid capture for colposcopic biopsy was 90% (47/52) and a specificity 50% (12/22); the corresponding values for *Papanicolaou smears* were 76% (54/71) and a specificity 77% (31/40). All the colposcopic biopsies evocative of invasive carcinoma, were positive for high-risk HPV presenting a pap smears of HSIL lesions.
Conclusion: HPV detection with the HC II assay represents an easy test for routine use, in molecular biology laboratory. In our experience, its sensitivity for the detection of HPV DNA is higher than classic cytological smears, 90% versus 76%, when compared with 'gold standard' of biopsies. This sensitivity makes this assay highly recomendable for routine detection of high and low-grade lesions, in association with cytological examination.

P1234 Detection of women at risk of developing vulvar cancer

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Background: The strong association of Human Papillomavirus (HPV) and cervical cancer makes important the study of these viruses in vulva, in women with HPV high risk (HR) in cervix.
Methods: We have made a cross-sectional study in 2338 cervical samples, in the last 3 years from women with any cytologic and/or colposcopic alteration. The DNA-HPV detection tests were: (1) a-PCR-based assay using the MY09–MY11 consensus primers. (2) Hybrid Capture II HPV microplate assay. The tests were performed without knowledge of the cytologic diagnosis and other clinical data. 816 patients had a positive results to HPV HR. In 515 of them, we made detection of HPV in vulva (Group I). We studied further 100 women without clinical symptoms (cytology and colposcopy negatives) and we have made detection of HPV in vulva (Group II).
Results: The positive rates were: Group I: 54.4% ($n = 280$) and Group II: 12% ($n = 12$), $P < 0.001$. The rate of positive HPV and the oncogenic risk, high risk (HR) and low risk (LR), in each group is shown in Table 1. In the Group I, coincident results between cervix and vulva were obtained in 242 patients (86.4%). The most frequent HPV HR was type 16 (31.5%) $P < 0.01$ and successive types were 31 (14.8%) and 61. We have made an OR = 5.6 to the patients exposed to HPV HR in cervix.
Conclusions: The presence of HPV in vulva in women with HPV HR in cervix was significantly higher ($P < 0.001$) than in the group without clinical symptoms, as the types of HPV HR. In our study, we met 5.6 times more HPV in vulva in those patients exposed to HPV HR in cervix.

Oncogenic risk of HPV	HPV(+), *n* (%)	HPV(+) HR, *n* (%)	HPV(+) LR, *n* (%)
Group I ($n = 515$)	280 (54.4)	236 (84.2)	44 (15.7)
Group II ($n = 100$)	12 (12)	5 (41.6)	7 (58.3)

P1235 Human papilloma virus testing in cervical cancer screening: results from a pilot experience in Italy

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Objective: Estimating HPV prevalence and type distribution in a study group representative of the population of Turin, Italy. In Italy no previous population-based study of HPV prevalence has been available so far.
Methods: From 6995 women enrolled in a population based screening program for cervical cancer in Turin, Italy, under informed consent (6556 women) a further cervical sample for liquid-based cytology was collected and, after slide preparation, the specimen was stored at −20 °C. A random study of 1048 samples, stratified by age, history of screening and screening unit was tested for HPV by PCR, in collaboration with IARC and the Free University, Amsterdam. Cytology was evaluated according to the Bethesda System. Samples were first prescreened by a B-globin PCR to check DNA quality, then HPV was detected by PCR with general consensus primers GP5+/GP6+0. Enzyme immunoassay was used to type high risk (HR) from low risk (LR) HPV with two specific cocktail probes. Final typing was achieved by a dot-blot hybridization test with specific probes complementary to the most common HR/LR HPV. Preliminary data are available for 727 samples (720 B-globin positive).
Results: HPV DNA was found in 57/720 (7.9%) samples. The majority of samples were HR positive (42/57 = 73.7%), while LR HPV was present in 9/57 (15.8%) and double HR and LR infection was detected in 6/57 (10.5%). HPV 16 was the most representative type (37.5% of HR-HVP infections). The prevalence of HR HPV infection was 8.72, 9.90, 3.80, 4.24 and 6.94% in age classes 25–34, 35–44, 45–54, 55–64 and >65, respectively. The majority of HPV infections (37/57 = 65%) were detected in women with normal smears.

Conclusions: HPV prevalence was consistent with available data from other Western European countries. Since the majority of HPV infection have been detected in cytological normal smears, the hypothesis of modifying the primary screening strategy by including HPV test is under evaluation.

P1236 Detection of papilloma virus infection in progressive cervical neoplasia by in situ hybridization

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Cervical cancer is one of the most frequently found cancers in women and appears to have a viral etiology. Since HPVs now comprise more than 100 different HPV types, the in situ hybridization (ISH) has been the preferred methodology for virus identification and typing on isolated DNA. We examined three groups of patients from Tehran region. Aged 21–27 years. The first group comprised 12 females with squamous cell carcinoma (SGG), the second group included six females who suffered from cervical intraepithelial neoplasia (CTN) and two dysplasia. Formalin fixed, paraffin embedded tissue specimens representing 20 cases of gynecology, oncology imam and mirza hospitals stage 2 and 3 were used. HPV DNA was detected by ISH with DNA probe kits (ENZO Inc.). All 18 carcinomas were positive for HPV 6/11 11(6101%), HPV 16/18, 10 (55.5%) and 31/33/51 14 (77.7%). In two patients (10%) HPV types could not been further classified and these cases were therefore categorized as HPVX. These findings are consistent with the hypothesis that HPV infection is the primary cause of cervical neoplasia. Furthermore, they support HPV vaccine research to prevent cervical cancer and efforts to develop HPV DNA diagnostic tests.

P1237 Chronic genital infection and antisperm antibody production

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Objectives: Our aim was to determine the prevalence and clinical significance of genital infection in antisperm antibody (ASA) production.
Methods: ASA production using capillary tube agglutination test was investigated in 126 women presenting with chronic genital infection and 120 healthy controls in IVF center of Yazd province-Iran.
Results: 31.8% of women presenting with genital infection showed *Ureaplasma urealyticum*, 23.5% *Chlamydia trachomatis*, 21.8% *Mycoplasma hominis*, 19% *Listeria*, 2.2% *E. coli*, 1.1% *Gardcnella vaginalis* and 0.6% *Klebsiella pneumoniae* infection. The percentage of isolation of ASA pathological titer (1/16 and >1/16) in patients compared with healthy controls were 74.8 and 0.8%, respectively.
Conclusions: These data suggest that chronic genital infection could increase ASA production level in patients compared with healthy controls.

P1238 Occurrence of *Ureaplasma urealyticum* and *Mycoplasma hominis* in patients with genito-urinary tract infections in Gdansk, Poland

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Objectives: To evaluate the incidence and antibiotic susceptibility of *U. urealyticum* and *M. hominis* in outpatients with genitourinary tract infections from January to August 2001.
Methods: Mycoplasma Duo Kit (Bio-Rad) and Mycoplsama IST (bioMerieux) were used to culture *U. urealyticum* and *M. hominis*. Antibiotic susceptibility to ofloxacine, tetracycline, doxycycline and erythromycin was determined with SIR Mycolasma (Bio-Rad) and Mycoplsama IST (bioMerieux). Ciprofloxacin activity was evaluated by broth macrodilution method.
Results: A total of 512 specimens were obtained from 382 patients, 286 females and 96 men, from 14 to 68 years old. The clinical material was as follows: 152 cervical swabs, 131 vaginal swabs, 112 urethral swabs, 89 urine and 28 semen. *U. urealyticum* was isolated from 80 specimens and *M. hominis* from 13. From 76 positive patients, 60 were female and 16 men. All *M. hominis* were susceptible to ciprofloxacin, ofloxacine, tetracycline and doxycycline, only 50% to erythromycin. Doxycycline was the only uniformly active agent against *U. urealyticum*. There was 4–8% resistance rate among other agents against *U. urealyticum*.
Conclusion: *U. urealyticum* and *M. hominis* were cultured from 20% of outpatients suffering from genitourinary tract infections. Doxycycline proved to be the most active agent in vitro. Other antibiotics were less active depending on the organism and the agent.

P1239 New perspectives of povidone-iodine used for prophylaxis against *Ophthalmia neonatorum*

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Objectives: In central Europe, neonate conjunctivitis is mainly caused by staphylococci, *E. coli* and chlamydia, whereas *N. gonorrhoeae* infections occur rarely. With neonate keratitis nosocomial pathogens predominate, the main ones being *P. aeruginosa*. Prophylaxis against *Ophthalmia neonatorum* is still necessary since it reduces the risk of infections that can impair vision and even lead to blindness. However, silver nitrate as the former agent of choice should be replaced because of the local irritation potential after instillation and the lack of antimicrobial efficacy against chlamydia. Povidone-iodine (PVP-I) was evaluated as possible agent of choice for the Credé prophylaxis.
Methods: The bactericidal efficiency of PVP-I (Betaisodona(R) solution) was measured in a quantitative suspension test according to the guidelines of the German society for hygiene and microbiology. The antichlamydial activity was determined by the reduction of inclusion bodies in McCoy cell monolayers detected with immunofluorescence microscopy. The local tolerability was assessed using the HET-CAM (chorioallantois membrane) test as well as the tissue explant test carried out on the peritoneum of neonatal rats [1].
Results: 1.25% PVP-I has a bactericidal effect in vitro within 30 s with a reduction factor of >5 lg against *N. gonorrhoeae*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. trachomatis*. By contrast, 1% silver nitrate and 0.1% erythromycin had no effect at all in 30 s against *C. trachomatis*. In the HET-CAM test and in the explant test the relevant PVP-I concentrations were found to be well tolerable. With healthy volunteers, 2.5% PVP-I solution caused pronounced burning; a 1% solution was tolerated without pain.
Conclusion: Because of the antimicrobial efficacy and the tolerability 1.25% PVP-I can be currently considered as remedy of choice for the prophylaxis against ophthalmia neonatorum.

Reference

1. Kramer A, Adrian V, Rudolph P, Wurster S, Lippert H. *Chirurg* 1998; 69: 840–5.

Toxoplasmosis

P1240 Toxoplasmosis: serologic control in pregnant women

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Introduction: *Toxoplasma gondii* is an obligate intracellular parasite of universal distribution. It may be the cause of subclinic infection in healthy individuals, of congenital toxoplasmosis and of severe infections in immunocompromised patients. Oral and transplacental routes used to be the most frequent mechanisms of transmission. Primoinfection is related to low avidity of specific IgG.
Objectives: To know the prevalence of infection in pregnant women in our population and to evaluate the usefulness of avidity of IgG for the detection of primoinfection.
Methods: All 2754 pregnant women attending our hospital during 1999 were screened for toxoplasmosis. First line test was anti-*Toxoplasma* IgG, and, in case it was positive, a specific IgM determination was also performed. If these antibodies were positive, a test for avidity of specific IgG was then done

(positive IgM were previously evaluated in order to eliminate possible residual cases). A second sample of serum obtained 3–4 weeks after the first one was studied in order to know the evolution of IgG titer. Both IgG and IgM antibodies were determined by means of a MEIA test by the Axsym system (Abbott). Tests for avidity of IgG were performed at the National Reference Center at Majadahonda (Spain).

Results: A total of 679 IgG tests were positive (prevalence: 24.6%). Only 22 out of these 679 sera had positive IgM (0.79%). Three of these patients with positive IgM were excluded because they had positive values in previous pregnancies, which discarded a recent infection. The remaining patients with positive IgM had high avidity of specific IgG, except one of them who showed a low avidity in her last control of a twin's pregnancy. Previous controls had been at 8 and 11 weeks (these two determinations were carried out in another center) and the third and positive one was at 32 weeks. IgG titers were 315, 322 and 198 IU/mL.

Conclusions: The prevalence of gestational infection was 0.03% in our population (one primoinfection every 2754 pregnancies). As our seroprevalence is 24%, strict prophylactic hygienic and alimentary measures are recommended in order to prevent infections in seronegative women. The test for avidity of specific IgG is useful in order to discard infection in suspected pregnant women (those with positive IgM), eliminating useless doubts. It is also a tool for beginning treatments in case of proven primoinfection.

P1241 Congenital toxoplasmosis: incidence and consequences in a prospective cohort of 1460 pregnancies monitored in Lyon, France

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Objectives: Estimate the incidence and consequences of congenital toxoplasmosis in a cohort of 1460 pregnancies with proven *Toxoplasma* infection.

Methods: Pregnant women and their children were managed between 1988 and 2000 according to a standardized protocol. Dates of maternal infection, types and duration of maternal treatments, results of prenatal, neonatal and postnatal work ups and postnatal treatments, prospectively collected, were available for all patients.

Results: Out of the 1460 pregnant women with proven *Toxoplasma* infection, 602 (41%) were infected in the first trimester, 473 (33%) in the second and 385 (26%) in the third. Prenatal treatment consisted of spiramycin alone in 1060 (73%) women, pyrimethamine-sulfadiazine alternating with spiramycin in 203 (14%), pyrimethamine-sulfadiazine alone in 94 (6%), and 103 (7%) were not treated. Out of the 51 nonlive births, 25 fetus spontaneously aborted or were stillborn, 12 fetus were terminated without proof of fetal infection, seven were terminated due to hydrocephalus or ventricular dilatation on ultrasound scan and seven after positive analysis of the amniotic fluid despite the lack of ultrasound lesions. Among the 1409 live born children, 1327 (94%) were followed up until infection was ruled out (987; 74%) or in (340; 26%). Based on live born children, the overall risk of fetal infection ranged from 24 to 30%, with estimates ranging from 3 to 10% in the first trimester, 21–26% in the second, and 58–63% in the third. All infected children were treated postnatally. At their last examination (mean age: 71 months; range: 12–169), 252/340 (74%) were free of any lesions. The remaining 88 children had intracranial calcification (29 cases), chorioretinis (69), hydrocephalus (two cases) or microphthalmia (two cases). None, but one child who died immediately after birth, had neurological impairment. Only two children with bilateral ocular lesions had significant visual impairment.

Conclusion: In our experience (antenatally treated pregnant women), the consequences of congenital toxoplasmosis are less severe than generally admitted. This information should be clearly given to future parents. Termination of pregnancy is only warranted in the event of fetal lesions on ultrasound scan. Follow up during the first year of life is crucial to exclude or prove congenital toxoplasmosis. Life-long surveillance of infected children is equally essential for secondary and tertiary preventions.

P1242 Evaluation of the new Platelia Toxo IgM TMB test kit

J. Franck, C. Masson, N. Lambert and M. H. Guindre
Marseille, Marnes La Coquette, F

Objectives: Toxoplasmosis, caused by *Toxoplasma gondii* and usually benign, can be severe following transplacental contamination (congenital toxoplasmosis) and in immunocompromised patients. Serologic testing is the primary tool for toxoplasmosis screening and diagnosis. The aim of this study is to compare sensitivity and specificity of Platelia Toxo IgM TMB to the VIDAS Toxo IgM test in the conditions of use of a hospital laboratory.

Methods: Prospective study: 607 samples destined to be done routinely were simultaneously tested when received, all the samples coming from either pregnant women, newborns or immunocompromised patients. Retrospective study: 262 samples were analyzed, 100 samples from recent infections, 48 samples from 14 seroconversions and 100 cord blood samples. Discrepant results are resolved with ISAGA Toxo IgM. The tests have been performed following the manufacturers' recommendations.

Results: Prospective study: no false positive sample was observed that confirmed the specificity of 100%. Sensitivity study gives a good result of 92%. Among the four platelia false negative samples: in three cases, IgM detected by ISAGA were residual IgM, while in one case IgM were nonspecific. These results were validated on a second sample. The predictive positive value of the test is 100.0% for a confidence interval of 95% (90.4–100.0) and the predictive negative value 99.3% for a confidence interval of 95% (98.1–99.8). Retrospective study:

- Samples from recent infections: no discrepant result was observed.
- Seroconversions: four samples give discrepant results.

The Platelia results, nearer to the ISAGA than VIDAS results show a rapid dynamic of platelia during seroconversions, with a precocious detection of specific IgM, but also a fast decrease when followed up. Cord blood samples: no discrepancy was observed in the 100 samples taken from healthy newborns (mother with negative serology), all samples being found negative. Out of the 14 samples from infected newborns, four of them found positive or doubtful with platelia are found negative with VIDAS, but positive by ISAGA. The results obtained with platelia are identical to those obtained with ISAGA and show the very good sensitivity of Platelia Toxo IgM TMB for the screening of children with congenital toxoplasmosis.

Conclusion: This test, along with Platelia Toxo IgG TMB, is perfectly appropriated for toxoplasmosis screening and diagnosis.

P1243 Evaluation of the new Platelia Toxo IgG TMB test kit

J. Franck, C. Masson, N. Lambert and M. H. Guindre
Marseille, Marnes La Coquette, F

Objectives: Toxoplasmosis, caused by *Toxoplasma gondii* and usually benign, can be severe following transplacental contamination (congenital toxoplasmosis) and in immunocompromised patients. Serologic testing is the primary tool for toxoplasmosis screening and diagnosis. The aim of this study is to compare sensitivity and specificity of Platelia Toxo IgG, to the IMX Toxo IgG test in the conditions of use of a hospital laboratory.

Methods: Prospective study: 787 samples destined to be done routinely were simultaneous tested when received, all the samples coming from either pregnant women, newborns or immunocompromised patients. Retrospective study: 162 samples were analyzed, 48 serums from 14 seroconversions, 14 cord blood samples from infected newborn and 100 cord blood samples from healthy newborn whose mothers had a negative serology during the pregnancy. Discrepant results were resolved with dye test. The tests were performed following the manufacturers' recommendations.

Results: Prospective study: no false positive sample on 787 random samples was observed, that confirmed the specificity of 100%. Sensitivity study gives a very good result of 98.2%. The predictive positive value of the test is 100.0% for a confidence interval of 95% (98.3–100.0) and the predictive negative value 99.0% for a confidence interval of 95% (97.5–99.6). Retrospective study:

- Seroconversions: in some cases, we observed a delay on specific IgG detection compared to IMX technique. This delay is less than 1 month in four cases and between 1 and 2 months in two cases. This weakness is compensated by the precocity of detection of specific IgM with Platelia Toxo IgM TMB, a very recent contamination being confirmed on all these samples by the high titer of specific IgM. The choice of a positive cut off at 9 IU/mL and of a gray zone from 6 to 8 IU/mL seems to be perfectly justified.
- Cord blood samples: no discrepancy was observed on the cord blood samples from healthy newborns, all samples being found negative with Platelia Toxo IgG TMB, nor on the ones from newborns with congenital toxoplasmosis, all being found positive with Platelia Toxo IgG TMB.

Conclusion: This test, along with Platelia Toxo IgM TMB is perfectly appropriated for toxoplasmosis screening and diagnosis.

P1244 Low incidence of *Toxoplasma* infection during pregnancy and in newborns in Sweden

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Stockholm, S

Objectives: To estimate the burden of disease due to congenital toxoplasmosis in Sweden, the incidence of primary infections during pregnancy and birth prevalence of congenital toxoplasmosis in 40 978 children born in two regions in Sweden was determined.
Methods: Women possibly infected during pregnancy were identified based on: (1) detection of specific IgG based on neonatal screening of the PKU card blood spot followed by retrospective testing of stored prenatal samples to detect women who acquired infection during pregnancy and follow up of their children to 12 months; (2) detection of specific IgM on the PKU blood spot.
Results: The birth prevalence of congenital toxoplasmosis was 0.73/10.000 (95% CI: 0.15–2.14). The incidence of primary infection during pregnancy was 5.1/10.000 (95% CI: 2.6–8.9) susceptible pregnant women. The seroprevalence in the southern part was 25.7% and in the Stockholm area 14.0%.
Conclusions: The incidence of infection during pregnancy was low, as the birth prevalence of congenital toxoplasmosis. Neonatal screening warrants consideration in view of the low cost and feasibility.

P1245 Serum profile of IgG and IgM antibodies towards *Toxoplasma gondii* in reproductive-age women: a laboratory view, 1995–1999

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Introduction: Toxoplasmosis, a disease of global dispersion, is caused from protozoan *Toxoplasma gondii*. With the normal transmission through placenta – from the pregnant woman to the embryo – causes congenital toxoplasmosis with con results, polymorph and is often fatal for the embryo.
Aim: The detection of specific IgG and IgM abs towards *T. gondii* in 18–40-year-old women who were examined in our laboratory before and during pregnancy (there was no clinical picture of toxoplasmosis in any of the cases). Also there was no patient with AIDS; neither under chemotherapy or immunorepressive therapy.
Material and method: We examined serums from 8100 women for IgG and IgM abs towards *T. gondii* with ELISA and direct agglutination and under suspicion of acute toxoplasmosis and for IgA anti-*Toxoplasma*.
Results: See table.

$IgG^{(-)}$, $IgM^{(-)}$	$IgG^{(+)}$, $IgM^{(-)}$	$IgG^{(+)}$, $IgM^{(+)}$	$IgM^{(+)}$, $IgG^{(-)}$
5.151	2.850	97	2
63.59%	35.18%	1.2%	0.03%

Conclusion: Although in our samples, an important percentage of women have been infected from this parasite, the small percentage (1.2%) that corresponds in recent infection is of great importance, because of the serious consequences for the embryo. Therefore, there is a necessity of control during pre-pregnancy period.

P1246 IgG avidity in acute symptomatic toxoplasmosis: multicenter evaluation of the reliability of five commercial kits

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Objective: Primary toxoplasmosis in gestation poses a hazard as it can be transmitted to the unborn child with severe sequelae. Gestational age (GA) at contamination is a critic factor, as there is a shift in transmission rate after the 10th week towards increasing incidence, while clinical severity decreases with GA. IgG avidity is reported as a useful test to date maternal contamination when seroconversion cannot be documented; furthermore, interassay variability among commercial kits is far to be defined. The clinical usefulness of five commercial kits in differentiating acute from latent toxoplasmosis has been assessed in a multicenter study.
Methods: Overall 131 serum samples were obtained from 54 patients with clinical and serological diagnosis of acute symptomatic toxoplasmosis and 49 control patients with clinical and serological diagnosis of chronic or latent toxoplasmosis according to routine confirmatory tests in collaborating centers. All of them were retested for IgM presence and absence, respectively, by using MEIA IgM Abbott, Vidas ELFA IgM BioMerieux, IFI IgM BioMerieux and for IgG pattern by time by using ELFA IgG, BioMerieux. IgG avidity test has been carried out by R.V. by using BioMerieux, Bouty, Dasit, Diesse and Radim commercial kits according to manufacturers' instructions in a single session.
Results: All but one (Radim) of the commercial tests seemed comparable in defining latent phase of the infection; in fact, high IgG avidity has been found in 98–100% chronic phase patients. False low IgG avidity results were shown in 0–1 of 49 control patients. Unfortunately, worse concordance has been shown among test kits in discriminating acute phase patients, as 64.3–100% of the tests carried out displayed low.
Conclusions: Most of commercial kits to carry out IgG avidity against *Toxoplasma gondii* showed comparable clinical usefulness to exclude a contamination occurring less than 3–4 months before the sample has been taken. Unfortunately, clinical effectiveness of low IgG avidity in discriminating acute infection seemed questionable, at least using some commercial kits.

P1247 Use of IgG avidity analysis versus IgA-specific detection for serodiagnosis of toxoplasmosis

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Objectives: The ability to discriminate between recently acquired *Toxoplasma gondii* infection and infection that occurred prior to pregnancy is crucial during the first trimester of pregnancy. As IgM antibodies may remain for more than 1 year after initial infection, it is necessary to use other methods to determine when the infection occurred. Detection of acute stage specific antigen is useful, but the reagent is not commercialized. Detection of specific IgA more or less IgE and IgG avidity analysis are possible in a not specialized laboratory. In the first part of this work, we assess IgG avidity analysis on sera with specific IgG and IgM. In the second part, we compared the ability of IgG avidity versus specific IgA detection to discriminate recently and ancient acquired toxoplasmosis.
Methods: A total of 220 routinely sera studied with Platelia Toxo IgG and Platelia Toxo IgM (Biorad) with specific antitoxoplasmosis IgG and IgM were classified as chronic (170 sera) or recent (50 sera) toxoplasmosis and IgG avidity analysis done with an adaptation of Platelia Toxo IgG. For 174 of these sera, the detection of specific antitoxoplasmosis IgA was made by immunosorbent agglutination assay (personal antigen).
Results: The study of the 220 classified sera showed that an avidity index of 0.45 or more was inconsistent with recently acquired toxoplasmosis. A total of 139 sera (82%) from the 170 classified as chronic toxoplasmosis had an avidity index more than 0.45. For the 174 sera with data of IgA and avidity, 75 sera (43%) are IgA-negative, considered as chronic toxoplasmosis (but two are from acute toxoplasmosis, without IgA); 110 sera (63%) have an avidity index more than 0.45, considered as chronic infection. In our data, 58 sera with specific IgA are chronic infection.
Conclusions: IgG avidity analysis is easily done with the Platelia IgG Toxo (Biorad). For sera with specific IgG and IgM at screening, IgG avidity is more contributive than IgA-specific detection to assess the patient as chronic toxoplasmosis. In our laboratory, we will change our algorithm, doing first avidity for pregnant women with specific IgG and IgM. IgA detection will be done in second intention for sera with low avidity. Further studies are necessary to simplify the avidity protocol which, actually, requires many dilutions for one serum.

P1248 Comparison among different strategies for microbiological diagnosis of *Toxoplasma gondii* infection in pregnant women, fetuses and newborn infants

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Objectives: Case definition of maternal or congenital toxoplasmosis is still problematic. Chemotherapy during pregnancy may modify the serological status and affect the detection of parasites in body fluids. The aim of this study was to evaluate the different strategies for microbiological diagnosis of *Toxoplasma* infection in pregnant women, fetuses and newborn infants.
Methods: Eighty-three pregnant women, two HIV positive, and their newborns were evaluated from 1996 to 2000 for suspected *Toxoplasma* infection. Specific IgG, IgM, IgA and IgG avidity were detected in maternal serum samples at different times during pregnancy. In newborns, specific IgG, IgM, IgA were evaluated at birth and monthly until negativization. A total of 54 amniotic fluids, 9 placentas, 32 cord blood samples and 15 urine samples from neonates were assayed by PCR for detection of *Toxoplasma* DNA *B1* gene. Thirty-one biological samples were assayed at Istituto Superiore di Sanità (Rome, Italy) for parasite culture and DNA detection by PCR-targeting *P30* gene and rDNA.
Results: Six newborns with congenital infections, classified as definite according to the Lebech et al. classification system, were identified; two were from HIV-positive mothers. All newborns were asymptomatic, but one manifested chorioretinitis. Serological markers of congenital infection were not detectable at birth when the mother had undergone chemotherapy during pregnancy, but serological rebounds were observed when the postnatal treatment was interrupted. In contrast, IgM and IgA were positive in infected newborns of untreated mothers. In one case, *Toxoplasma* DNA was detected in cord blood; the mother was HIV positive. All other biological samples (cord or peripheral blood, urine, placenta) resulted negative, even in presence of congenital infection. The mothers of infected newborns seroconverted during pregnancy, while no case of congenital infection was observed when stable titers of IgG and high or intermediate values of IgG avidity were obtained.
Conclusions: Treatment of mother and newborn improves the outcome of infection with increased asymptomatic congenital toxoplasmosis. This study supports the validity of serological and molecular strategies for diagnosis of *Toxoplasma* infection in pregnant women, but it points out the complexity of neonatal diagnosis. Serological monitoring of the newborns up to 1 year of age appears the most reliable procedure in the absence of suitable, not invasive biological samples.

P1249 Diagnosis of toxoplasmosis on spontaneous abortion by immunohistochemistry

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Objectives: To evaluate immunohistochemistry method for diagnosis of spontaneous abortion caused by toxoplasmosis based on sensitivity and specificity.
Methods: The study was conducted as a cross-sectional study at the Department of Obstetric and Gynecology, Sardjito Hospital, Yogyakarta, Indonesia, during May 1999 till April 2000. A total of 70 pregnant women with spontaneous abortion were included in this study. The inclusion criteria were as follows: the women had risk factors of toxoplasmosis during the first or second trimester and were willing to participate by putting signed in inform consent. An amount of 1 mL of each subject had been taken for detection of antibodies. The presence of IgM and IgG antibodies were detected by Toxo ISAGA and Toxo Screen-DA kit (BioMerieux), respectively. One gram of abortion tissues were collected from each subject and processed by standard methods, also subsequently embedded in low-melting paraffin. Sections of approximately 6 microns in thickness were cut and stained by immunohistochemistry using monoclonal antibodies against RH-strain of *Toxoplasma gondii*. Data were analyzed using sensitivity and specificity of Herrman formulas (1995).
Results: The results of detection of antibodies showed that eight (11.4%) out of 70 subjects had both IgM and IgG in their sera, and 33 (47.1%) had only IgG. The results of detection antigens by immunohistochemistry showed that 35 (50%) out of 70 subjects had positive antigens in the abortion tissues. Thirty-three (47.2%) out of 70 subjects had positive antibodies in the sera and positive antigens in the abortion tissues. Eight (11.4%) subjects had positives antibodies in the sera, but negative antigens in the abortion tissues. Two (2.8%) subjects had negative antibodies in the sera, but positive antigens in the abortion tissues. The sensitivity and specificity of immunohistochemistry diagnostics were 80.5 and 93.1%, respectively. The positive predictive value and negative predictive value were 94.3 and 77.1%, respectively.
Conclusions: Immunohistochemistry method has a high sensitivity and specificity, therefore serological diagnosis for spontaneous abortion caused by toxoplasmosis must be confirmed with immunohistochemistry.

Bordetella

P1250 The sero-epidemiological survey of immunity against pertussis in adult population in Poland

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Introduction: In Poland during 1997–2001 an unexpected increase of pertussis incidence was observed with the highest numbers in older children and adults. Immunity after primary vaccination lasts only few years. There is no effective currently available vaccine against pertussis used for booster in adults. In the first step of the program, base-line serological status against *Bordetella pertussis* should be assessed.
Objective: To evaluate the serological status of healthy adult population measured by antibody titer against pertussis toxin IgG-PT and IgA-PT.
Materials and methods: Blood samples from healthy adults were collected after informed consent. Pertussis antitoxin IgG and IgA were tested in total of 184 blood samples by ELISA calibrated by the WHO standard.
Results: The highest percentage of persons with IgG-PTAb < 50 EU/mL (70%; 42/60) and the lowest GMT for IgG-PTAb was observed in group of 35–50 years (31.8 EU/mL). The highest titer of IgA-PTAb was found in persons above 35 years of age. Detailed results are shown in the Table 1.

Table 1 GMT of antibody against pertussis toxin IgG-PTAb and IgA-PTAb in study population

	Age		
GMT [EU/ml]	19–34 years	35–50 years	>50 years
IgG	39.4	31.8	35.7*
IgA	76.8**	124.8**	130.9**

*ns: NOT significant.
**$P < 0.05$.

Conclusions: In individuals above 35 years of age high IgA-PTAb with rather low IgG-PTAb antibody titers were observed. These findings could reflect natural contacts with *Bordetella pertussis*. It suggests that booster immunization in adults should be considered.

P1251 Relative role of pertussis among acute respiratory infections in adults

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Objective: To assess the relative proportion of infection with *Bordetella pertussis* and *Bordetella parapertussis* in prolonged coughing in adults aged more than 18 years.
Methods: Patients were recruited from sentinel physicians (general practitioners) in Krefeld, Germany. The main inclusion criterion was coughing for more than 7 days. Clinical information was gathered by questionnaires. For diagnostic purposes, nasopharyngeal swabs, pharyngeal washings, and peripheral blood were sampled from every patient. Direct detection of antigen or nucleic acid (PCR) was done for *B. pertussis*, *B. parapertussis*, adenovirus, influenza virus A and B, parainfluenza viruses, and respiratory syncytial virus (RSV). IgA/IgM and IgG antibodies against *B. pertussis* antigens, adenovirus, *Chlamydia pneumoniae*, coxsackie virus, ECHO-virus, influenza virus A and B, *Mycoplasma pneumoniae*, parainfluenza viruses, and RSV were measured by ELISA.
Results: 143 patients were recruited, with a continuous age distribution between 18 and 80 years; 58% were female and 42% were male. They were coughing for a mean of 24 days. Positive laboratory results were obtained in 27% for adenovirus, 25% for influenza virus A/B, 20% for RSV, 11% for Bordetellae, 10% for parainfluenza viruses, and 10% for *C. pneumoniae* infection. Signs for infections with more than one agent were frequent, and serological diagnosis of *M. pneumoniae* infection was hampered by polyclonal stimulation.
Conclusions: *Bordetella* infections were one of the frequent causes for prolonged coughing in adults in this German population.

P1252 Investigation of the population structure of *Bordetella pertussis* in the UK: a comparison of pre- and post-vaccination strains

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Objectives: To investigate the dynamics of the UK *B. pertussis* population using IS1002-based restriction fragment length polymorphism (RFLP), by comparing pre- and post-vaccination isolates.
Methods: A total of 47 UK clinical isolates isolated between 1940 and 1999 were analyzed. The number of isolates, grouped by decade was as follows: 1940s (12), 1950s (12), 1960s (12), 1990s (11). Primers targeting the IS1002 were used to generate digoxigenin-labelled PCR products. Genomic DNA was digested using SmaI and the products separated by field-inversion gel electrophoresis together with molecular size markers. DNA was transferred onto nylon membranes and probed with the labeled PCR products. Immunological detection of hybridized products was performed using the DIG DNA Detection Kit. Developed Southern blots were scanned and analyzed using BioNumerics (Applied Maths).
Results: IS1002-RFLP analysis of all 47 isolates resulted in the detection of 4, 5 or 6 bands. The 47 strains produced 15 distinct RFLP patterns, 4 of which were present in more than one decade. Six different patterns were seen in the 1940s and 1950s, seven in the 1960s and four in the 1990s. RFLP pattern 1 dominates the *B. pertussis* population, occurring in 21/47 isolates, is seen in 2/12 of the 1940s strains, 7/12 in the 50s, 5/12 in the 60s, and 7/11 of the 1990s.
Conclusions: Previous studies in the Netherlands indicate that genetic diversity has decreased after vaccination was introduced in 1953, reflected in the reduction of frequency of RFLP types over time. Our preliminary data can neither support nor refute that this has occurred in the UK. However, since the introduction of vaccination in the UK in 1953, the predominant RFLP pattern 1 has increased in the *B. pertussis* population from 2/12 in the 1940s to 7/11 in the 1990s. This RFLP pattern, also found in Germany and North America, has increased in the Dutch *B. pertussis* population following vaccination. This clonal expansion of *B. pertussis* strains is suggested to be due to vaccine-driven evolution. It has also been hypothesized that clonal expansion of *B. pertussis* strains that are antigenically different from the vaccine strains is responsible for the occurrence of pertussis epidemics. IS1002-RFLP and other methods allow the analysis of the population structure of *B. pertussis* over time, and may give warning or aid in the investigation of epidemics and outbreaks.

P1253 *Bordetella pertussis* adenylate cyclase toxin: a potential epidemiological marker and diagnostic tool for pertussis infection

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Background: Adenylate cyclase toxin (ACT) is an essential virulence factor of *B. pertussis* and a protective antigen in mouse models of pertussis infection. As well as being a possible component of future acellular pertussis vaccines, it is also being investigated for its use in multipurpose vaccines, as a vehicle for intracellular delivery of foreign epitopes to antigen presenting cells. Before the toxin can be used in such applications its properties should be fully characterized, including its potential for antigenic variation. The presence of the AC enzymic activity in clinical specimens from pertussis-infected individuals has been used in diagnosis of pertussis. Conventional assays for AC use radioisotopes and are time-consuming and expensive. A novel conductimetric assay that is rapid and inexpensive has been developed to detect AC enzymic activity.
Objectives: To determine possible sequence variation in the region of the *cyaA* gene encoding the C-terminal region of ACT of *B. pertussis* and develop a rapid conductimetric assay of AC enzymic activity for pertussis diagnosis.
Methods: Twenty clinical isolates of *B. pertussis* were selected from the period 1920–1999. Oligonucleotide primers were designed to target two areas of the *cyaA* gene encoding the immunogenic C-terminal region of the protein and PCR products of 570 and 620 bp were produced. Nucleotide sequences were determined using an automated DNA sequencer (CEQ 2000 XL Beckman Coulter). Conductimetric assay involving a pyrophosphatase-coupled reaction and a simple apparatus for measuring changes in the associated electrical conductance have been developed. This assay has been optimized for the detection of low levels of recombinant AC enzymic activity.
Results: Amplified products of expected size were obtained with all 20 clinical isolates and reference strain. No sequence variation was evident in the two regions examined compared to the available sequence data. Simulated clinical samples with different numbers of *B. pertussis* added (wild-type strain 18323 and BP348 pRMB1 were used), different lysis methods for release of AC enzyme, and the need for an enrichment step prior to assay have been investigated.
Conclusions: Preliminary results indicate that there is little or no variation in the *cyaA* gene of *B. pertussis* isolates and thus it will not be a useful target for genotyping this organism. The potential of the conductimetric assay as a diagnostic tool will be reported.

P1254 Native and genetically inactivated pertussis toxins are potent inducers of human monocyte-derived dendritic cell maturation and secretion of type 1 cytokines: implications for induction of protective immune responses

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Objectives: Pertussis toxin (PT) is a critically protective antigen present in all pertussis vaccines, but the immunological mechanisms underlying protection have not yet been elucidated. Since dendritic cells (DC) exert a crucial role in the induction and regulation of immune response, we investigated the capacity of PT to induce maturation and function of human monocyte-derived DC and to act as adjuvant in LPS induced DC maturation.
Methods: Immature DC were obtained by colturing monocytes in presence of IL-4 and GM-CSF for 6 days. DC maturation was studied by cytofluorometric analysis and antigen presentation ability in MLR. The cytokine production was assessed by quantitative RT-PCR, ELISA, cytometric bead array, and intracellular staining. Th1/Th2 polarization was studied by using naive cord blood T cells.
Results: Both the native (nPT) and a genetically detoxified (dPT), but not a heat-inactivated PT, efficiently promoted the expression on DC of CD80, CD86, HLA-DR and CD83 markers, the alloreactive antigen presentation and the cytokine production. In particular, both nPT and dPT, while not affecting IL-10 production by LPS-stimulated DC, strongly synergized with LPS for IL-12 production. DC stimulated by PT plus LPS secreted soluble factors fostering IFN-gamma but not IL-4 and IL-5 production by naive T cells. This Th1 polarization, as well as the alloreactive antigen presentation, was markedly inhibited by anti-IL-12 mAb.

Conclusion: Overall, the findings support the notion that nPT is a potent Th1 adjuvant, and that dPT has fully preserved this adjuvanticity. The synergic interaction between PT and LPS in IL-12 production might be relevant for the mechanisms of pertussis vaccine-induced protection.

P1255 Isolation of *Bordetella pertussis* and *B. holmesii* from blood cultures of three patients in the UK and identification using SSU rRNA gene sequencing

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Background: Bacterial isolates from blood cultures obtained from three patients were referred for identification. patient 1 was a 75-year-old immunocompromised female; patient 2, was a 53-year-old male with endocarditis, fever and rigors; and patient 3, was a 39-year-old female with fever and abdominal pain. Preliminary tests indicated that all isolates belonged to the genus *Bordetella*.
Objectives: To obtain a definitive identification of the three isolates.
Methods: Isolates were subcultured onto blood charcoal, Columbia blood, MacConkey, and nutrient agar plates and Gram stains were performed. Fatty acid analysis was performed using the Sherlock Microbial Identification System (MIS). Genomic DNA extracts were prepared, PCR performed using primers targeting conserved regions of ribosomal DNA (rDNA) and amplicons cloned and sequenced. Sequence data was assembled, aligned manually, and phylogenetic analyses performed.
Results: All three isolates grew on blood charcoal, Columbia blood and nutrient agar and were small Gram-negative coccobacilli. Only two isolates (from Patients 2 and 3) grew on MacConkey agar. Fatty acid results yielded scores of 0.698, indicating identification as *B. pertussis* (patient 1), 0.496 (patient 2) and 0.263 (patient 3) indicating identification as *B. avium* from the latter. DNA sequence analysis confirmed the identity of isolate from patient 1 as *B. pertussis* (100%) and those from Patients 2 and 3 as *B. holmesii* (99.9–100%).
Conclusion: Definitive identification of all three isolates was achieved using SSU rRNA gene sequencing. In all three cases, the contribution of *Bordetella* spp. in causing disease cannot be determined. However, further investigation of the role of *Bordetella* spp. in disease, particularly when isolated from nonrespiratory sites, is clearly warranted. Preliminary studies using PCR have shown that *B. pertussis* maybe carried by asymptomatic people. The prevalence of *B. holmesii* carriage is unknown, although in one study active surveillance for *B. pertussis* by culture resulted in an increase in isolation of *B. holmesii*. *B. holmesii* is capable of causing severe infection in both immunocompromised and immunocompetent hosts. *B. holmesii* has been previously isolated from patients with splenectomy or endocarditis. In this investigation patient 2 has endocarditis and patient 3 had previously had a spenectomy, indicating that these conditions may predispose to infection with this organism.

P1256 Detection of *Bordetella pertussis* virulence factor pertussis toxin gene variants using LightCycler™ real-time PCR and fluorescence resonance energy transfer hybridization probe melting curve analysis

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Objectives: Antigenic variation in two important virulence factors of *Bordetella pertussis*, pertactin and pertussis toxin (PT), has been reported. The polymorphism in the PT operon is mainly found in the gene encoding the S1 subunit. Four alleles have been described, namely S1A, S1B, S1D and S1E. Most of the recent isolates represent S1A. So far, the only method to determine these alleles has been PCR-based sequencing, a relatively time-consuming and expensive method. Here we describe the rapid detection of PT gene variants by LightCycler™ real-time PCR and fluorescence resonance energy transfer (FRET) hybridization probe melting curve analysis.
Methods: A total of 57 Finnish clinical isolates, two vaccine strains and one reference strain (18 323) representing PT S1A, B, D and E alleles were tested. Bacterial genomic DNA was used. The primers and probes were designed on the basis of the published sequences and synthetized by TIB Molbiol, Berlin. The probes QJ 3 BDE and QJ 4 BDE are specific for alleles S1B, S1D and S1E. The probes QJ 5 E and QJ 6 E are specific for S1E and thus differentiate S1E from S1B and S1D. To each run a negative control, without template DNA was included. To the runs using the QJ 3 BDE and QJ 4 BDE probes, DNA extracted from strains representing S1A, B, D and E was included. To the runs using QJ 5 E and QJ 6 E probes, DNA from strain representing S1E was included. The amplification reactions and the FRET hybridization probe melting curve analysis were carried out in LightCycler™ (Roche Diagnostics GmbH, Mannheim).
Results: The probes QJ 3 BDE and QJ 4 BDE correctly hybridized to and gave signal for the PCR products derived from strains harboring S1B, S1D and S1E alleles. The probes QJ 5 E and QJ 6 E hybridized to and gave signal for the PCR products derived from the strains representing S1E allele. The specific mean of melting temperature (T_m) for QJ 3 BDE and QJ 4 BDE was 66.43 °C (SD 0.41). The specific mean of T_m for QJ 5 E and QJ 6E was 66,43 (SD 0,17). All intra- or interassay variations of T_m for both sets of probes were low ($\leq$0.29 °C).
Conclusions: The assay correctly identified the PT gene variants. The assay was simple, rapid (both assays can be carried out within 2 h) and reliable when compared to sequencing and is therefore suitable for large scale screening of *B. pertussis* clinical isolates.

P1257 Isolation and characterization of *Bordetella bronchiseptica* strains from clinical health workers

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Bordetella bronchiseptica is a common pathogen of the upper respiratory tract in many animals species and causing diverse illnesses. In humans this microorganism has been isolate from immunosupressed individuals or with other pathologies but in each case the individuals had been in touch with domestic animals. So, the aim of this work was the isolation of *B. bronchiseptica* strains from healthy people who had no contact with domestic animals for at least 6 months prior to the studies.
Methodology: Pharyngeal swabs from individuals with the characteristics described above and from dog breeders were grown onto MacConkey agar. Biochemical and serological tests with antiserum against *B. bronchiseptica*, *B. pertussis* and *B. parapertussis* were employed. The PCR technique was used in order to identify *B. bronchiseptica* with DAL 1 and DAL 3 as primers. After *B. bronchiseptica* strains were identified we probed for antibiotic resistance and the assay of crystal violet stain was used in order to identify if the strains were in phase I or not; red blood cells were also used from bovine, equine and sheep in blood agar 5% for hemolysis test. Dermonecrotoxin was detect in guinea pigs with raw extract of the bacterial suspension; and for pertactin detection immnunoelectrotransfer was used with two monoclonal antibodies (BB07 and BB05).
Results: We isolated four *B. bronchiseptica* strains from clinical health individuals, three strains from people without animal contact and 1 strain from dog breeder. The four strains were identified by biochemical, serological and PCR methods. The isolated strains were not in phase I. Neither expressed dermonecrotoxin or pertactin; also they had no lysed red blood cells from bovine and the antimicrobial susceptibility assay were similar among the strains.
Conclusions: Conditions settled for this work reduced the number of possible candidates to examine, nevertheless we isolated three stains from three individuals with the mentioned characteristics. It is important to say that titer antibodies of these three individuals were lower than dog breeders who did not yield *B. bronchiseptica*.

Isolated strains showed less pathogenicity, because they did not remain in phase I; also some virulence factors were not expressed and titer antibodies were low. These results show the possibility that *B. bronchiseptica* remained in the respiratory tract for a long time and when the individual suffered immunological abatement this microorganism came into view.

Tissue protozoans and *Pneumocystis*

P1258 Prevalence of *Plasmodium* among travelers and immigrants in a Madrid hospital

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Objectives: Study of the prevalence of imported malaria concerned in travelers and immigrants coming from endemic areas.
Methods: We studied 720 individuals from January 2001 to October 2001. All patients came from countries with high incidence in the disease (mainly Equatorial Guinea). The diagnosis was realized by microscopy with Field—stained thick and thin blood smears.
Results: We found 58 cases positives of malaria (8%). We obtained 47 cases of *P. falciparum* (81%), 7 cases of *P. ovale* (12%) and 4 cases of *P. vivax* (7%). None case of *P. malariae* was seen. The geographical distribution was the following: *P. falciparum* (46 from Equatorial Guinea and 1 from Conakry Guinea); *P. ovale* (6 from Equatorial Guinea and 1 from Cameroon); *P. vivax* (2 from India, 1 from Papua New Guinea and 1 from Ecuador).
Conclusions: As a result of the arise of travelers found in the last years, an increase between the cases of malaria has been observed. The result of our study show a prevalence of 8% of cases of imported malaria, therefore, we due to consider when a person has traveled to a zone with malaria and to make the correct diagnosis and later processing.

P1259 Imported malaria at an infectious disease unit in Vienna, Austria: a review of 332 cases between 1990 and 2000

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Objectives: To study the epidemiological, clinical and laboratory features in imported malaria, and thus increasing physician awareness of the variability in its presentation and improve clinical management and health outcomes.
Methods: Medical records were reviewed for patients with microscopically confirmed malaria diagnosed at our department between 1990 and 2000. Patients with relapsing or recrudescent infections were counted only once.
Results: Of a total of 332 cases, 208 were caused by *P. falciparum*, 94 by *P. vivax*, 13 by *P. malariae*, 5 by *P. ovale*, 11 patients had dual infection, and in 1 case plasmodia were unspecified. 78% of the patients were male, median age was 36 years (range, 14–79). 16% were classified as having severe falciparum malaria according to WHO criteria. There were 5 fatal cases, 4 of them were female. 94% of falciparum cases were acquired in subsaharan Africa, 6% in Asia, and 1 case in the Americas. *P. vivax* infection was acquired in Asia, Africa and the Americas in 62, 21 and 17%, respectively. 33% of the falciparum cases were immigrants, most of them returning from a journey to their country of origin. 61% of patients with falciparum malaria, and 50% of the vivax cases had taken no chemoprophylaxis at all. 100% of vivax cases, and 99% of falciparum patients gave a history of fever. 11%, however, were afebrile at the time of their presentation. While 85% of the falciparum cases fell ill within 2 weeks after their return to Austria, and none later than 2 months, 51% of the vivax cases developed symptoms later than 2 months after their return. Other commonly reported but unspecific symptoms were headache, shaking chills, and myalgia. Thrombocytopenia ($<140\,000/mm^3$) was the most prominent laboratory feature occurring in 79% of patients with counts $<100\,000/mm^3$ in 63%. Hemoglobin levels below 12 g/dL were observed in 41%, a subnormal Quick's test (<70%) in 65%. Leukopenia ($<4000/mm^3$) was noted in 46%, leukocytosis in 9%. 64% of the patients had an elevated LDH (>240 U/L), GPT (ALT) was elevated (>22 U/L) in 52%. 10% of the falciparum cases had hyperparasitemia, 13 of these patients had clinical jaundice, 11 cerebral malaria (*sensu lato*), 7 showed signs of ARDS, 6 acute renal failure, 5 had DIC and 5 MOF. 12 patients with falciparum malaria required treatment at the ICU.
Conclusion: Malaria has to be ruled out or proven promptly in any symptomatic patient with a history of travel to a malaria-endemic area.

P1260 Presumption of malaria infection by using the Cell-Dyn 4000 hematology analyzer

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Objective: Malaria mortality in nonendemic areas of the world, where malaria is a relatively rare disease, is almost always due to a missed or late diagnosis. Clinicians may not initially consider malaria as part of the differential diagnosis of pyrexia (especially if the clinical/travel history is inadequate) and may only institute general screening tests such as a Full Blood Count. A number of reports were received about unusual patterns of dots in the automated leukocyte differential plots obtained on the Cell-Dyn 4000 analyzer (Abbott Diagnóstics, Santa Clara, CA) from patients with malaria. The purpose of our work was to go to this potential method for diagnosis of malaria.
Methods: We studied peripheral blood samples, collected on EDTA tubes, from 40 patients who had active malaria. Samples are processed in parallel using Field stain of thick and thin smears as reference method and by the Cell-Dyn 4000 hematology analyzer. The three principles used by the Cell-Dyn 4000 (impedance, light scatter and fluorescence) allow to have a complete cell count of white and red blood cells, including reticulocytes (via RNA detection) and leukocyte viability index obtained by DNA detection.
Results: The main abnormal patterns of greatest relevance to malaria were when either populations of purple and/or blue leukocyte events (representing neutrophiles on normal samples) depolarize light, or when the green-coded depolarizing events fall outside of the normal eosinophil 'footprint'. Other findings consist on DNA material detection not associated to any cell fragility phenomenon and a reticulocyte distribution showing a small peak corresponding to infected reticulocytes.
Conclusion: Due to the increasing flow of travelers and immigrants coming from endemic areas with malaria, cytometric methods should be considered as a promising tool in malaria diagnosis. Thus, it can be very significative in those nonendemic areas where clinicians are not aware of this disease and the diagnosis could be delayed or even missed.

P1261 Status of malaria in Iranshahr county (Sistan and Baluchistan province, south-eastern of Iran) in the past decade (1991–2000)

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Malaria is a public health problem today in more than 90 countries, inhabited by a total of some 2400 million people—40% of the world's population. Malaria is one of the 10 most prevalent and deadly disease in the world. About 300–500 million clinical cases occur every year with over 1.2–1.7 million deaths. Relative risk of acquiring malaria in Iran is below 1:50 000. Over the past 80 years in Iran, numerous researchers have worked on malaria. The disease has become well controlled in the most of the country only in the south-eastern corner of Iran. This area includes provinces of Sistan and Baluchistan, Hormozgan, the tropical areas of Kerman, and is characterized by 'refractory malaria'. Iranshahr county has a high morbidity of this disease in Iran. A retrospective study on malaria cases of the last decade was conducted in order to definite the status of the disease in Iranshahr county. According to the results 'API' decreased from 85.73 in 1991 to 29.11 in 1994, following a peak about 67.80 in 1995 decreased again to 10.14 in year 2000. Trend of 'ABER' after a relative increase in the years of 1993–1994, decreased from 145.56 in 1991 to 19.35 in the year 2000. Except decreases in the years of 1993–1994, 'SPR' had a relatively constant trend and reached from 5.89 in 1991 to 5.24 in year 2000. Annual *Plasmodium falciparum* Index 'AFI' and Annual *Plasmodium vivax* Index 'AVI' had no much differences, but in the last 2 years of 1999–2000 this difference was higher. Notwithstanding the effective activities of the primary health care system that provoked notable reduction in trend of malaria cases, the disease control programs have not achieved their goals in this area. The following factors are considered responsible for this failure:
1 The partially exophilic habits of the main common vector of malaria—*Anopheles culicifacies*.

2 Existence of four secondary vectors (*A. d'thali, A. fluviatilis, A. superpictus, A. stephensi*) in addition of *A. pulcherrimus* as a doubtful vector.
3 The great dispersion of villages.
4 Low socioeconomic/health status of local communities.
5 High illiteracy rate.
6 Socialization of local communities with Pakistani neighbors.
7 The most Afghan immigrants (over 1 out of 6 of over 360 000 population number of Iranshahr).

P1262 Visceral leishmaniasis: difficult on treatment

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Objectives: The recognition of frequency and cause of difficult leishmaniasis to be treated.
Methods: Eighty patients of age 18–56 years were studied and observed during 1987–2001.
The treatment: Glucantim—61 cases, 80 mg/kg/die i.m. for 15 days; Lomidini—4 cases, 7 doses; Allopurinol—10 cases 25 mg/kg/die for 4 weeks and Glucantimi + Allopurinol—5 cases. The end of treatment was decided by parasitological criterion. We identified the frequency and cause of difficult cases for treatment and mortality rate.
Results: The difficulty of treatment was related to: (a) the etiological treatment. It failed on nine cases with Glucantime: four immediate deaths, one febrile vasculitis and four severe myocardiopathie; five cases with Allopurinol: five severe vasculitis; two cases with Lomidini: one death and one hypotension and renal insufficiency; 1 case with Glucantime + Allopurinol: severe vasculitis. The fever was normalized on the third day of treatment in 2.5%; on the fourth day 10%; on the fifth day 32.5%; on the sixth day 20%; on the seventh day 13.7%; on the eighth day 5% of the cases and after that in 13 cases 16.25% (the last resulted leishmania—like pathologies with leishmania in bone marrow aspiration). Continuing the follow up on 65 cases, we noted parasite presence after the first cycle in 7.46%, after second cycle in 20% and after third cycle its disappearance. (b) The necessity of supportive treatment on 15% of cases (antishock, antianemic, antiseptic). The mortality rate was 6.25%.
Conclusions: We did not seen leishmaniasies resistance toward etiological treatment, but a delay of its action after 6 days on 18.75% of cases. The Leishmaniasies 'difficult' to be treated were identified in 36.25%.

P1263 Epidemiology of cutaneous leishmaniasis (reservoir hosts and human infection) in Jarghooyeh rural district, Isfahan county, central Iran

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Objectives: This study was carried out to determine the reservoir hosts and human infection of cutaneous leishmaniasis in Jarghooyeh rural district, south-east of Isfahan county.
Methods: This study was conducted over a period of 12 months (from 4 April 1997 to 20 March 1998) in Jarghooyeh rural district, 60 km from south-east Isfahan county, central Iran. To study the characteristics of the human disease, four villages were selected, in each of which all the inhabitants were examined by house to house visit. The number and the places of scars and sores were noted for each individual. In each case with cutaneous lesion, scrapings were taken from the sores and examined for the presence of *Leishmania* parasites. Rodents were caught in 20 live traps, 15 day intervals. After capturing of rodents, smears were prepared from the edge of ears. All smears were stained by Giemsa by routine methods and examined under a light microscope.
Results: In four villages of the Jarghooyeh rural district, two species of rodents (*Rhombomys opimus* and *Meriones libycus*) were identified. *R. opimus* was the predominant species and its leishmanial infection rate was 6.6%. Study of prevalence among 5237 inhabitants showed a rate of 3.87% for ulcers and 42.84 for scars. Based on number of scars, 51.56% of previously affected people had only one scar, 22.5% had 2 scars, 10% had 3 scars and 15.94% had 4 scars or more. Hands, legs and face were the affected parts of the body, with 44, 33.8 and 13.8% of the active lesions, respectively.
Conclusion: These investigations showed that ZCL is endemic in this area. Active disease is usually found in childhood, and older people usually have scars of old lesions. The highest and least infection rate were observed in fewer than four and over 25-year-old age groups, respectively. There is a non-significant difference in disease based on sex ($P < 0.05$).

P1264 Recurrent visceral and disseminated cutaneous leishmaniasis caused by *Leishmania infantum* Mon-1 during HIV infection

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Introduction: Leishmaniasis is emerging as a common and serious opportunistic disease in patients with human immunodeficiency virus (HIV) infection in endemic areas, such as southern Europe. In HIV-infected patients this protozoan disease can occur with various clinical presentations, ranging from typical visceral forms to asymptomatic or atypical cases. A 32-year-old i.v. drug abuser male patient, with advanced HIV infection was first hospitalized owing to persisting hyperpyrexia, weight loss, asthenia, systemic lymphadenopathy, hepatomegaly, splenomegaly, pancytopenia, and hypergammaglobulinemia. His CD4+ lymphocyte count was 101 cells/mm^3, while plasma HIV viral load was 3000 copies/mL; ongoing antiretroviral therapy included zidovudine, lamivudine, and nelfinavir since 25 months. High titer (1:8192) anti-*Leishmania donovani* antibodies were detected by an indirect immunofluorescence assay, but our patient refused both bone marrow and liver biopsy. On the ground of a strong suspicion of visceral leishmaniasis, therapy with i.v. liposomal amphotericin B was administered for 21 days, leading to a complete clinical remission. Thirteen months later, our patient developed a fixed, nonevolving rash involving the chest, back and abdomen, with disseminated nonulcerate, nonscabby, maculo-papular lesions, painless and not pruriginous, approximately 3–8 mm in diameter. The histopathologic study, May Grunwald-Giemsa staining and culture of a skin biopsy specimen prompted a diagnosis of cutaneous leishmaniasis, and the zymodeme Mon-1 of *Leishmania infantum* complex was identified by an isoenzymatic characterization. A novel 28-day attack course of liposomal amphotericin B was administered, leading to a partial reduction of cutaneous lesions. Three months later, in spite of a secondary prophilaxis with the same drug associated with oral itraconazole, disseminated maculo-papular cutaneous lesions appeared again with initial features. Therapy with i.v. pentamidine isethionate was started and continued for 8 weeks, leading to a complete resolution of cutaneous rash.
Conclusion: Zymodeme Mon-1 of the *Leishmania infantum* complex is the most common causal agent of HIV-associated visceral leishmaniasis in the Mediterranean basin, and it could also rarely be responsible for localized cutaneous forms. However, disseminated cutaneous leishmaniasis sustained by Mon-1 zymodeme described in our HIV-infected patient is an exceedingly uncommon presentation.

P1265 A successful control program for zoonotic cutaneous leishmaniasis in a new focus of disease, Ardakan county, Yazd province, central Iran

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Objectives: An epidemic focus of zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* has established in Ardakan county, Yazd province, central Iran, during 1998–1999. Because of its public health importance, and the high risk of disease transmission to the other parts of the province or the country, which are susceptible for disease establishment, we decided to control ZCL with assistance of Yazd province public health center in 2000.
Methods: The control program was conducted in two phases: rodent control via poisoning with Klerat, and indoor residual spraying with Propoxur 50% WP 2 g/m^2.
Results: Our findings show a five-fold reduction in disease incidence in treated area, meanwhile we observed a 1.3 time increase in this rate in control area. The decrease of ZCL incidence was significant in treated area ($P < 0.0001$). In addition, incidence rate of disease in control area was significantly greater than that of the treated area ($P < 0.01$).

Conclusion: Based on this study and other similar surveys, the rodent control seems to be an effective method for ZCL control. Although, indoor residual spraying can help the reduction of cases in epidemics.

P1266 Cutaneous and visceral leishmaniasis in Aydin, Turkey, January–November, 2001

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Objectives: Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) are endemic in the western and south-eastern parts of Turkey. The aim of this study was to determine clinical and demographic characteristics of VL and CL.
Methods: The records of patient with VL and CL of Central Healthy Service of Aydýn and Adnan Menderes University Medical Faculty have been evaluated retrospectively.
Results: Forty-five patients were found infected with CL and four patients had VL in Aydýn. Rate of sex in patients' who had CL was in female 57.8% and male 42.2%. CL was more frequent in the age group of 0–19 years (40%) (range 1–81 years). Four patients' who had VL ages ranged from 1 to 12 years. When the number and localization of CL lesions evaluated, it has been found that 21 (46.7%) cases had one lesion, 11 (24.4%) cases had two lesions and 13 (28.9%) cases had three or more lesions. The face was the most frequent site of involvement (53.3%). The incidence rate while minimum in August to November (2.2%), increased to maximum of 84.4% in January–April.
Conclusion: Interestingly, there was both of VL and CL in Aydýn area and leishmaniasis may still continue as endemically. For a successful fight against Leishmaniasis, it is necessary to control vectors and reservoirs also to detect and record the clinical and demographic characteristics carefully.

P1267 Field trial for the control of zoonotic cutaneous leishmaniasis in Badrood, Iran

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Tehran, IR

Objectives: Due to the resurgence of zoonotic cutaneous leishmaniasis (ZCL) in some nonendemic areas of Iran, extensive studies have been conducted on the epidemiology of the disease in different parts of the country in recent years. The objective of this study was to determine the effects of rodent control on the reduction of the incidence of ZCL in an endemic area in Badrood, Iran.
Methods: A survey was carried out in two villages, in a circle around the villages during April 1997–January 1999. The control strategy adopted in 1997 consisted of destruction the colonies of gerbilline rodents by digging in a radius of 500 m from houses in the intervention area. Opened burrows were baited with zinc phosphide. One village was kept as control. Evaluation was made in 1998 and no other control measures was carried out in the area. Case finding was done by house-to-house visit once in every season during 1997–1998 and all of the inhabitants of the selected villages were examined.
Results: The average reduction of rodent holes was calculated 87.4% 1 year after the first baiting in the intervention area. Changes in the number of rodent holes and the incidence of the disease in the intervention and control villages were statistically significant (P-value < 0.000001).
Conclusions: Our evaluation demonstrated that, the program reduced the incidence of ZCL 12-folds in treated village when compared with that of the control one at the end of the first year of operations and more than one-fifth of its original level through 2 years. The results show that, the method is very effective for destroying rodents and reducing the incidence of ZCL in a small scale and special circumstances.

P1268 Investigation of *Pneumocystis carinii* trophozoites and cysts and *Cryptosporidium parvum* oocysts in respiratory tract specimens of patients with pulmonary disorders

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P. carinii is a unicellular eukaryotic organism causing serious opportunistic pneumonia in immunosuppressed hosts, specially with AIDS and in cancer and transplant patients. Pulmonary colonization in HIV-negative patients and carriage in immunocompetent patients with underlying pulmonary disease may also exist. *C. parvum* is a protozoan of serious pathogenicity, the cause of gastrointestinal and extraintestinal infections involving lungs, bile ducts, pancreas in immunocompromised patients including AIDS, cancer and transplant patients who are receiving immunosuppressive therapies and also rarely in immunocompetent hosts. Asymptomatic carriers are considered to have an important role in establishing infection. We investigated the presence of *P. carinii* trophozoites and cysts and *C. parvum* oocysts in bronchial lavage ($n = 50$) and sputum specimens ($n = 69$; total $n = 119$) from patients ($n = 90$) with pulmonary disorders between 28 June and 28 November 2001. Defined major symptoms includes chronic cough (6), fever (2), dyspnea (3), hemoptysis (6), sputum secretion (75). Radiological abnormalities were found in all patients. The patients did not receive antifungal and/or anti-*P. carinii* prophylaxis or treatment before obtaining the specimens. Direct microscopical examination was performed from fresh specimens using acid fast (Ziehl-Neelsen and Kinyoun) and Giemsa staining. Direct immunofluorescent antibody detection (Meriflour *Pneumocystis carinii*; Meridian Diagnostics, Inc.) procedure was also used in sputum specimens and the results were assessed under fluorescent microscope. The underlying diseases and predisposing factors were lung and other organ cancers ($n = 69$), past or active tuberculosis ($n = 9$) Hodgkin lymphoma ($n = 1$) rheumatoid artritis ($n = 2$), Behçet's disease ($n = 1$) *Pamphigus vulgaris* ($n = 1$) long-term and large spectrum antibiotic usage ($n = 37$) corticosteroids ($n = 13$). Age range was16–85, median age was 66. Neither *P. carinii* trophozoites and cysts, nor *C. parvum* oocysts were found in the present study. This result is in parallel with the very low *P. carinii* frequency found in two previous study and one pulmonary cryptococcosis case reported in Turkey. Pneumocystosis and cryptosporidiosis have become emerging life-threatening infections in immunocompromised individuals. They can be detected with routine techniques used for identification of mycobacteria and fungi in respiratory secretions and lavage specimens so their investigation may be useful in patients at risk.

P1269 *Pneumocystis carinii* pneumonia in HIV seronegative oncologic patients

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Objectives: To assess the incidence and the predisposing factors of *Pneumocystis carinii* pneumonia (PCP) in oncologic patients without the acquired immunodeficiency syndrome.
Methods: Bronchoalveolar lavage (BAL) or induced sputum specimens from 131 consecutive oncologic, HIV seronegative patients (55 M, 76 F of mean age 53 years) were studied for the detection of *P. carinii* cysts and trophozoites, using a direct immunofluorescent procedure. Hematologic malignancy in 72 (55%) and solid tumors in 59 (45%) of our patients were the underlying malignancies. Ninety out of the 131 patients were undergoing chemotherapy during the last 6 months.
Results: PCP was diagnosed in 30 (22.9%) out of 131 of our patients. There was no significant difference in the incidence of PCP between the patients with hematologic malignancy (20.8%, 15/72) and those with solid tumors (25.4%, 15/59). All 30 of our patients with PCP had undergone chemotherapy during the last 6 months but 24 patients (80%) were also receiving long-term therapy with corticosteroids at the time of diagnosis (mean duration 3 months). All but one of our patients with PCP had a successful outcome with a trimethoprime–sulfamethoxazole treatment.
Conclusions: PCP is quite frequent in oncologic patients. Chemotherapy and long-term treatment with corticosteroids are the main factors predisposing to this infection.

Intestinal parasites

P1270 Antiamoebic in vitro activity of pefloxacine

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Objectives: Amebiasis is a disease caused by the intestinal protozoan *Entamoeba histolytica*. It has a worldwide distribution affecting 10% of the world population and has become more and more common also in non tropical areas. Metronidazole remains the first choice treatment but various studies have demonstrated that cure rates with this drug do not reach 100% moreover the lack of a comparable drug to use as alternative treatment significantly narrows the therapeutic options in people who cannot take metronidazole due to side-effects and allergic or idiosincrasic reactions. For these reasons the identification of new amebicidal drugs is important, and this is made all the more evident by the growing scientific interest on the topic. In this study, we examine the antiamebic activity of pefloxacine in vitro. A quinolone was chosen because, similarly to metronidazole, it is effective against anaerobe bacteria and acts primarily against DNA interfering with its synthesis.
Methods: Xenic cultures on Robinson's media of a strain of *E. histolytica*, isolated in May 1997 from a symptomatic carrier coming from Ruanda and cryo-preserved, were used. Pefloxacine was tested at the doses of 10; 15; and 5 μg/mL, that correspond to the therapeutic concentrations in vivo and its effect on parasite growth was compared to that of cultures of the same strain and of comparable initial amebic density that were either drug-free (negative control) or exposed to 10 μg/mL of metronidazole (positive control).
Results: As seen in Fig. 1, metronidazole showed a marked effect between the 48 and the 72 h, with parasitic clearance. The drug-free test did not show significant modifications of the normal growth rate in cultural conditions. Pefloxacine, in all the concentrations examined, determined a gradual decrease of the parasite density throughout the experiment, showing an enhanced amebicidal activity between the 48 and the 72 h.

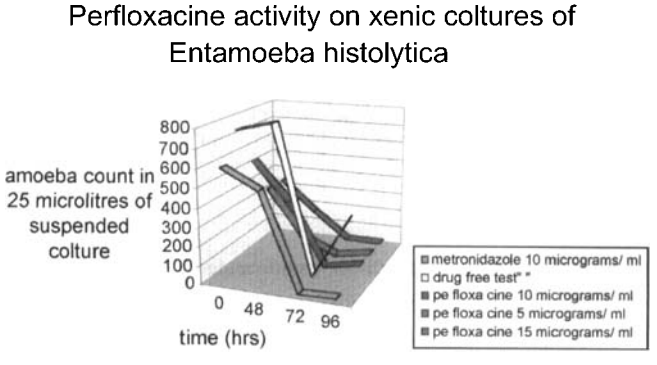

Figure 1

Conclusions: The promising in vitro activity of pefloxacine against *E. histolytica* confirms the activity of quinolones against amebic infection and is a good starting point for further in vivo randomized studies of these drugs in the treatment of symptomatic amebiasis.

P1271 Extraintestinal amebiasis—an emerging infection

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Objectives: Clinical presentation of extraintestinal amebiasis, with several pulmonary abscesses and splenic abscess.
Methods: The author present the clinical case of the patient M.P., 56 years, from the district of Arad, Romania, with the following symptoms of disease: abrupt onset with shivering and septic fever, cough with muco-purulent expectoration and, occasionally hemoptysia, wasting syndrome (15 kg weight loss), respiratory impairment and toxemia. The radiologic examination shows several opacities with large dimensions in both lungs. After antibiotic therapy with third generation of cephalosporines, the clinical signs and the radiologic aspects are unmodified. The performed bronchoscopy is followed in few hours by vomica with discharging of brown, chocolate-like pus. The native microscopical examination of the bronchial aspirate and of the expectoration shows extremely frequent clusters of vegetative, magna forms, of *E. histolytica*. Abdominal echography and computer tomography confirm the presence of a splenic abscess, without involving the liver and the central nervous system. There were either epidemiologic argumentations nor other cases of infections with *E. histolytica* in the territory.
Results: After two cycles of treatment with the combination hidroxiclorquine + metronidazole, the author succeeded the sterilization of the pulmonary abscesses with the tendency of normalization of the radiologic aspect and the disparition of the splenic abscess.
Conclusions: Extraintestinal amebiasis is nowadays not any more only a tropical disease and physicians can be confronted with diagnose difficulties, if a singular case appears in Central Europe regions.

P1272 Outbreak of *Cyclospora cayetanensis* gastroenteritis associated with consumption of imported Thai Basil, Vancouver, British Columbia, May 2001

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Objectives: *Cyclospora cayetanensis*, a coccidian parasite, is a recently identified cause of protracted and recurrent gastroenteritis associated with certain fresh produce. In May 2001, an outbreak of *C. cayetanensis* gastroenteritis was identified in British Columbia (BC). By June 15, a total of 17 cases were reported to the BC Center for Disease Control by the respective health authorities. A majority of these patients were either of Vietnamese origin or had frequented Vietnamese restaurants in the Greater Vancouver area. This report presents the findings of the outbreak investigation.
Methods: We conducted a case–control study involving a total of 17 case patients that were identified and reported for positive *C. cayetanensis* on stool microscopy. We interviewed patients using a structured questionnaire about symptoms and dietary habits within the 2-week period before the onset of symptoms. Controls were asymptomatic volunteers nominated either by the patients or their physicians. Controls were asked using the same questionnaire about the same 2-week period as their matched case patient. Both patients and controls with a travel history in the 4-week period prior to developing symptoms were excluded from this study.
Results: 17/17 (100%) of patients were exposed to Thai Basil, an essential ingredient in popular Vietnamese cuisine, either at home or at a restaurant, compared to 3/17 (18%) of the controls ($P = 0.004$). Fresh bean sprouts were also implicated ($P = 0.04$). Although incomplete to date, our trace-back investigations revealed that the Thai Basil was imported via Hawaii while bean sprouts were locally grown.
Conclusion: This outbreak in Canada is the first documented sporadic outbreak of cyclosporiasis linked to Thai Basil. As there is limited knowledge of the pathogenesis and modes of transmission of *C. cayetanensis*, this outbreak provides the opportunity to increase our understanding of this emerging pathogen and to improve on our prevention and control of future outbreaks.

P1273 Genotyping of human isolates of *Giardia lamblia* from Portugal

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Genetic characterization of a total of 28 *Giardia lamblia* isolates from cysts and from axenic cultures were performed by polymerase chain reaction to amplify segments of gene encoding the enzyme triosephosfate isomerase (tpi), coupled with the detection of restriction-fragment-length polymorphisms (PCR-RFLP). DNA extraction for cysts was performed from whole faeces and from samples concentrated using two methods: (i) the cyst samples were suspended in resuspension solution (500 mM Tris–HCl, 10 mM EDTA and 100 mg/mL Rnase A) and cell lysis solution (0.2 M NaOH and 1% SDS) provided in the Wizard Plus Minipreps DNA purification Systems (Promega) and were treated for 2 min in a Mini-Beadbeater (Stratech Scientific) using

0.5 mm zirconium beads. DNA was purified from the lysate using the procedure provided in the kit; (ii) the cyst samples were mechanically disrupted in the presence of 10 M guanidinium thiocyanate in 0.1 M Tris–HCl (pH 6.4)—0.2 M EDTA (pH 8.0)—2% Triton X-100 together with 0.5 mm zirconium beads and isoamyl alcohol. DNA was purified from the lysate using coarse activated silica. Genomic trophozoites DNA was extracted in vitro by axenic cultures in log late phase using TBE buffer (10 mm Tris–HCl, 1 mM EDTA), lysis buffer (8% triton X-100, 0.25 M sucrose, 50 mM EDTA, pH 8.0) and proteinase K. DNA was purified from the lysate using the Prep-A-Gene DNA purification Kit (Biorad). Two sets of primers were used to amplify segments of gene encoding the enzyme triosephosfate isomerase (tpi), and the amplified PCR products were cut by *Xho*I and *Hind*III. Isolate Portland 1 (ATCC 30888) (assemblage A, group 1), B-36 (assemblage A, group 2) and Ad-28 (assemblage B) were used as genotype standards. The present methodology identified genotypes belonging to the two previously described human genetic groups and is the first molecular characterization of *G. lamblia* from Portugal. The sensitivities of PCR procedures increased with the increasing numbers of cysts as observed by fluorescence microscopy. Our results point that human *G. lamblia* isolates from Portugal were typed to the two genetic groups: assemblage A 90% (Nash group 1 and group 2) and assemblage B (Nash group 3) 10%. There were no significant differences in the distribution of the genotypes by *G. axenic* cultures and cysts.

P1274 *Giardia lamblia*: a re-emerging infectious disease in the child population of Crete, Greece

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Background: Giardiasis has been considered to be uncommon in the child population of Crete, Greece, however, more recently several cases have been observed. The present study was undertaken to review the epidemiology and the clinical manifestations of *Giardia* infection in children seen at the University General Hospital of Crete.
Methods: Medical and microbiologic records of all children with stool specimens and serology positive for *Giardia* were retrospectively reviewed during the 8-year period from January 1994 through October 2001.
Results: 559 stool specimens were examined for the presence of *Giardia lamblia* during the study period. In seven children cysts of the parasite were demonstrated and the diagnosis of giardiasis was serologically confirmed. All children were diagnosed during the last 3 years of the study period (1999–2001); not a single specimen was found positive before 1999. The children were aged 6–11 years. Six were boys, all belonging to immigrant families and born in other Balkan countries. The remaining child was a girl born in Crete, without a history of travels abroad. All children had presented with recurrent abdominal pain. Two children had undergone appendectomy and further two were investigated for failure to thrive. Following the diagnosis of giardiasis, all were treated with metronidazole and had a full recovery.
Conclusions: Giardiasis is a re-emerging infectious disease for the children of Crete. This re-emergence seems to be related to the recent migration of families to the island. *Giardia* infection should be included in the differential diagnosis of children with chronic recurrent abdominal pain even in areas where the disease is considered to be eradicated.

P1275 Prevalence of certain Enteropathogens among apparently healthy children in a slum area of Istanbul

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Background: A prospective study was carried out to investigate the prevalence of certain medically important protozoa, helminths, and bacterial agents in an apparently healthy child population with 4–12 years of age from a slam of Istanbul during summer period of 2001.
Methods: Stool samples from 1069 children were collected and processed for parasitic and bacterial examination. Ethyl-acetate sedimentation technique was used for parasitic examination in stool samples. Cell-tape method was also carried out for the detection of *Enterobius vermicularis*. Bacterial examination was performed by standard methods for the isolation and identification of *Salmonella* spp. and *Shigella* spp. from stool samples.
Results: Five hundred twenty (48.6%) children had one or more intestinal parasites. *E. vermicularis* and *Blastocystis hominis* were found in high rates (24%), followed by *Giardia lamblia* (11.8%). In 109 (10.2%) of children, more than one parasite were present. The incidence of the other intestinal parasites (*A. lumbricoides*, *H. nana*, *T. trichiura*, *T. saginata* varied between 1 and 3%. Bacterial pathogens including *Salmonella* spp. and *Shigella* spp. were found in 0.6% of children.
Conclusion: The high prevalence of *E. vermicularis*, *G. lamblia* and *B. hominis* in the apparently healthy children was the most significant result of this study. In addition to the high prevalence of *G. lamblia* and *B. hominis*, the occurrence of *Salmonella* spp. and *Shigella* spp., even in low incidence among apparently healthy population may be indicative for poor hygienic conditions of water supplies and food sources in this region of Istanbul Metropolis with poor living standard.

P1276 The rate of *Oxyuris vermicularis* infection and vulvitis in children 2–5 years of age in Sari kindergarten, Iran

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Sari, IR

Objective: *Oxyuris vermicularis* is one of the main hygienic problems causing in the world specially in children under 2 years of age. Over 40 million Americans specially children are infected by *O. vermicularis*. One of the complications of parasite is it's role in causing vulvitis. Since the parasite is more prevalent in girls than boys, this study was conducted to show the prevalence rate of *O. vermicularis* infection and vulvitis in children between 2 and 5 years of age in Sari township.
Methods: Samples were collected by scotch tape method from all children of 2–5 years of age. Vulva of the children were examined by a midwife under the supervision of a specialist. Information about itching, edema, erythema and vulvar itching, vaginal excretion, puritis and bed wetting were recorded in a questionnaire. Other informations like age, education, occupation of parents, family members, the number of siblings, the number of children in each class and their baby sitters, cleanliness of nails, beds and their informal habits, bathing, disinfection of fomities and bed sheets, the presence of physicians in kinder garden, hygienic card for baby sitters and the children infected with *O. vermicularis* were recorded in a questionnaire and the children infected to *O. vermicularis* were examined by scotch tape method.
Results: From the total number of 216 children under study, 64% were infected to *O. vermicularis*, 38 (13.5%) persons had vulvitis, and 13 (6%) persons had vulvitis along with *O. vermicularis*. There was no significant relationship between *O. vermicularis* and vulvitis by X statistical analysis. 34.2% who had vulvitis, were infected to *O. vermicularis* too. There were significant relationship between the age and *O. vermicularis* infection ($P < 0.001$), between vulvitis and age group ($P < 0.05$), between *O. vermicularis* and using fomities ($P < 0.01$) and between vulvitis and itching of anus ($P < 0.05$).
Conclusions: Regarding the prevalence of *O. vermicularis* infection and its role in vulvitis and the limitation of the studies in this field, it is necessary to have a proper planning for the improvement of health and the agents of causing vulvitis.

P1277 Simultaneous *Enterobius* and *Toxocara* infection in a closed collective: some immunological findings

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Objectives: The aim of this study was to define the peculiarities of immune status of patients with *Enterobious* + *Toxocara* infection.
Patients and methods: A total of 135 young men closed collective were involved in study. *Enterobious* ova were found in anorectal scrapings. Sera were tested for *Toxocara canis* antibodies by ELISA. Total, T-, B-lymphocyte, CD4, CD8, total NK counts and helper/suppressor (H/S) ratio in peripheral blood were measured by laser flow cytometry.
Results: Enterobiosis alone was found in 17 patients, toxocarosis alone in 6, *Toxocara* carriers—19; 7 of patients were infected with both *Enterobius* and *Toxocara*. A tendency to increase of H/S ratio was observed in patients with simultaneous *Enterobius* and *Toxocara* infection (1.42 ± 0.21) if compared with enterobiosis alone (1.15 ± 0.01, $70\% < P < 80\%$) and *Toxocara* carrying alone (1.14 ± 0.08, $70\% < P < 80\%$). A tendency to decrease of NK-lymphocyte count was registered in simultaneous enterobiosis and toxocarosis (272 ± 45 cells/mm^3) if compared with enterobiosis alone (355 ± 47 cells/

mm^3, $70\% < P < 80\%$). No significant differences were observed amongst these groups for total T-, B-lymphocyte, as well as CD4 and CD8 counts. Obtained data revealed increasing of total B-lymphocyte counts (325 ± 52 cells/mm^3) and H/S ratio (1.28 ± 0.18) in patients with toxocarosis if compared to *Toxocara* carriers (246 ± 22 and 1.14 ± 0.08 cells/mm^3, respectively; $80\% < P < 90\%$ and $50\% < P < 60\%$, respectively).
Conclusions: Immunosuppression in so-called aggravated groups of patients (enterobiosis + toxocarosis, toxocarosis alone) is not so expressed as in patients with enterobiosis or *Toxocara* carrying alone. This finding was confirmed too by higher antibody level to *T. canis* in simultaneous enterobiosis and toxocarosis, as well as in toxocarosis than in *Toxocara* carrying alone.

P1278 The treatment of hymenolepiasis

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Background: Hymenolepiasis is the most frequent taeniasis in Albania. Its complete cure is not always immediately realized. The aim is the recognition of the real efficacy of its current etiological treatment.
Methods: We studied 150 patients with hymenolepiasis of age 10–62 years, accumulated during the period of 1986–2000. We separated them in two groups. Group I—100 patients were treated with Niclosamide [Yomesan, Radeverm]: first day 2 g/die, followed by 1 g/die for 6 additional days. Group II—50 patients were treated with Praziquantel [Biltrycini, Cesol] 25 mg/die in a single dose. The parasitological control was executed 15, 30 and 60 days after treatment. The positive cases resulted by coproparasitological examination were treated up to three cycles as above mentioned.
Results: In the first group, with niclosamide, we identified *Taenia nana* elimination in: 73% out of 100 patients after the first cycle; 62.9% out of 17 patients after the second cycle and 60% out of 6 patients after the third cycle with niclosamide. Totally, from 100 patients, the niclosamide purified 96% of them using on cases without effect up to three weekly cycles treatment. In the second group with praziquantel were identified: purification after the first cycle; 78% out of 50 cases treated after the second cycle, 72.7% out of 8 patients treated and after the third cycle 66.6% out of 3 patients using the antiparasitic drug. We cured 98% of 50 cases using three cycles of paraziquantel treatment. It is obvious that the efficacy of niclosamide and praziquentel on hymenolepiasis treatment is very high, but not absolute. The remaining of a sensible positive percentage, repeated after each cycle treatment with those drugs, indicates that reaching the complete cure on hymenolepiasis is not easy. We think that except the therapeutical factor, there should intervene other factors related to self-infestation or *Taenia nana* circulation in the patient life environment.
Conclusions: The etiological treatment of hymenolepiasis is easier to be realized with praziquantel because of its comfortable application; niclosamide remains a very good alternative.

P1279 Re-infection rate of *Ascaris lumbricoides* in the rural areas of Hamadan, West Iran: report of one year follow-up

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Hamadan, IR

Objectives: This study was carried out to determine the post-treatment re-infection rate (RIR), for evaluating the infection pressure that used in the control programs.
Methods: A total of 187 individuals rendered *Ascaris* egg negative by drug treatment were used in the re-infection study done 3-monthly for 12 months. Stools from 187 individuals, from different villages distributed in the all provinces level, were examined to determine post-treatment re-infection rate for *Ascaris*. All positive cases treated with albendazole and stool examination follow-up was done during 5–7 days after treatment. Those who were rendered negative by treatment and those originally negative were included in the RIR study.
Results: Re-infection rate at the 3-month intervals were 5.5, 3.2, 6.15 and 6.4% for first, second, third and fourth period of follow-up, respectively. Commulative RIR was 21%. Most of infected individuals (48%) passed unfertilized eggs.
Conclusions: Because this study was done after a mass treatment program, the infection pressure changed to an acceptable level and considered as a good guide index for surveillance of ascariasis in the control program area. On the basis of results of this study, we recommend frequency of mass treatment only one time a year for next 3 years.

P1280 A multicentre retrospective study of domestic intestinal parasitosis in Italy in the 1990s

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On behalf of the Association of Italian Clinical Microbiologists, Committee of Parasitology (AMCLI-CoSP), Milan, I

Objective: To focus the attention of Microbiologists on home intestinal parasitosis, following specific indications provided with previous 'ad hoc' Courses and the distribution of a preliminary Manual of Diagnostic Parasitology. For this study, eight Laboratories of Clinical Microbiology were recruited, of whom the responsible guaranteed a standardized stool parasitological investigations.
Methods: The data of 31 years, between 1990 and 1999, were collected, with a total of 18.182 subjects for standard stool parasitological examination, 2.151 for cellophane-tape test, 1.106 for *C. parvum*—acid fast stain (only three Labs per 18 years), 411 for *S. stercoralis*—Baermann/larvae culture methods (only one Lab per the last 5 years), 545 for *D. fragilis*—Giemsa stain (only one Lab per the last 2 years). Five Labs were of north Italy, two of middle Italy, one of south Italy. Two Labs supplied data of the last year, two Labs of the last 2 years, one Lab of the last 3 years, one Lab of the last 5 years, one Lab of the last 7 years, one Lab of 10 years.
Results: Helminths ova were reported only in 0.6% of subjects, with prevalence of *Taenia* spp. (0.3%). No pathogen trematodes were observed; among nematodes only *A. lumbricoides* (0.05%) and *T. trichiura* (0.05%) were observed (also very few casula larvae of *S. stercoralis* and eggs of *E. vermicularis* were reported); among cestodes very few cases of *H. nana*, *H. diminuta* and *D. latum* (all in 0.005% of cases) were observed too. Forty cases of *S. stercoralis* (9.7%) and 256 cases of *E. vermicularis* (11.9%) were instead reported when specifically investigated. Protozoa were reported in 3.2% of subjects: *G. intestinalis* in 1.9%, other not pathogens (*B. hominis* included) in 1.3%. *C. parvum* prevailed in 0.7% of investigated cases, *D. fragilis* in 2.1%.
Conclusions: AMCLI-CoSP concludes that few Labs are still organized for good fecal diagnostics and are available to perform adequate data collection. Anyway, faecal parasites and particularly helminths are very rare in Italy; so, the investigation for these pathogens could be reserved to organized Labs. Moreover, more Labs must investigate for *D. fragilis* and other protozoa using at least a Giemsa stain, must research specifically *S. stercoralis* when at risk factors are present, many stool specimens must be analyzed for excluding *G. intestinalis*.

HCV

P1281 Ortho total HCV core antigen assay can aid early prediction of response in patients treated with interferon/ribavirin

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Objectives: To evaluate the predictive value of Total HCV Core Antigen Assay and viral kinetics in patients with chronic HCV.
Methods: A total of 122 patients (pts) infected by genotype 1, 4, 5 or pretreatment viral load (bDNA 2.0, Chiron) >3 Meq/mL, with no previous treatment, received 6 mU IFN during 12 months (M). Ribavirin was given with IFN after 3 months therapy, for 9 months in pts with detectable RNA (Cobas Monitor 2.0, Roche; Quantiplex HCV-RNA 3.0, Bayer). Viral load was expressed as log(IU/mL) and HCV Ag as log(pg/mL × 10 000).
Results: Sustained response (SR) was obtained in 40% of the pts, relapse (RR) in 18%, non-response (NR) in 42%. Fifty-two pts had detectable RNA at M2.

Pre-treatment Ag values were correlated with viral load ($r^2 = 0.779$). The HCV Ag level was significantly lower in SR ($n = 32$) than other groups (5.47 vs. 6.07, $P < 0.001$). We observed a rapid decrease of Ag (5.2 log pg/mL) and viral load median (5.1 log IU/mL) after M1 in SR. In pts who relapsed after IFN alone ($n = 20$), the fall was less (2.6 log pg/mL, 3.6 log IU/mL) during M1. In SR ($n = 29$) and RR ($n = 10$) two-combination therapy, the decrease of Ag and viral load median at M1 were, respectively (Ag: 1.2 and 1.4 log pg/mL; RNA: 2.4 and 1.5 log IU/mL). Ribavirin increased the fall of Ag and RNA load to undetectable levels for SR and RR at M4 and M6, respectively. We did not observed significant variation of Ag and viral load in NR (31). The negative predictive value of HCV-RNA and Ag after M1 of treatment was 100%, and positive predictive values were 81 and 82%. After one month of IFN alone, it was highly predictive of SR, correlated with RNA negative and early reduction of HCV-RNA (>2 log).

Conclusion: Early measurements of total HCV core antigen are useful to predict long-term response to treatment.

P1282 Genetic variability of E1/E2 regions of HCV genotype 4

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Six genotypes of HCV have been identified that show different geographical distribution. High heterogeneity was found, from our studies performed in Africa for genotypes 2 and 4. This latter genotype is highly prevalent in Egypt. The present work was aimed to characterizing viral variants of HCV genotype 4, from 141 hepatopathic patients in Egypt (141) by the analysis of E1 and E2 genome regions. These regions are relevant for future vaccine development. Thirty-six isolates were studied by phylogenetic analysis (DNA DIST and Neighbour Joining methods). Twenty were assigned to subtype 4a, whereas four new subtypes were characterized, to which, respectively, 4, 2, 1 and 1 isolates were assigned. High heterogeneity was observed among isolates of 4a subtype in both genome regions (mean variability >10%; range between 14 and 30%). The analysis of similarity (nucleotide and protein sequences, Simplot program) and secondary prediction on protein sequences (Antheprot Package) demonstrated that also few amino acid changes could induce protein modifications of antigenic presentation. In conclusion, the high intra- and inter-subtype variability, both in nucleotide and protein sequences were observed for genotype 4 isolates. These findings, relative to genome regions applied for vaccine development, may be relevant for HCV prophylaxis particularly in highly endemic areas.

P1283 Genotype distribution to identify the source of HCV infection in Moroccan HD patients

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Hepatitis C virus (HCV) infection is highly prevalent and represents a major problem in hemodialysis patients (HD) worldwide. Little is, however, known about this high-risk group in Morocco. The principal objective of the present study was to determine the genotype distribution and to identify the source of HCV infection in Morocco HD patients. Our results show that 46.3% of the 615 screened HD patients, collected from different Moroccan cities are positive for HCV antibodies by ELISA. Our results also show that the frequency of HCV-positive HD patients decreased after 1994, the date at which the screening of Moroccan blood-donors started, indicating that screening of blood-donors' results in a decreased of HCV transmission in HD patients. However, the percentage of HCV infection in both transfused and untransfused HD patients remained elevated after 1994. These results are in favor a nosocomila infection as the main cause of HCV transmission in HD patients. HCV-RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in 108 sera of the 172 HCV-positive patients tested (63%). HCV genotypes were examined using the line probe assay. Subtype 1b was predominant (75%), whereas, subtypes 2a/2c, 1a and 3a were less common (13.9, 3.7 and 09%, respectively). This genotyping data strongly suggest a transfusional and nosocomial pathways of infection in this high-risk group in our country.

P1284 Evaluation of ortho total HCV core antigen assay in assessment and follow-up of patients treated for chronic HCV

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Introduction: Pretreatment viral load and response to IFN/ribavirin are correlated. An assay to quantitate 'Total' HVC core antigen in serum or plasma, in the presence of anti-HCV antibodies, has been developed by OCD. The test is an ELISA requiring pretreatment with denaturing solution at 56 °C for 30 s to dissociate immune-complexes and intact virions. Free Ag capture is via a MWP coated with two anti-HCV Mabs, each directed to a distinct region of the protein. Detection is via a different pair of anticore Mab's conjugated to poly-HRP. Total Core Ag assay is theoretically capable of measuring viremia, and may reflect viral load.

Objectives: We evaluated HCV Ag with two quantitative assays for HCV-RNA: bDNA 2.0 or 3.0 (Bayer) and Monitor 2.0 (Roche).

Methods: We studied 191 samples from untreated patients and 237 from 144 patients with chronic HCV treated with IFN or IFN/ribavirin, at 1 month (M1), M2, M3, M4, M6 and M12.

Results: Correlation of Ag and quantitative assays was high ($r = 0.85$ and 0.83, respectively), and we did not find any difference between the levels of RNA and Ag among HCV genotypes ($r = 0.82$–0.96). In untreated RNA positive patient samples ($n = 144$), HCV Ag was under the cut-off in eight patients. bDNA 2.0 and Monitor 2.0 failed to detect 5 and 1 samples, respectively. Ag values, before treatment, were significantly lower in sustained responders than in other groups (5.4 pg/mL vs. 6 pg/mL, $P < 0.001$). In patients treated with IFN or combination therapy, we found very good correlation between decrease or negative of Ag and viral load: 2 log IU/mL decline of HCV-RNA after M1 of interferon was significantly correlated with the negative of HCV Ag and response. A number of 38/41 of sustained responders had a RNA load decrease >2 log IU/mL and 39/41 had a negation of HCV Ag after M1.

Conclusion: Total HCV Core Ag appears to be a new tool for monitoring patients with HCV infection.

P1285 Efficacy and tolerability of interferon-α and ribavirin in the therapy of patients with chronic hepatitis type C

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Objectives: The combination of interferon-α and ribavirin is the current strategy of treatment of patients with chronic hepatitis type C. The aim of our study was to evaluate the efficacy and tolerability of interferon-α plus ribavirin combination therapy in patients with chronic hepatitis type C.

Methods: Eighty-four patients aged 20–65 years were enrolled to this prospective study; there were 54 naive patients, and 30 relapses after ineffective interferon-α monotherapy. Patients were anti-HCV(+), HCV-RNA(+) by PCR (cut off: 200 copies/mL) with histologically confirmed chronic hepatitis and abnormal activity of aminotransferases lasting at least 6 months before the start of therapy. Patients were treated with interferon-α 2b (Schering–Plough, 3 mU thrice weekly) plus ribavirin (Schering–Plough, 1.0–1.2 g/day). Blood morphology and biochemical tests were performed every month, HCV-RNA was assessed at baseline and after 24 and 48 weeks of therapy. HCV-RNA(–) at 24 weeks was defined as early response (ER), at 48 weeks as end of treatment response (ETR).

Results: After 24 weeks of treatment 31 patients (72%) eliminated HCV – achieved ER. Twenty-nine of patients (67% of all patients) were still negative for HCV-RNA after 48 weeks and achieved ETR; two patients (5%) had detectable serum HCV-RNA again. Hemoglobin decreased from 14.2 to 12.2 g/dL ($P < 0.05$) after 24 weeks, then increased to 12.9 g/dL after 48 weeks. Leukocytes count decreased from 5.25×10^9/L to 3.7×10^9/L ($P < 0.05$) after 24 weeks and increased to 4.36 × 109/L after 48 weeks.

Conclusion: The study confirms that interferon-α and RIBA combination therapy was effective in patients with chronic hepatitis type C as estimated at the end of treatment and was well tolerated.

P1286 Induction therapy with interferon alpha-2b in naïve chronic hepatitis C patients

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Objectives: Hepatitis C virus infection is a major health burden affecting estimated 200 million people worldwide. Interferon is the first drug shown to be effective in patients with chronic hepatitis C (CHC). Current data suggest that daily administration of interferon-α in CHC is more effective than the thrice weekly (tiw) scheme. The aim of the present study was to evaluate the efficacy of induction therapy in CHC patients.
Methods: A total of 56 naive biopsy proven CHC patients (25 males, 31 females) with ALT levels >1.5 × IU and positive HCV-RNA in serum were included. The therapy was administrated 5 mU INFα 2b every day for 1 month, followed by a maintenance period of 11 months with the same dose tiw.
Results: At the completion of the induction period, 37 of 56 (66.1%) patients had partial virological response. Among the patients studies, the virological response with clearance of HCV-RNA was achieved in 39.2% and the biochemical response with normalization of ALT in 44.6% at the end of 12-month therapy. Overall, sustained response rate was found as 37.5%. Interferon treatment was well tolerated without significant side effects.
Conclusion: Daily administration of 5 mU INFα 2b for 1 month, followed by a maintenance period of 11 month with the same dose tiw in CHC is associated with high rate of sustained response.

P1287 Side-effects of double therapy (interferonα plus ribavirin) in chronic hepatitis C

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The double therapy interferonα and ribavirin is the much more effective then therapy with interferon alpha only. Between 1996 and 1999 we realized a prospective, open, pilot study of efficiency and tolerability of double therapy in 29 patients with chronic hepatitis C: 21 males (72, 41%) and 8 females (27, 59%). For this purpose, we conducted the double therapy during 24 weeks and followed patients 24 weeks more. All patients were treated with interferon alpha 3 mU, three times/week and ribavirin 1200 mg/day divided in two doses. Based on maintaining HCV-RNA in serum (PCR method) and serum aminotransferase activity before, during and after the therapy, patients were divided in two groups:

- *SRP (sustain responders)*: 13 patients (44, 83%) with fast significant decrease and elimination of HCV-RNA in serum and normal levels of serum aminotransferase activity during therapy and following, and;
- *RRP (relapse responders)*: 16 patients (55, 17%) with significant decrease HCV-RNA in serum and normal levels of serum aminotransferase activities only during therapy.

Interferon-α plus ribavirin is generally well tolerated therapy. Side-effects are generally reversible. The most frequent side-effects of double therapy in our patients have been 'fly-like syndrome' (fever, headache, arthralgia, myalgia) during first 2 weeks of therapy in 13 patients (44, 83%) and 'late syndrome' (arthralgia, myalgia, nausea, fatigue and insomnia) in 16 patients (55, 17%). Our patients tolerated these side-effects well and therapy was not changed. Leukopenia (under 3.0×10^9) was observed in three patients (10, 34%); anemia (hematocrit under 30) in eight patients (27, 59%); leukopenia and anemia in four patients (13, 79%); depression in one patient (3, 45%) and suicidal ideas in one patient (3, 45%). All these side-effects required the change of therapy regime (reduction of doses or therapy break during 2 weeks at least). Early termination of the therapy was not necessary in any patient.
Conclusions: Side-effects of double therapy in our study were frequent but not dangerous. In all patients, side-effects have been solved by reduction of doses or by temporary break of the therapy.

P1288 Viral kinetics in Lithuanian patients with chronic hepatitis C virus infection during induction vs. standard interferon-α therapy

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Objective: To evaluate viral kinetics during induction and standard three times a week (tiw) dosing with interferon-α (Realdiron, Lithuania) for treatment of patients (pts.) with chronic hepatitis C virus (HCV) infection.
Methods: Thirty-six pts. with mean age of 37 years were entered consecutively to three treatment groups: standard IFN treatment 3 mU/tiw for 48 weeks (group I), induction IFN treatment with standard follow-up dosing 6 mU/day for 10 days followed by 3 mU/tiw until Week 24 (group II) and high follow-up dosing 6 mU daily for 10 days followed by 6 mU/tiw until week 24 (group III). All pts. were HCV-RNA-positive in serum at inclusion, and had had raised liver enzymes for more than 6 months (mo). Sixteen pts. had genotype 1 and 20 – genotype no. 1. HCV-RNA levels were analyzed at baseline, week 4, 8, 12, end-of-treatment and at follow-up the 24 weeks after treatment stop. A liver biopsy was performed in all pts. within 6 months prior to entry and at treatment stop. Histological evaluation was performed according to Ishak scoring system.
Results: Induction IFN mono-therapy followed by standard or high-dose tiw dosing of IFNα used as mono-therapy was not better then standard tiw treatment from onset in achieving a decline in HCV-RNA levels week 4, 8 or 12 during treatment. Patients with HCV genotype 2 or 3, on the other hand, had a much more pronounced decline in HCV-RNA levels, irrespective of treatment schedule, than genotype 1 pts., reflected by a mean 3 log and 1 log decline of the HCV-RNA levels already week 4, respectively. No patient treated with induction and a low follow-up IFN dose during only 24 week achieved sustained virological response (SR), whereas pts. treated with induction and a high follow-up dose achieved SR in 36%, a difference which nearly reached significance, $P = 0.067$. Pts. treated with standard scheme during 48 weeks had an overall SR of 42% not significantly different from the result in the combined induction groups 17%, $P = 0.1$. Adverse events were the commonly noted with IFN treatment. In the induction high-dose follow-up group nearly half of pts. had trombocytopenia (<75 × 109/L).
Conclusion: Genotype no. 1 infections respond with a much more pronounced decline in viral levels early during treatment than genotype 1 pts. and that a short-induction period does not translate in a higher sustained response rate. Adverse events were significantly more common in pts. treated with high-dose IFN therapy.

P1289 Daily interferon in chronic hepatitis B patients: a multicenter study from Turkey

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Objectives: Chronic hepatitis B (CHB) infection is a major health problem in Turkey. HBsAg positivity varies from 3 to 12.5% in Turkish population. In this study, response to the daily interferon treatment was evaluated in chronic hepatitis B patients.
Methods: The treatment consisted of interferon alpha-2b (5 mU/day) for 24 weeks. Patients were evaluated as to the clinical response and side-effects during and 1 year after termination of the treatment. Inclusive criteria for the study population were determined as such; HBsAg positivity for a minimum of 6 months prior to treatment, increased ALT levels, HBeAg or antiHBe positivity and HBV DNA positivity. Liver biopsy was performed prior to the treatment and evaluated according to Knodell scoring system. A total of 60 patients (female/male = 21/39) were included into the study. Mean age was 33 ± 9.2 (17–55). Four patients were discarded from the protocol because of side-effects of the therapy.
Results: At the end of the 6 months, the following responses were noted: biochemical 71.4%, virologic 46%, complete response (biochemical and virologic response) 41%, HBeAg negativity and antiHBe positivity 44%. One year after the treatment the following responses were noted: biochemical 44.6%, virologic 39%, HBeAg negativity and antiHBe positivity 50%,

sustained response (biochemical and virologic response) 33.9%. Loss of HBsAg was determined in 3.5% of patients.
Conclusion: Daily administration of 5 mU interferon alpha-2b for 6 months in CHB is associated with high rate of sustained response.

P1290 Viral dynamic of hepatitis C in patients treated with pegylated interferon-α plus ribavirin

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Objective: To study the influence of HCV genotypes, basal viral load, and viral dynamics as predictive factors in early viral response (EVR) in patients treated with pegylated interferona-α plus ribavirin (PEG-IFN + RBV).
Patients and method: Thirty-four patients with chronic hepatitis C treated with PEG-IFN + RBV were studied, 16 males and 18 females. The average of age was 43 years. We used a PCR technique (Monitor, Roche) to determinate the viral load at baseline, at 48 h, and 2, 4, 12 and 48 weeks. Genotypes were determined by reverse hybridization using InnoLIPA genotyping kit (Innogenetics). we defined three phases: (I) changes on viral load at 48 h; (II) changes at 4 weeks; and (III) changes between 48 h and 4 weeks. EVR is defined as negation of RNA HCV viremic or decrease of 2 logs on viral load from baseline to 12 weeks.
Results: Twenty-one patients showed EVR. Genotypes no. 1 were associated with EVR (52% of patients infected by genotype 1 versus 100% of infected by no. 1 genotype) but no viral load at baseline. The phase II of viral dynamics showed a higher decrease in responders than non-responders ($3.88 \log \pm 2.47$ vs. $0.61 \log \pm 0.5$, $P < 0.001$). Also, the phase II was statistically different according to genotypes (decrease of $2.3 \log \pm 2.2$ in genotype 1 vs. $5.3 \log \pm 1.9$ in no. 1 genotype, $P < 0.001$).
Conclusions: HCV genotypes and phase II of viral dynamic, but no viral load at baseline were associated with EVR. Also, no. 1 genotype showed a deeper decrease of HCV-RNA in phase II of viral dynamic. On the other hand, phase II were associated with the viral genotype. Therefore, the influence of genotype in EVR should be explained by a different viral dynamic.

P1291 Factors predictive of response to interferon-α (IFN) plus ribavirin in patients with chronic hepatitis C non-responders to IFN alone

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Objective: The aim is to assess if HCV genotypes, HCV-viral load, and pretreatment liver histology may be prognostic indicators in the therapy with IFN plus ribavirin of patients with chronic hepatitis C non-responders to IFN alone.
Methods: Thirty-two patients were studied. In all, individuals were evaluated the histological activity index (HAI), the HCV genotype and the HCV viral load. HAI was scored according to Ishak et al. Serum HCV-RNA was determined by qualitative (Amplicor–Roche) and quantitative (Amplicor Monitor-Roche) methods. HCV-genotypes were identified by Inno-LiPA HCV II-(Innogenetics). Biochemical response (normalization of serum alanine aminotransferase concentrations) and virological response (undetectable serum HCV-RNA) were evaluated at the end of therapy and after 6 months of follow-up (sustained response-SR).
Results: 19 patients were infected by genotype 1, 2 patients by genotype 2, 4 patients by genotype 3 and 7 patients by genotype 4. Virologic SR was 10.5% in genotype 1, 50% in genotype 2, 0% in genotype 3, and 14.2% in genotype 4. The table shows the mean HAI score and the mean viral load among different genotypes in patients responders and non-responders (see following table).

Patients (genotypes)	No. of patients	Mean HAI score (grading–staging)		Mean viral load	
		Responders	Non-responders	Responders	Non-responders
1	19 (59.4%)	7–1	6.8–1.9	200 000	700 000
2	2 (6.25%)	8–4	8–4	200 000	312 000
3	4 (12.5%)	–	6.45–2	–	265 250
4	7 (21.87%)	8–2	53–53	1 000 000	740 750

Conclusions: HAI score was not significantly different among responders and not responders. A high response rate was achieved in genotype 2, whereas genotypes 3 and 4 showed poor response. No patient with genotype 3 had a sustained virologic response. Another factor that may be predictive of response is the viral load before treatment (200 000 copies/mL vs. 700 000 copies/mL, in patients with genotype 1). These results might be useful to determine the best cost-effective therapeutic strategy in patients with chronic hepatitis C non-responder to IFN.

P1292 Interferon-α plus ribavirin combination therapy in patients with chronic hepatitis C, non-responders to interferon alone: response rates in different genotypes

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Objective: To assess the efficacy of therapy with interferon-α (IFN) plus ribavirin in patients infected with different genotypes of HCV non-responders to IFN alone.
Methods: In 1999–2000, we treated 111 naives patients with IFN alone. Sustained biochemical and virologic response was achieved in 12.7% of patients with genotype 1, 54.5 and 32.4% in patients infected by genotype 2 and 3, respectively. All patients with genotype 4 were non-responders to IFN alone. We studied 32 patients non-responders to previous treatment with IFN 3–5 mU three times weekly for at least 3 months. All patients had positive serum HCV-RNA as well as biochemical and histological evidence of chronic hepatitis. HCV genotype 1 was found in 19 patients (59.4%), and genotypes 2, 3 and 4 have been detected in 2 (6.25%), 4 (12.5%) and 7 (21.87%) patients, respectively. Patients were treated with IFN 3 mU three times weekly plus ribavirin 1–1.2 g daily for 6 months. Biochemical response (normalization of serum alanine aminotransferase concentrations) and virologic response (undetectable serum HCV-RNA) were evaluated at the end of therapy and after 6 months of follow-up (sustained response).
Results: The following table summarizes response rates among different genotypes.

Genotypes (no. of patients)	Response at end therapy (%)		Sustained response (%)	
	Biochemical	Virologic	Biochemical	Virologic
1 (19)	47.4	86.8	15.8	10.5
2 (2)	100	100	50	50
3 (4)	75	75	50	25
4 (7)	14.2	14.2	14.2	14.2

Conclusions: Re-treatment with IFN plus ribavirin is effective in about 20% of patients with genotypes 1 and 3 non-responders to IFN alone. Patients with genotype 2 are the only ones that show a high response rate to the combination therapy. Genotype 4 is more common than previously reported in Italy (7.3% vs. 3%), and shows no response to IFN alone; in patients with genotype 4 is mandatory the combination therapy IFN plus ribavirin.

P1293 Quasi-species in hepatitis C virus and their implications in antiviral therapy

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Introduction: Interferon-α eliminates hepatitis C virus (HCV) in only 15–30% of chronic infected patients. The aim of this study is to evaluate the relationship between the hypervariable region 1 (HVR1) quasispecies of hepatitis C virus and the evolution of therapy to determinate the significance of genetic complexity as a predictor factor of the responsiveness to interferon therapy.
Patients and methods: A total of 12 patients divided into three groups were analyzed (three patients with sustained response, three patients with transient response and six non-responders patients). Patients were infected with

genotypes 1a (one patient), 1b (seven patients) and 3a (four patients). The viral quasispecies were detected by nested RT-PCR by using primers flanking HVR1 mediated single-strand conformation polymorphism (SSCP) assay. The bands were visualized by silver staining.

Results: Two different genetic complexity patterns were obtained. The patients with sustained response showed a low quasispecies genetic complexity. In three non-responders patients and in one patient with transient response the genetic complexity pattern was high and all of them were 1b genotype. Into the rest of patients with transient response the appearance of new bands was detected. These patients were 3a genotype. The high genetic complexity pattern was detected only in patients infected with subtype 1b.

Conclusions: These results suggest that the presence of a low genetic complexity pattern at the beginning of the interferon therapy is necessary to achieve a sustained response. Similarly, a high genetic complexity pattern could be related with the lack of a positive response to the treatment. The presence of this pattern only in patients infected with genotype 1b may explain the poor response to the therapy. A high genetic complexity pattern could be an independent predictive factor to genotype, as we have obtained in transient responders infected with genotype 3a. Because of the low number of patients involved in this study, prediction of response to interferon based on the genetic complexity pattern was not possible. Large-scale studies are urgently needed.

P1294 Lamivudine enhances T-cell immune response, via a direct antiviral activity, in chronic hepatitis B patients

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Objective: Lamivudine is an oral nucleoside analogue with a potent antiviral effect on hepatitis B virus (HBV) due to inhibition of viral DNA-polymerase. B2-microglobulin (B2-m) constitutes a significant part of HLA class I, which plays a critical role in the T-cell immune response mechanism against viral infections. The aim of this study was to evaluate serum B2-m levels in HbeAg(–)/HBV-DNA(+) patients with chronic hepatitis B (CHB) before and after 6-month lamivudine monotherapy and to compare the results with those of healthy control subjects (HS).

Methods: Serum from 29 CHB patients [ages: 44 + 2 years, ALT = 188.7 + 104 IU/L, HBV-DNA (2.4 + 2.3) × 10^6 copies/mL, HAI = 6.9 + 2.8, stage 3.2 + 1.3)] was obtained prior to and after completion of 6-month lamivudine monotherapy, as well as serum from 26 HS of similar age and gender. Serum B2-m levels were determined by ELISA (IMX-Abbott). All the subjects were free of other acute or chronic illness and had not previously received any medication for hepatitis. All patients displayed normal liver function tests were HBV-DNA(–) (<400 copies/mL, Amplicor, Roche Monitor) after the end of the 6-month therapy. Statistical evaluation was based on Wicoxon-test (P-value < 0.05).

Results: Serum B2-m levels in CHB patients after the end lamivudine therapy were 1966.05 + 341.48 IU/L, significantly higher than those prior to therapy (1765.75 + 405.1 IU/L, $P = 0.001$). The corresponding serum B2-m levels in the HS were 1419.5 + 307.36 IU/L and remained in similar levels after the 6-month period (1407.5 + 295.45 IU/L, $P > 0.05$).

Conclusion: Six-month lamivudine monotherapy results in a significant reduction of viral load and possibly enhances the HLA class I mediated immune response, thus implying a possible indirect immunoregulatory effect of this primary antiviral drug.

P1295 The effect of interferon therapy on patient with chronic active hepatitis B and C

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Objectives: Many drugs have been used for treatments of hepatitis but they were not effective. Interferon is an effective drug and causes response to 40 and 25% chronic hepatitis B and C, respectively, in the USA. The aim of present study was to evaluate the effect of interferon in Iranian patients.

Methods: The effect of interferon alpha-2b on 20 patients were evaluated. Of these patients 11 patients (55%) had chronic hepatitis B and nine patients (45%) had chronic hepatitis C. In patients with chronic hepatitis B, 10 mu Interferon alpha-2b was administrated subcutaneously three times a week for 16 weeks, in chronic hepatitis C patients 3 mu Interferon alpha-2b was injected subcutaneously three times a week for 24 weeks. Regarding the interferon complications (bone marrow suppression) the patients were examined in the first, second, fourth weeks and then monthly after beginning the treatment. Before beginning of the treatment, virus markers (HBS Ag, Hbe Ag, HBV DNA, HBC Ab, HCV Ab, HCV-RNA) and liver enzymes (Ast, Alt, Ap) were recorded and liver biopsy were taken in all patients. These experiments were repeated after treatment too and the results were analyzed by student t-test.

Results: Histological findings in patients with chronic hepatitis B showed response in four patients (36.3%) four patients (36.3%) without any changes, three patients (27.3%) progression of the disease. In patients with chronic hepatitis C, the response was (33.3%), without any changes was (33.3%), progression was (33.3%). Biochemical response (decreased Alt level) before and after treatment was compared in chronic hepatitis B and C patients. The results corresponded with cytological findings in chronic hepatitis B patients (36.3%) but in chronic hepatitis C patients, biochemical response (44.5%) was higher than cytological response.

P1296 Markers for hepatitis C nosocomial transmission in dialysis

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Objectives: To know the hepatitis C (HCV) prevalence and to identify factors related to higher risk of seroconversion for HCV at Dialysis Units (DU).

Methods: 155 DU have been surveyed by the Infectious Control Team of CADRA, from 1/2/1998 to 7/30/1999 on field. Compliance with standard precautions, rules and procedures, prudent use of Vancomycin, cleanliness, reuse process, sterilization and disinfection, transfusions, pathogenic waste management, were surveyed. Relationship between these markers and HCV seroconversion is analyzed. In order to identify independent markers, stepwise logistic regression (SLR) was used.

Results: HCV prevalence was 27.8% in this survey. DU with anti-HCV (+) patients represented 58.7% in this survey, and 34.8% of them registered seroconversions (SC+) during the last 2 years before the visit. According to the model identified by SLR, a predictive score was made. When the characteristic was present, the following scores were given: Transfusions: 2.5 points, absence of Right Dialyzers ID: 2 points, vancomycin prophylaxis: 1.5 points. The probability of seroconversion was predicted by adding the score accumulated at each DU. DU with no scoring had 1.5% probabilities of seroconversion. DU with 6 points (maximum score) had 89.3% probabilities of seroconversion.

Conclusions: A pattern has been identified which allows to predict the risk of seroconversion at each DU. The continuous surveillance of dialysis practices, educational programs and supervised use of antibiotics are essential in order to improve quality of care and reduce the impact of nosocomial infections.

Variables	SC+ ($n = 54$)	No SC ($n = 101$)	P
Decontamination clamps	19 (35.2%)	66 (65.4%)	0.0003
Transfusions*	50 (92.6%)	53 (52.5%)	<0.0001
Solid soap	28 (51.8)	31 (30.7%)	0.01
Abundant sink (HD room)	7 (12.9%)	38 (37.6%)	0.001
No sink (HD room)	19 (35.2%)	9 (8.9%)	0.0001
Right waste management	11 (20.4%)	47 (46.5%)	0.001
Proper disposable	6 (11.1%)	25 (24.8%)	0.04
Bad cleaning procedures	20 (37.0%)	16 (15.8%)	0.003
Right Dialyzers ID*	13 (24.1%)	80 (15.8%)	<0.0001
Vancomycin prophylaxis*	33 (61.1%)	13 (12.9%)	<0.0001

*Independent markers in SLR.

P1297 Risks of hepatitis C among Jordanian population

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Objectives: To study the seroprevalence positive of hepatitis C among Jordanian population.
Methods: *Study population*: recruitment to the study was conducted at various primary health care centers and various governmental and private hospitals in Jordan. 7866 sera were collected (December 1996 through December 2000). A questionnaire was completed for subject concerning personnel and epidemiological data relating to hepatitis C. Apart from demographic data, such as age and sex, the questionnaire asked for details of the home environment, previous contact with jaundice, external environments and travel abroad.

Test method: a sample of blood (3–10 mL) was taken from each subject and stored at 20 °C prior to serological analysis. Serum samples were analyzed using ELISA kit (Modified HCVab, Abbott Laboratories), a sample being considered positive for HCV IgG titer result was 10 mIU/mL or greater.

Statistical analysis: the results were expressed as the proportion of subjects in each group or subgroup, positive for HCV with a 95% confidence interval (CI). All data were summarized using SPSS (Version 10). Confidence intervals were not calculated for subgroups of less than 10 subjects. Evaluation of the relationship of each variable with HCV status was determined using the chi-squared or Fisher's exact test. Statistical significance was assumed at the conventional level of $P < 0.05$ and all quoted P-value are two-sided.
Results and conclusions: The prevalence of anti-HCV antibody among Jordanian population was tested at clinics and hospitals. A blood sample was taken to test the presence of IgG (indicating past infection) and a questionnaire concerning personnel and epidemiological data relating to hepatitis C was completed. In total 7866 samples for persons aged <10 and <60 years were suitable for the analysis. There were 2007 subjects who positive for HCV (25.5%). The proportions of subjects positive varied significantly with age ($P = 0.001$); 32.9% in the <35 years were positive compared with 25.6% aged 35 and below. There was a significant association between a positive-HCV test and social level ($P = 0.004$), with a higher proportion positive in the low social level. Persons with jaundice, live with infected persons, infected mother during pregnancy, blood transfusion or travel abroad were significantly more likely to be HCV positive ($P = 0.001$, $P = 0.004$, $P = 0.020$, $P = 0.001$, respectively).

P1298 Hepatitis C virus patient serotyping in Belgium

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Objectives: Given that both pathogenicity and the response to treatment are associated with HCV serotype, it would seem sensible to establish the prevalence of the different HCV types in Belgium. The HCV serotypes were determined in Belgian patients and possible associations with age and sex were investigated.
Methods: The serotype was identified in 68 HCV-RNA positive samples.
Results: In 55 of the 68 samples (80.9%), antibodies were identified with serotype 1 (58.8%) and serotype 3 (19.1%) showing the highest prevalence. Seventeen samples contained several serotypes with serotype 1 being detected in 82.4% of cases. There was no significant difference in the distribution of HCV types in relation to sex. Serotype 1 was detected among older patients compared with serotype 3 ($P < 0.01$) and serotypes 2 and 4 ($P = 0.05$).
Conclusion: Serotypes 1 and 3 are the most prevalent types in Belgian patients. The data suggest that serotype 1 spread earlier than serotypes 2, 3 and 4. This corroborates previous European studies.

P1299 Endogenous levels of mRNA for type I and II IFNs and IFN-related genes in liver biopsies of patients with chronic HCV infection

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Objective: To measure endogenous expression of type I and II, IFNs, IFN receptor (IFNAR-1), IFN-stimulated genes (IRF-1 STAT-1, PKR, and oligoA-S) as well as ICE and IL-18 in liver biopsies of naive patients with chronic HCV infection and in patients affected by non-infectious liver pathologies.
Methods: Liver biopsies from 24 HCV-infected chronic patients negative for HBV and HIV infection, and from six patients with NASH and one with autoimmune hepatitis were analyzed. Histological evaluation was performed by Knodell score. Gene expression was measured at mRNA level by semi-quantitative RT-PCR, and normalized to β-actin.
Results: In liver tissue from HCV-infected patients mRNA levels of Type I IFN were decreased, while those of IFN-γ, IFNAR-1, IRF-1, and IL-18 were up-regulated as compared to tissues from non-infectious hepatitis patients. In HCV-infected patients IFN-γ was positively correlated with IFNAR-1, IRF-1 and IL-18. No correlation was observed between viremia and the other parameters. ALT levels were correlated with IFN-γ, but not with IFN type I expression. Liver fibrosis was absent or mild (F0–1, $n = 13$) in the biopsies with higher IFN type I mRNA levels, while it was moderate or severe (F3, $n = 11$) in those biopsies with lower IFN mRNA levels ($P = 0.024$ for IFNα and 0.047 for IFNβ).
Conclusions: Several genes acting downstream the IFN pathway are up-regulated in the hepatic tissue from patients with chronic HCV infection, as compared to non-infectious hepatic diseases. Therefore, it is likely that this phenomenon is determined by virus-driven mechanisms. The degree of tissue damage, in terms of fibrosis, seems to be counteracted by the expression of endogenous type I IFN, which is, in turn, down-regulated in HCV-infected patients. Because HCV has evolved escape mechanisms against the IFN system, our observation can have pathogenic implications.

P1300 Long-term follow-up of a newborn with immunotolerance to HCV infection

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Objective: We presented the long-term follow-up of a vertically HCV-infected, seronegative but RNA-positive infant.
Methods: Sera markers of HCV were determined in a baby, born to an anti-HIV-negative intravenous drug-user prostitute aged 28 years. Baby was born by vaginal delivery at 38 weeks of pregnancy. His mother was anti-HCV-positive with RIBA bands for NS4(2+) and c33c(2+). His aunt was also anti-HCV-positive and presented the same RIBA profile than the mother. Serum anti-HCV was determined by using MEIA (AXSYM HCV 3.0 Abbott), whose antigen includes 4 recombinant proteins related with structural and nonstructural regions of virus genome (HCr43, c200, c100–3 and NS5). Serological results were confirmed by RIBA 3.0 (Chiron) which includes 5 peptides, 2 recombinant (c33c and NS5) and 3 synthetic (c100, 5-1-1 and c22), the first four being nonstructural proteins and the fifth a core protein of virus. Viral RNA was assayed by RT-PCR (Cobas Amplicor HCV Monitor V2.0, Roche). HCV genotype was determined with a line probe assay (InnoLIPA HCV II, Innogenetics).
Results: Infant sera results are expressed in the table. Infant was anti-HIV-negative during all follow-up. No immunodeficiency was detected and his response to measles, mumps and rubella vaccines, administered at 18 months, was positive, with appearance of antibodies against three viruses (IgG EIA indexes: 3.4, 2.6 and 2.6, respectively). Patient presented hepatomegalia during follow-up with elevated AST and ALT levels, but no increase of serum bilirubin level was detected. The infant was also tested

to exclude other infectious, metabolic or immunological causes of chronic liver diseases. Liver biopsy was refused. Mother HCV-RT-PCR was positive (2.4×10^5 UI/mL). Infant, mother and aunt were HCV genotype 4.

	Anti-HCV		RIBA 3.0							
Age (months)	Results	Index	Results	NS4	c33c	c22	NS5	RT-PCR (IU/L)	AST (U/L)	ALT (U/L)
Birth	POS	59.4	POS	4+	4+	–	–	NEG		
3	POS	18.7	POS	4+	3+	–	–	5.6×10^5	27	26
5	POS	4.9	POS	3+	2+	–	–	4.8×10^5	41	48
8	POS	1.1	NEG	±	±	–	–	2.6×10^5	102	179
11	NEG	0.2	NEG	–	–	–	–	$>8.5 \times 10^5$	192	303
16	NEG	0.2	NEG	–	–	–	–	2.6×10^5		
21	NEG	0.2	NEG	–	–	–	–	2.3×10^5	205	270
25	NEG	0.2	NEG	–	–	–	–	2.6×10^5	85	117
33	NEG	0.2	NEG	–	–	–	–	$>8.5 \times 10^5$	78	82

Conclusions: Passively acquired maternal antibodies were negative before 9th month. Infant developed a chronic HCV infection with elevation of ALT and AST levels and high viral load. HCV specific antibodies were absent as result of an infant immunotolerance to viral antigens.

P1301 Clinical, virologic characteristics and liver histology of anti HCV-positive subjects according to aminotransferases levels

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Objective: The aim of the study was to find out if the clinical and virologic findings and liver histology in patients with increased ALT levels are different as compared with patients with normal ALT levels.
Material and methods: We retrospectively studied 81 anti HCV-positive patients, HIV seronegative for which the hepatic biopsy was perform in addition to the serum HCV-RNA detected by PCR. Most of patients were women (55.6%), the mean age being 44.7 ± 11.9 years. We excluded concomitant infection with HBV, an autoimmune cause of hepatitis and hepatotoxic drug intake. The persistence of normal ALT values (in 22 patients, 27.2%) was documented by repeated testing with at least three values taken over a 6-month period. We used Ishak score to appreciate liver histology. Data were analyzed using Epi-Info version 6, the significance level being 5%.
Results: We did not find any statistically significant difference between men and women with normal ALT levels (22.2%, respectively, 31.1%). Surprisingly we found an older average age in persons with elevated ALT levels, but the mean age was under 50 years for both groups (39.7 ± 12.2 years in normal ALT group and 46.6 ± 11.3 years in elevated ALT group). The frequency of symptoms was similar in anti HCV-positive patients with normal ALT levels than in those with elevated levels (72.7%, respectively, 76.3%). Consumption of alcohol was significantly greater in the elevated ALT levels group than in patients with normal ALT levels (49.2% vs. 9.1%, OR 9.67, $P < 0.05$). Biochemical features were not different in the two groups as well as virologic characteristics of patients with chronic hepatitis C. The genotype 1 was predominant in the two groups. The histological findings (staging and grading) in normal ALT level group were mild (20/22, respectively, 21/22) and severe staging or cirrhosis was rare. The fibrosis was significantly greater in patients with elevated ALT ($P < 0.05$), while the necro-inflamation was significantly greater when ALT level was superior to two fold the normal. We did not found any statistically significant difference between the severity of histological characteristic and the presence of symptoms, either with consumption of alcohol.
Conclusion: An important number of patients infected with VHC have normal ALT levels despite having detectable HCV-RNA in serum. It seems important to evaluate the response rate to IFNα as well as the long-term outcome without treatment in these patients.

P1302 Cryoglobulinemia in Greek patients with chronic HCV infection and association with liver histology

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Objectives: To determine the prevalence of cryoglobulinemia and to identify any association between cryoglobulinemia and liver histology in HCV-infected Greek patients.
Patients and methods: 80 HCV-RNA-positive patients (44 males, 36 females, mean age 50.1 ± 16.6 years) were studied. The presence of cryoglobulins was assayed by collection and coagulation of blood at 37 °C, centrifugation and incubation of serum at 4 °C for 7 days. Liver biopsy samples were evaluated according to the staging and grading system described by Ishak et al.
Results: Cryoglobulins were detected in 22/80 (27.5%) patients. Only two patients presented an unmistakable cryoglobulinemic syndrome (vasculitis, glomeluronephritis, arthralgia). When croglobulinemic patients were compared with non-cryoglobulinemic patients, grading of necro-inflammatory activity (6.27 ± 2.21 vs. 5.84 ± 2.48, NS) and staging score (3.41 ± 1.62 vs. 3.29 ± 1.92, NS) were not found different.
Conclusions: The prevalence of HCV related cryoglobulinemia was 27.5% in our patients, although only two patients manifested a clear clinical syndrome of cryoglobulinemia. There was no association between the severity of the histological lesions and cryoglobulin presence.

P1303 Prevalence of cryoglobulinemia in HCV-infected Greek patients on chronic hemodialysis

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Objectives: To determine the prevalence of cryoglobulinemia and assess the frequency of auto-antibodies in HCV-infected Greek patients on chronic hemodialysis.
Patients and methods: 73 HCVAb(+) patients (49 M, 24 F, mean age 57.8 ± 13 years) on maintenance hemodialysis were studied. Serum HCV-RNA was detected by RT-PCR. The presence of cryoglobulins was assayed by collection and coagulation of blood at 37 °C, centrifugation and incubation of serum at 4 °C for 7 days. Rheumatoid factor (RF), antinuclear (ANA), antimitochondrial (AMA) and anti-smooth-muscle antibodies (ASMA), were detected in serum.
Results: Cryoglobulins were detected in 22/73 (30.1%) patients but the cryocrit value was very low: 2.4% in two patients, 1.4% in one, 1% in two and <1% in 17. HCV-RNA was detected in 56/73 (76.7%) patients. Rheumatoid factor was found in 21/73 (28.8%) patients, ANA in 16/73 (21.9%), AMA in 1/73 (1.4%) and ASMA in no one. There was no association between the presence of cryoglobulins and patients' age, sex, RF, ANA or HCV-RNA positivity.
Conclusion: This proportion of cryoglobulinemic patients was not different from that found in a Greek report of 87 HCV patients without renal disease.

Cryoglobulinemia is quite frequent in HCV patients on maintenance hemodialysis but cryocrit values are exceptionally low. On the contrary to what is known for HCV patients with normal renal function, no correlation was found between cryoglobulinemia and age or RF positivity.

P1304 Prevalence, genotypic profile and risk factors for HCV infection in a population of Lebanese hemodialysis patients

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Objective: Hemodialysis patients are considered at high risk of developing HCV infection. The aim of this study was to determine HCV-genotype distribution and to analyze the risk factors for HCV infection in this group of patients.
Methods: Two hundred and thirty-two patients on maintenance hemodialysis were routinely screened for HCV Ab by MEIA HCV 3.0 (AxSym, Abott). The HCV-positive samples were further analyzed using nested RT-PCR, followed by hybridization to HCV subtype core region-specific biotinylated oligonucleotide probes immobilized onto avidin coated wells. DNA/probe hybrids were detected by DNA enzyme immunoassay (DEIA). An analysis of the risk factors including transfusion history and date of starting hemodialysis was performed.
Results: Out of 232 patients, 27 (11.6%) were HCV positive, 17 were males and 9 were females, the mean age was 49 (SD 15.6 years). HCV infection was more prevalent among older patients (>50 years vs. <50 years, $P=0.001$) and those who started hemodialysis before implementation of routine HCV screening in 1993 ($P=0.001$). Gender and transfusion history was not found as a significant risk factor. Seven patients (26%) had mixed HCV infection and 16 had nona-nonb genotype 2 infection. The table below shows the genotypic profile of our study population.

	Single genotype infection $n=20$ (74%)									
Genotype	1a	1b	2	2a	2b	3a	4	5	6	Mixed infection
Nb	1	1	16	0	0	2	0	0	0	7
%	3.7	3.7	59.2	0	0	7.4	0	0	0	26

Conclusion: The prevalence of HCV infection in hemodialysis patients in our study population remains within the range usually reported (11.6%). The fact that the majority of cases (59.2%) were infected with nona-nonb HCV genotype 2 suggests a nosocomial transmission of HCV before implementation of routine HCV screening in 1993; however, further subtyping by direct sequencing is necessary to confirm this pathway.

HBV

P1305 Effect of IL-12 and IL-18 on anti-HBcAg response of Th lymphocytes in children with chronic hepatitis B

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Objectives: The typical lymphokine pattern in chronic hepatitis B (CHB) was characterized by a weak or absent antigen-specific IFN-γ production. The aim of this work was to evaluate effects of IL-12 and IL-18 on the secretory anti-HBcAg response of peripheral blood Th lymphocytes in children with CHB.
Methods: The studies included 12 children, aged 5–16 years with documented diagnosis of chronic active type B hepatitis. T CD4 lymphocytes were isolated from peripheral blood using the biomagnetic technique (Dynal) and rHBcAg stimulated cytokine production (IFN-γ, IL-4, IL-5 and IL-10), estimated by ELISPOT assays (Mabtech) in 24 h cultures. In parallel, cultures of TCD4 lymphocytes were set up, stimulated with rHBcAg in presence of IL-12 and/or IL-18.
Results: In response to rHBcAg, IFN-γ producing cells were not observed or very weak IFN-γ production was disclosed, with preserved secretion of IL-5 and IL-10. In turn, IL-4 producing cells could not be detected. After costimulation with IL-12 and rHBcAg a significant number of IFN-γ producing cells was detected with no significant changes in secretion of the remaining cytokines. A most pronounced increase in the number of IFN-γ producing cells was observed following rHBcAg stimulation of Th lymphocyte cultures in presence of IL-12 and IL-18.
Conclusion: IL-12 and IL-18 synergically activate a strong, antigen-specific IFN-γ production in Th lymphocytes of CHB-affected children.

P1306 Cellular gene activation by HBV integration in or close to chromosomal regulative elements: a hypothesis from the WHV/Woodchuck model

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Aim: In the WHV/Woodchuck model for HBV-induced HCC, the N-*myc2* proto-oncogene can be activated by WHV integration either close to the gene or in the b3n and win noncoding loci located 10 and 150 kb downstream, respectively. A matrix attachment region (MAR) is located in b3n. MARs are noncoding regulative elements of chromosomal DNA. Aim of this work was to identify and characterize further cases of WHV insertion in b3n in naturally occurred HCCs to better clarify the possible involvement of the MAR element in mediating N-*myc2* activation.
Methods: Search for integration in b3n was carried out by Southern blotting/hybridization. Integration was characterized by PCR and sequencing. N-*myc2* transcription was assayed by Northern blotting.
Results: Fifteen Woodchuck tumors were screened for integration in b3n. One tumor showed an additional, WHV positive, fragment. Sequencing showed WHV insertion to be located a few nucleotides adjacent to the MAR, closer than previously described integrations. Tumor-specific N-*myc2* transcripts indicated activation of the gene.
Conclusions: The data reinforce the hypothesis that N-*myc2* activation by integration in b3n might be mediated by deregulation of the noncoding MAR element located nearby WHV insertions. This mechanism has never been investigated in human HCC. Several HBV insertions occur in noncoding regions, whose function(s) cannot easily be investigated because suitable approaches are unavailable. Clarification of the mechanisms involved in N-*myc2* activation by WHV insertion in the b3n and win noncoding loci may significantly contribute to elucidate basic mechanisms for better understanding the role of HBV integration in HCC.

P1307 Peginterferon α-2a (40 kD) (PEGASYS) therapy for HBeAg-positive chronic hepatitis B (CHB): 48-week end of follow-up results and evaluation of the prognostic factors of response

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Background: Recipients of peginterferon α-2a (40 kD) have recently been shown to exhibit improved 24-week end of treatment responses compared with patients given standard interferon (Roferon A). (Cooksley WGE et al. Hepatology 2001; 34: 349 A. Abstract 710.)
Objectives: To assess the 48-week end of follow-up responses following 24 weeks' treatment with peginterferon α-2a (40 kD) or Roferon A, and to evaluate of the prognostic factors of response, in the treatment of HBeAg-positive CHB.
Methods: A total of 194 adults, who had not previously been treated with interferon-α or had received <6 months of nucleoside analogue therapy in the preceding 6 months were enrolled into a Phase II, multicenter study. All patients were serum-positive for HBsAg, HBeAg, HBV DNA and demonstrated biopsy-proven CHB with ALT > 2× ULN. They were randomized (1:1:1:1) to receive Roferon A (4.5 MIU tiw) or peginterferon α-2a (40 kD) (90, 180 or 270 μg qw) for 24 weeks and followed-up for an additional 24 weeks. Efficacy was assessed by measurement of HBeAg and HBV DNA (AMPLICOR HBV MONITOR test), but the study was not powered to assess the effects of the individual doses of peginterferon α-2a (40 kD). A logistic regression model was used to estimate the effects of baseline HBeAg, HBV DNA and serum ALT on response rates at 48 weeks.
Results: An HBeAg positive response (defined qualitatively as 'negative' or quantitatively as '<0.280 peiu/mL') occurred at 48 weeks in 25.5, 36.7, 34.8 and 29.2% of patients given Roferon (4.5 MIU tiw) and peginterferon α-2a (40 kD) (90, 180 or 270 μg qw), respectively. The greatest drop in HBV DNA was produced by peginterferon α-2a (40 kD) 180 μg qw; $\log_{10}$ 3.5 at the end of treatment. Preliminary analyzes suggest that certain prognostic factors associated with standard interferon therapy such as baseline ALT may be less important with the use of peginterferon α-2a (40 kD).
Conclusion: Peginterferon α-2a (40 kD) at doses of 90 and 180 μg qw, demonstrated similar sustained responses that were substantially higher than those produced by Roferon A (4.5 MIU tiw). However, peginterferon α-2a (40 kD) dosed at 180 μg qw produced the greatest drop in HBV DNA and quantitative eAg during treatment period. The reasons for the significant improvement in efficacy in the use of peginterferon α-2a (40 kD) over standard interferon in chronic hepatitis B will be discussed.

P1308 Differential immunogenicity of a recombinant hepatitis B vaccine in Iranian neonates: influence of ethnicity and environmental factors

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Objectives: To compare immunogenicity of a recombinant hepatitis B (HB) vaccine in two groups of neonates born in two cities of Iran with different geographic and ethnic backgrounds.
Methods: Ten micrograms of a recombinant HB vaccine (Heberbiovac) was administered under field condition to Iranian healthy neonates at 0, 1.5 and 9 months intervals. The subjects consisted of two groups of 290 and 231 neonates selected from two cities located at north-west (Uromia) and south-east (Kerman) of Iran, respectively. The level of anti-HBs antibody was quantitated in serum 2–4 weeks after administration of the last vaccine dose, by sandwich ELISA.
Results: A higher seroprotection rate (anti-HBs > 10 IU/L) (98.3% vs. 96.1%) and significantly increased serum anti-HBs antibody titer (11869 vs. 6104 IU/L) ($P < 0.001$) were induced in vaccinated neonates from Uromia city, compared to those born in Kerman.
Conclusion: These findings suggest contribution of ethnic and/or environmental factors in the antibody response to recombinant HB vaccine in human.

P1309 Revaccination with a single low dose of a recombinant hepatitis B vaccine induces protective antibody response in the majority of healthy nonresponder neonates

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Objectives: To evaluate the influence of supplementary vaccination with a single dose of 2.5, 5 or 10 μg of a recombinant hepatitis B (HB) vaccine in healthy nonresponder neonates to primary vaccination.
Methods: Three different doses (2.5, 5 and 10 μg) of a recombinant HB vaccine (Heberbiovac) were administered i.m. at 0, 1.5 and 9 months of age to three large groups of healthy Iranian neonates. Serum samples were collected 2–4 weeks after completion of vaccination and anti-HBs was quantitated by sandwich ELISA. Immediately, the fourth vaccine dose of the same concentration was administered to nonresponder neonates and anti-HBs was also measured in their serum.
Results: Administration of fourth vaccine dose induced seroprotection (anti-HBs > 10 IU/L) in 10/12 (83%), 10/12 (83%) and 21/24 (88%) of nonresponder neonates in 10, 5 and 2.5 μg groups with geometric mean titer of 8012, 4421 and 1850 IU/L, respectively.
Conclusion: These results indicate that a significant proportion of nonresponder neonates can be induced to develop a protective anti-HBs response following administration of a single supplementary dose.

P1310 Long-term investigation of new-borns of HBsAg positive mothers vaccinated against hepatitis B

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Objectives: Protective titers of anti-HBs antibodies after immunization, vanishing of anti-HBs in long-term period and breakthrough infections after immunization were investigated in 533 neonates of HBsAg-positive mothers vaccinated against hepatitis B.
Methods: Combine passive-active immunization was commenced in 1988. The number of immunized children gradually increased and the group included 533 new-borns in November 2001. All mothers were HBsAg-positive during pregnancy, 27 of them were also HBeAg positive. The children received hepatitis B immunoglobulin at birth and three 10 μg doses of plasma-derived or recombinant vaccine at interval 0, 1 and 6 months (only 18 children of HBeAg-positive mothers at interval of 0, 1 and 2 months). The immunization schedules were completed in 497 children, serologic investigations after immunization were performed in 478 children. Blood samples were obtained after completion of immunization schedule, at 2 years of age, and biennially thereafter. Samples were tested by ELISA for HBsAg, anti-HBs, anti-HBc, anti-HBe and anti-HBcIgM.
Results: Anti-HBs antibodies were tested in 354 children during 2 months after immunization. Protective titres of anti-HBs were proved in 335 of 354 children (94.6%). Vanishing of protective anti-HBs antibodies were detected in 137 of 478 children (28.7%) with serologic investigation after immunization. Disappearance of anti-HBs was observed in 7.9, 16.5 and 23.6% children during second, fourth and sixth year of life. Only one child suffered from mild, acute hepatitis B in the beginning of the second year of his life. The child was infected with variants of HBsAg with substitutions at residues 137 and 139. Asymptomatic infections with presence of anti-HBc antibodies were observed in four children since fourth till seventh year of life. The increase of anti-HBs without revaccination was observed in 54 children.
Conclusions: Protective titers of anti-HBs after immunization in 94.6% children, vanishing of protective anti-HBs during 8 years in 28.7% children, one hepatitis B and 4 asymptomatic infections were proved in 533 children vaccinated against hepatitis B during 14-years monitoring.
Grant support: Ministry of Health, Czech Republic, IGA No. NI6047-3.

P1311 Vaccination of patients with chronic renal failure against hepatitis B

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Objectives: Anti-HBs antibodies response after immunization against hepatitis B and breakthrough infections were investigated in 540 patients with chronic renal failure from 4 dialysis units and their out-patients departments. Of these patients, 182 died during investigation.
Methods: Active immunization against hepatitis B was commenced in 1988. The number of immunized patients gradually increased and the group included 540 patients in November 2001. The vaccination schedule was 0, 1, 2 months for dialysis patients and 0, 1, 6 months for predialysis patients. Plasma-derived or since 1990 recombinant vaccine were administered intramuscularly, each vaccine contained 40 μg of HBsAg, but for predialysis patients only 20 μg till 1998. The immunization schedules were completed in 367 patients. Blood samples were obtained after third or next dose of vaccine and biannual thereafter. Samples were tested by ELISA for HBsAg, anti-HBs, anti-HBc, anti-HBe and anti-HBcIgM. The patients without protective anti-HBs level after vaccination were once or twice revaccinated. The patients with vanishing of anti-HBs antibodies were also revaccinated.
Results: Anti-HBs antibodies after vaccination were investigated in 309 patients. Protective anti-HBs level were proved in 152 of 309 patients (49.2%) after immunization and in 184 (59.5%) or 206 (66.7%) patients after fourth and fifth dose of vaccine. The new HBsAg positive status was proved in 28 dialysis patients, most of them suffered from acute hepatitis B. These breakthrough infections were more frequent after initiation of vaccination program, the latest breakthrough infection was proved in 2000 and previous four infections in 1994. Asymptomatic infections with new appearance of anti-HBc antibodies were proved in nine patients. The increase of anti-HBs without revaccination was observed in 38 patients.
Conclusions: Vaccination of patients with chronic renal failure is not satisfactory, only 66.7% of patient developed protective anti-HBs level after five doses of vaccine, but long-term vaccination considerably reduces hepatitis B incidence in this group of patients.
Grant support: Ministry of Health, Czech Republic, IGA No. NI6047-3.

P1312 Seroprevalence of hepatitis B and D in rural Egypt: a prospective study

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Introduction: The consequences of acute and chronic hepatitis B virus (HBV) infection represent a major health problem in Egypt. Individuals with chronic HBV infections are predisposed to an increased risk of developing hepatocellular carcinoma. In 1992, HBV vaccine was integrated into the infant immunization schedule throughout Egypt.
Objectives: We describe a prospective study done in the summer of 1999, in two villages, one in Qena governorate, Upper Egypt (1462 individuals in 235 families) and the other in Sharquiya governorate, the Nile Delta (1273 individuals in 279 families), to evaluate the prevalence of HBV and HDV and to assess associated risk factors.
Methods: Sera collected from both cohorts were tested by ELISA for total anti-HBc and HBsAg. Positive HBsAg sera were tested for anti-HDV IgM. All tests were performed using commercial kits.
Results: There is an age-dependent increase in anti-HBc antibodies with an overall seroprevalence of 42.5% ($n=621$) and 18.2% ($n=232$) for Qena and Sharquiya, respectively. However, chronic infection rate as measured by positive sera for total anti-HBc only, showed prevalence rates (PR) of 32.7 and 16.5% (Qena and Sharquiya, respectively). Overall, seroprevalence of HBsAg was 11.6% ($n=170$) in Qena and 2.8% ($n=36$) in Sharquiya. Carrier rate, however, as indicated by positive HBsAg alone was 1.9% ($n=28$) and 1.1% ($n=14$) for Qena and Sharquiya, respectively. The PR for anti-HBc and HBsAg were 8.5% ($n=11$) and 7.7% ($n=10$) in Qena and 2.4% ($n=3$) and 1.6% ($n=2$) in Sharquiya. There was no significant difference for both markers between children who have or have not received vaccination. Anti-HDV PR, for HBsAg positive sera, was 16.5% ($n=28$) and 5.5% ($n=2$) for Qena and Sharquiya, respectively. None of the individuals with both HBV markers reported jaundice signs. Among the families surveyed in Qena and Sharquiya, 42% ($n=50$) and 24% ($n=14$) of these families had more than one HBsAg positive individual. Comparison between HBsAg positive and negative individuals for potential risk factors, emphasize the role of blood transfusion in HBV transmission in both sites ($P=0.0001$) when compared to injections, surgeries, dental work and shaving ($P=0.35$, 0.20, 0.45, 0.22, respectively)
Conclusion: Although chronic and/or past infection rates for HBV are high in both study communities, these results suggest that active transmission is ongoing and prevention and control measures should be strictly enforced.

P1314 Ten year study of histological variation of liver and laboratory findings in 100 healthy hepatitis B carrier

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Objectives: Hepatis B (HBV) is the most common cause of chronic hepatitis worldwide. One of the potential dangers of healthy HBV carriers, a part from afflicting others is the emergence of chronic hepatitis and eventually liver cirrhosis, liver cancer and, the resultant death. The objective of this study was to investigate the variations of liver histology along with other laboratory findings.
Methods: In a descriptive study 100 healthy HBV carriers whose positive HBsAg was longer than 6 months were studied. First they underwent liver Enzymes, sonography and biopsy. If the tests were normal, the patients underwent repeated tests except for liver biopsy, every 6 months. In the event of an increase in liver enzymes or α-phetoprotein, and sonographic changes, they underwent liver biopsy and the results were recorded.
Results: thirty percent of the healthy HBV carriers experienced no specific laboratory changes in periodic tests in 10 years. Seventy percent of the patients did experience lab changes consisting of an increase in ALT and AST and bilirubin and three cases of increase in α-phetoprotein and some sonographic changes like liver coercing. Nodule formation and in one case, liver mass were reported. The aforementioned patients underwent liver biopsy. In this group 30 persons under 5 years and 40 persons over 5–10 years experienced lab changes. The results of their liver biopsy are as follows. In 30-subject group: 5 persons (16%) afflicted with C.P.H Grade 1, 15 persons (50%) afflicted with mild C.A.H Grade 3, 7 persons (23.3%) afflicted with moderate C.A.H. Grade 3, 3 persons (10%) had no abnormal histological variations. In 40-subject group: 25 persons (62.2%) afflicted with severe C.A.H. Grade 4, 2 persons (5%) afflicted with C.P.H. Grade 1, 2 persons (5%) afflicted with early cirrhosis stage 3, 1 person (2.2%) afflicted with heptoma, 10 persons (25%) afflicted with mild C.A.H. Grade 2
Conclusion: With regard to the fact that healthy HBV carriers can potentially endanger others in the society and cause high costs for taking care of such patients, and vain attempts to cure them, the best thing to do is to train people how to prevent the disease and how to avoid passing the virus to others. Afflicted people are recommended to undergo clinical and paraclinical tests at least every 6 months so that the progression of the disease towards chronic hepatitis, cirrhosis and liver cancer can be checked.

P1315 Seroepidemiological study on hepatitis B infection prevalence in Bulgaria

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Introduction: Bulgaria is a country with intermediate endemicity of hepatitis B viral (HBV) infection. As a result of the universal infant immunization program successfully implemented since 1992, the incidence rate of acute

hepatitis B in the targeted children age group was dramatically reduced. However, in the older age groups and especially in adolescents and young adults, the annual incidence rate of acute clinically manifested cases retained its constant level varying between 45.4 and 63.2/100 000 in the age group 15–19 and between 32.9 and 42.3/100 000 in the age group 20–29 in the recent 5 years.

Objectives: To perform a seroepidemiological analysis of the HBV prevalence in the general population of 14–60 years of age and to evaluate the HBV burden on that part of the population unprotected by vaccination.

Methods: A multicentre study was conducted in the biggest cities in Bulgaria: Sofia, Plovdiv, Varna, Pleven and Stara Zagora in the period 1999–2000, comprising a total of 11 597 healthy nonimmunized persons distributed in 5 age groups: 14–19; 20–29; 30–39; 40–49 and 50–59 years of age. After an informed consent, blood samples were obtained and tested for HBV markers: HBsAg, anti-HBs and anti-HBc using commercially available enzyme immunoassay (Sorin Biomedica).

Results: Seronegative for HBV markers were 72.54% from a total of 11 597 persons. HBsAg carriage was proven in 3.86% on the average, varying from 1.96 to 5.26% in the different study sites, and within significantly broader limits in the different age groups (1.52 to 5.72%). A total of 27.46% of the tested subjects showed evidence of previous or present HBV infection (from 17.77% in Sofia to 38.57% in Plovdiv), and from 8.48% in the 14–19 age group to 40.70% in the 40–49 age group, results corresponding to the surveillance data of the annual incidence rate of acute HBV cases.

Conclusions: The results obtained confirm the data of previous observations, which proves that the HBV endemicity in Bulgaria is characterized by an intermediate prevalence of HBV. The distribution of HBV infection is unequal in the different areas of the country as well as in the different age groups.

P1316 Hepatitis B in Catalan adopted or immigrant children

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Introduction: Hepatitis B is an important public health problem in many parts of the world. About 300 million people (5% of world population) are infected, which allows an endemic maintenance of the virus. More than 10% of south-east Asia and tropical Africa inhabitants are viral carriers. A great number of children born in these areas attend our hospital, which is a Reference Center for Pediatric Imported Diseases in Catalonia, after arrival to our country. In our community, systematic hepatitis B vaccination is carried out during the early adolescence since 1990.

Objectives: To know the prevalence of hepatitis B infection in adopted or immigrant children attending our hospital, as well as their infective status.

Methods: Two hundred and seventy-five adopted or immigrant children aged one month to 13 years (16.3% less than 1-year-old and 57% with ages between 1 and 2 years) were controlled at the Pediatric Imported Diseases Unit from January 2000 to October 2001. Hepatitis B surface antigen (HBVsAg) was determined in all them as a screening test. In case of positivity, hepatitis B e antigen (HBVeAg) and hepatitis B e antibody (HBVeAb) were also studied. All tests were performed by MEIA methodology in an Axsym System (Abbott).

Results: HBVsAg was positive in 15 patients (5.45%), aged 11 months (one patient), 1–2-year (12 patients), 3 years (one patient) and 4 years (one more patient). All of them had positive HBVeAg but negative HBVeAb. Children came from India (nine from Calcutta, one from Bombay and one from New Delhi), China (one), Ukraine (two) and Ivory Coast (one). There was none declared case of acute hepatitis B in children less than 5-year-old in Catalonia during 1999 and four cases in children aged 5–19 years.

Conclusions: There was a high infection rate in the studied pediatric population compared to that of Spanish children in a similar age range. The presence of HBVeAg in all cases demonstrates their infectivity. The introduction of children at risk of transmission of hepatitis B among our autochthonous population, which is not systematically vaccinated at early or adult ages, is a reason for vaccination of close contacts of these children.

P1317 Prevalence of HBsAg, anti-HBs, anti-HBc total, anti-HBc IgM, anti-HCV and anti-HAV IgG in hemodialysis patients

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Objective: To investigate the prevalence of HBsAg, anti-HBs, anti-HBc total, anti-HBc IgM, anti-HCV and anti-HAV IgG positivity in hemodialysis patients.

Methods: The positivity of HBsAg, anti-HBs, anti-HBc total, anti HBc IgM, anti-HCV, anti-HAV IgG were determined in 59 patients undergoing hemodialysis (the average age 47.2 ± 16.9, 31 men and 28 women).

Results: The positivity of HBsAg, anti-HBC total, anti-HBs, anti-HCV and anti-HAV were percentage 6.8, 45.8, 62.7, 81.4 and 94.9, respectively. While all patients with HBsAg positivity have anti-HBc total positivity, no anti-HBc IgM positivity was determined. Co-positivity of both HbsAg and anti-HCV were found in four patients (6.8%). The average duration of hemodialysis was 7.3 months (1 month–5 years) in 11 anti-HCV negative patients. The average duration between the beginning of hemodialysis and positivity of markers was 22.3 months (2 months–3 years). Anti-HBs positivity was due to seroconversion in 22 patients and vaccination in 15 patients.

Conclusion: High seroprevalance of hepatitis markers were found in hemodilaysis patients. Hepatitis markers must be checked regularly in hemodialysis patients and infection control protocols should be strictly used in hemodialysis units.

P1318 Intrafamilial transmission of hepatitis B virus

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To determine the possible route of hepatitis B virus (HBV) transmission among family members, 240 family members (42 spouses, 32 offsprings, 34 mothers, 35 fathers, and 97 siblings) of 84 HBsAg carriers, and 384 healthy individuals (control group) were screened for HBV markers by ELISA method. The median age was 33 ± 14.62 in the study group, and 35.0 ± 10.08 in the control group. The χ^2 and Fisher's exact tests were used for statistical analysis. The prevalences of HBsAg and seropositivity among the family members (29.6 and 43.8%, respectively) were significantly higher than the control group (9.6 and 29.7%, respectively, $P < 0.05$). Seropositivity was observed to increase by age. The prevalences of HBsAg and seropositivity were higher among the spouses (70.0 and 90.0%, respectively) and offsprings (66.7 and 100%) of female index cases, and siblings (40.2 and 49.5%) of all index cases than the other family members. Among the 37 offsprings of the 14 families with an HBsAg positive father, 16 (43.2%) were HBsAg, and 3 (8.1%) were anti-HBs positive. Among the 21 offsprings of the five families with an HBsAg positive mother, 9 (42.8%) were HBsAg and 1 (4.8%) was anti-HBs positive. However, in the five families with both parents HBsAg positive, 4 (44.4%) of 9 offsprings were HBsAg, and 4 (44.4%) were anti-HBs positive. A total of 23 siblings of the index cases who both parents negative, 7 (30.4%) were HBsAg-positive and 4 (17.3%) were anti-HBs positive. These data suggest that transmission of HBV to offsprings was very high in case both of the parents were positive, but there was no difference in case only mother or father was positive. As well as mother, HBV infection of the father had increased the prevalence of HBsAg. Despite a high HBsAg prevalence in the offsprings of female index cases, HBsAg and anti-HBs prevalences in the mothers of index cases were not higher than the control group ($P > 0.05$). According to these data, mother alone has not a considerable role to acquire of HBV infection. In addition to these results, a high seropositivity rate among siblings of index cases suggested that both parents-to-child and sibling-to-sibling horizontal transmission might be the main route of intrafamilial spread of HBV infection. The number of HBeAg positive patients (four index cases, and three family members) was not sufficient to determine the role of HBeAg in the intrafamilial transmission.

P1319 Economic evaluation of preventive therapy in neonates from HbsAg seropositive mothers

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Introduction: Human-HBIG is administered to neonates, born from HbsAg seropositive mothers, in order to achieve passive immunization against HBV infection. The Human-HBIG preparation used was packed in 5.0 mL bottles. The dose given once to every mother carriers' newborn is 0.5 mL. If no other Human-HBIG is needed for another case, the 4.5 mL of Human-HBIG is rejected for safety reasons (Company's recommendation).
Objectives: The purpose of our study was to evaluate the cost of preventive HBV therapy in neonates from HbsAg positive mothers.
Methods: In 'Elena Venizelou' Maternity Hospital all women in labor are tested for hepatitis B whether they were tested during pregnancy or not. The method we used to test the serum samples for HbsAg, was Abbott microsomatic ELISA. In the total of neonates from HbsAg seropositive mothers, who were born from 1/1/1997 until 31/8/2000 were determined: (a) The total number of 5 mL bottle of Human-HBIG used. (b) The total quantity of administered Human-HBIG. (c) The total quantity of rejected Human-HBIG. (d) The total cost of the administered Human-HBIG quantity. (e) The total cost of the rejected Human-HBIG quantity, based on the medicine's price (90.815 GDR per bottle for the year 2000).
Results: In our hospital during the period from 1/1/1997 to 31/8/2000, 20.064 births took place. 691 births were from HbsAg seropositive mothers (3.44%). From the above-mentioned seropositive women were born 685 babies, 10 twin and a stillborn child.595 bottles of Human-HBIG were used in total. (Five bottles for the 10 twin, 95 bottles for 190 babies born in the same day and 495 bottles for the 495 babies.) The total administered quantity of Human-HBIG was 347.5 mL. The total rejected quantity of Human-HBIG was 2.6275 mL. The total cost was 54 034 925 GDR 6 311 643 GDR (11.68%) was the value of the administered quantity and 47 723 281 GDR (88.31%) was the value of the rejected quantity of the medicine.
Conclusion: Strategy to reduce these costs should be directed at using smaller package of Human-HBIG (since 1 mL bottle is available), in order to achieve passive immunization in neonates born from HbsAg-carrier mothers.

P1320 Effects of interferon-α therapy on cardiac functions in patients with chronic hepatitis B infection

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Various types of cardiovascular complications, such as myocardial infarction, ventricular fibrillation, cardiomyopathy, myocarditis and atrioventricular block, attributed to interferon therapy, had been reported previously. The aim of this study was to evaluate the cardiac effects of interferon-α therapy in patients with chronic hepatitis B (CHB) infection. Forty-five patients with CHB (42 male and 3 female; mean age 34.2 ± 11.5 years) were included in the study, and 10 MU interferon-α-2β was administered three times a week for 6 months to these patients. The cardiac evaluation (detailed medical history, physical examination, electrocardiography and systolic, diastolic functions parameters by the echocardiography) was performed at the beginning and 1st, 3rd and 6th months of the therapy. No patient had any cardiac symptoms before and during interferon therapy. Systolic and diastolic blood pressures, heart rate were not significantly affected during therapy ($P > 0.05$). None of the patients revealed cardiac rhythm disturbance on electrocardiography before and during therapy period. No significant changes were detected in systolic (ejection fraction, fractional shortening, preejection period, left ventricular ejection time, the ratio of pre-ejection period/ejection time, Q–V peak) and diastolic (E peak, A peak of transmitral flow velocity, E/A ratio, deceleration time, isovolumic relaxation time by the conventional echocardiography, and E peak, A peak, deceleration time of E wave at the medial and lateral corners of mitral annulus by tissue Doppler echocardiography) left ventricular function parameters between at the beginning and at the 1st, 3rd, 6th months. The results of this study suggest that interferon-α therapy do not significantly deteriorate cardiac functions in patients with CHB infection, and it may be used safely in patients who have not previously cardiac disease.

Hepatitis

P1321 Changing seroepidemiological patterns of HAV infection in Konya, Turkey between 1998 and 2001

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Objective: The seroepidemiology of hepatitis A virus (HAV) was assessed among the patients with prediagnosis of viral hepatitis.
Methods: Between the period of 1998 and 2001, 9638 sera from the patients with the prediagnosis of viral hepatitis were tested for the presence of anti-HAV total antibodies and 9519 sera for the presence of anti-HAV IgM by using ELISA method (DiaSorin s.r.l. 13040 Saluggia (VC), Italy).
Results: Between the period of 1998 and 2001 anti-HAV IgM seropositivity was 12.9, 10.2, 6.5 and 5.9%, respectively. Infection rates decreased significantly with years from 1998 to 2001 ($P < 0.05$). It was observed that the majority of acute HAV infections were taking place between the months October and March. Anti-HAV total seropositivity was 71.9, 79.8, 82.5 and 88.5% during the years 1998–2001. Anti-HAV antibodies rates increased significantly with years from 1998 to 2001 ($P < 0.05$). No difference in the incidence rates for anti-HAV antibodies found between males and females ($P > 0.05$).
Conclusion: These observations indicate that HAV infection is endemic in Konya in Turkey. It is also observed that the incidence rate of anti-HAV IgM antibodies is decreasing and the incidence rate of anti-HAV total antibodies is increasing with years from 1998 to 2001. The increasing incidence of anti-HAV total antibodies may be related to active immunization against HAV infection and decreasing incidence of anti-HAV IgM antibodies may be related to sanitary improvements and also active immunization in recent years. Ongoing vaccination of children may prevent future outbreaks.

P1322 Decline of maternal HAV antibodies in a neonatal cohort

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Introduction: The anti-hepatitis A virus (HAV) vaccination in infants would guarantee the control of infection. The vaccine is safe and well-tolerated in 5-month-old babies. However, the immunogenicity of the HAV vaccine in infants could be impaired by the presence of passively acquired maternal HAV antibodies.
Objectives: In the present study, we evaluated the prevalence of HAV antibodies in women at delivery in a low-endemicity area and the decline of passively acquired maternal HAV antibodies in the first year of life in infants born by HAV-positive mothers.
Methods: We enrolled 103 women at delivery and evaluated their babies in the first year of life (at birth, 3, 6, 11 and 12 months), after obtaining informed consent. Anti-HAV antibody levels were determined by MEIA test (HAVAB 2.0 Abbot Italia). Anti-HAV levels >10 mIU/mL were considered protective.
Results: Eighteen out of the 103 enrolled mothers (17.5%) had anti-HAV serum level >10 mIU/mL. At birth infants exhibited anti-HAV antibody

levels that were similar to the levels of their mothers. In the 18 positive infants, the anti-HAV levels declined after birth during their first year of life. The geometric mean diminished from 1388.9 mIU/mL at birth down to 15.3 mIU/mL at 12 months. Anti-HAV level was still >10 mIU/mL in 6 out of 6 infants, in 12 out of 13 and in 11 out of 18 babies, respectively, at 3, 6 and 12 months. Two out of 85 infants born by anti-HAV-negative mothers and negative at birth, were found positive at 5 months of age (210 and 2480 mIU/mL) and remained positive until 12 months (12 and 42 mIU/mL, respectively).

Conclusion: There seems to be no reason for not including anti-HAV immunization in childhood vaccination programs, even in low endemic HAV areas. We propose to screen all women at delivery for anti-HAV antibodies: children born by anti-HAV-negative mothers could be vaccinated early during the first year of life, children born by anti-HAV-positive mothers could procrastinate vaccination, if necessary.

P1323 Hepatitis A virus infection and coronary artery disease

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Over the past decade, awareness of possible association between atherosclerosis and infections has steadily increased. Specific organisms that have been implicated include *Chlamydia pneumoniae, Helicobacter pylori,* herpes simplex virus, cytomegalovirus, and hepatitis A virus (HAV). However, several prospective seroepidemiological studies failed to demonstrate an association between these infections and atherosclerosis. The aim of this study was to evaluate an association between HAV infection and coronary artery disease (CAD) by using serologic tests. A total of 360 individuals undergoing coronary angiography, included in the study. Two hundred and sixty-four patients who had angiographic evidence of atherosclerosis included in CAD group, and 96 patients who had no angiographic evidence of atherosclerosis included in control group. Serological status of the cases was assessed by ELISA test measuring IgG antibodies to HAV. Risk factors for CAD (age, sex, smoking, diabetes, dyslipidemia, hypertension and C-reactive protein (CRP) levels) were analyzed. Association of HAV infection with CAD, risk factors, and other characteristics were investigated using t-test, χ^2-test, and regression modeling. Mean age was 56.9 (range, 35–89 years) in CAD group, and 54.4 (range, 37–74) in control group. Two hundred and sixty-three (99.6%) of the 264 subjects in CAD group, and 94 (97.9%) of the 96 subjects in control group had IgG antibodies. There was no difference between the groups in respect to HAV seropositivity ($P < 0.05$). The odds ratio was 0.18, and 95% CI; 0.016–1.99. Due to a very high seroprevalence, we could not perform further analysis for seronegative and seropositive individuals. There was a higher prevalence of smoking, diabetes and hypertension in CAD group than in control group ($P < 0.05$). In addition, mean LDL (132.1 ± 31.6 mg/dL vs. 92.3 ± 31.9 mg/dL, $P < 0.001$), and CRP levels (1.15 ± 3.2 mg/dL vs. 2.41 ± 5.24 mg/dL, $P < 0.01$) were higher in CAD group than in control group. Smoking, elevated LDL, trygliceride and total cholesterol levels were also associated with increased levels of CRP ($P < 0.05$). However, no significant association were found between CRP levels and HAV seropositivity ($P > 0.05$).

In conclusion, it is impossible to evaluate the possible association between HAV infection and CAD by using the seroepidemiological method, especially in the population who have high seroprevalence rates.

P1325 Trends in the epidemiology of acute hepatitis in northern Greece during the 1990s

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The objectives of this retrospective study were to present and analyze the acute hepatitis cases admitted to the Medical Clinic of Infectious Diseases Hospital of Thessaloniki, Greece during the last decade.

Methods: In this study, 1898 adult patients with acute hepatitis (mean age [mean ± S.D.]: 31.16 ± 13.0 year), 1244 males and 654 females were included. Type of hepatitis, gender, age, duration of hospitalization and disease outcome were recorded and estimated during a period of 10 years.

Results: (1) As to the type of hepatitis: HAV infection represented 21.6%, HBV 60.6%, HCV 6.06%, acute HBV superimposed on chronic HCV 2.64%, acute HCV on chronic HBV 0.93%, acute HAV on chronic HBV infection 1.2%, and HDV 0.50%, while of unidentified type 6.4%. (2) As to the gender, in the females, as compared with the males, the proportion of hepatitis B and the unidentified type was greater ($P < 0.01$ and $P < 0.05$, respectively). On the contrary, men had hepatitis C at a higher ratio ($P < 0.01$), as well as coinfections ($P < 0.001$). (3) As to the age, elder people (>40 year), as compared with the younger ones, had a longer duration of hospitalization ($P = 0.000$), much higher mortality ($P < 0.001$), in a much higher ratio hepatitis B ($P < 0.001$) and of unspecified type ($P < 0.001$), but in a much lesser proportion hepatitis A ($P < 0.001$) and coinfections ($P < 0.001$). (4) As to the year of admission, during the 2nd 5-year period (1996–2000) a significant decrease in the total hepatitis cases was observed, in comparison with the 1st period (1991–1995) (871 vs. 1027, $z = 3.514$, $P < 0.000$). This was mainly due to the decrease of hepatitis B cases ($P < 0.001$), while an increase in hepatitis A cases and coinfections was indicated ($P < 0.01$ and $P < 0.001$, respectively). (5) As to the outcome, there were 55 severe (fulminant + subacute) hepatitis cases (2.89%), out of which 40 succumbed (72%), the unidentified cases having proportionally much higher mortality rate ($P < 0.001$).

Conclusion: HBV infection still remains at the top of the list, with preference to the females and the elder people, while HAV infection is more common at the younger ages and HCV infection in the males. During the 2nd 5-year period of the 1990s, a reduction in HBV infection was noticed, due presumably to the vaccination schedule. On the other hand, the increase in the HAV cases during the same period, could be attributed to the rise of the susceptible elder individuals, after the continuously improving sanitary conditions in our country.

P1326 Five-year prevalence of viral hepatitis B and C

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Objectives: To assess the prevalence of infection with viral hepatitis B and C agents in the patients of a medical department of a tertiary hospital in a period of 5 years.

Methods: Retrospective study of the notes of all patients admitted in our department in the last five years, during which all patients were screened for hepatitis B and C. The HBV marker used was HbsAg, detected by third generation ELISA and HCV antibodies were detected with a third generation anti-HCV ELISA, followed by immunoblotting (RIBA 3) if the test was positive.

Results: Anti-HCV positivity was found in 1.8% of the patients and 2% were positive for HBsAg. There was no difference in prevalence of hepatitis B or hepatitis C between males and females. In multivariate analysis, the variables significantly associated with HCV seropositivity were age older than 40 years and history of blood transfusion. A decline in anti-HCV prevalence was observed in patients over 85 years of age. Seropositivity of both HBsAg and anti-HCV was strongly associated with a history of intravenous use of illegal substances. Prevalence of HBsAg was significantly higher (20.4%) among patients who were immigrants.

Conclusions: The higher prevalence of both chronic HBV and HBC in a hospitalized population than the estimated prevalence below 1% in the general population through studies on blood donors and army recruits, is due to the increased age of the patients admitted in medical departments.

P1327 A hospital outbreak of hepatitis E in Karachi, Pakistan with possible parenteral transmission

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Objective: To investigate a nosocomial outbreak of hepatitis E involving 6 serologically confirmed and 12 presumptive cases that occurred on a neurosurgery ward at a public teaching hospital.

Methods: A case–control study. Health care practices were assessed by interviews of ward staff, and selected laboratory investigations were performed on patients, health care workers (HCWs), and water samples. Age-matched controls were randomly selected from patients admitted for more than 2 weeks to the ward during October 2000–February 2001.
Results: Water samples from ward sources ($n = 10$) were free of fecal coliforms. Due to a shortage of nursing staff, the practice of sharing an intravenous administration set among patients was reported for dexamethasone and mannitol infusions. Females were more likely to develop hepatitis E than males (OR 2.8; 95% CI, 0.96–5.20), and a cluster of cases occurred in one corner of the female section of the ward. Mean length of hospital stay for cases was 42 days versus 27 days for controls ($P = 0.0009$); death rates among cases and controls were 5/18 (28%) and 6/75 (8%), respectively. None of the HCWs reported illness or clinical signs of jaundice. Cases more frequently received i.v. dexamethasone (61% vs. 19%, respectively; OR 4.6; 95% CI, 1.4–15.9); $P = 0.0001$), i.v. mannitol (100% vs. 49%, respectively; OR 3.8; 95% CI, 1.6–18.9), and blood transfusions (OR 2.67; 95% CI, 0.61–10.64; $P < 0.01$) than controls.
Discussion: In conclusion, i.v. dexamethasone and mannitol infusion and blood-component therapy were significant risk factors for the development of hospital-acquired hepatitis E on this neurosurgical ward, and parenteral transmission via sharing of i.v. administration sets could be an explanation.

Molecular diagnostic methods II

P1328 PCR on microchip: development of a multiplex PCR-based microchip assay for identification of clinically relevant bacterial species

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Objectives: Speed coupled with accuracy is of greatest importance in diagnostics of infectious diseases. PCR-based methods offer the potential to meet these goals, however, individual target amplification is usually performed in separate reactions to identify and characterize bacterial species. Microchip-based technologies allow to parallel numerous assays by assigning specific targets to elements on the microchip. As a proof of principle, we showed that bacterial species can be identified accurately from blood cultures using isolated bacterial genomic DNA directly without preamplification in a single multiplex PCR reaction on a microchip.
Methods: On the basis of own sequence data of suitable regions of the bacterial genome from clinically relevant in-house strains, specific primers were designed for the identification and characterization of 15 different clinically relevant bacterial species. Additionally three group-specific primers detecting the major groups of Gram-positive bacteria, enterococci, staphylococci and streptococci, as well as primers identifying antibiotic resistance genes like the *MecA*-gene of staphylococci were implemented. Amino-linked primers were immobilized in pairs (forward and reverse) per spot onto glass-slides. Solid phase amplification assays were carried out in a sealed microchamber by using bacterial genomic DNA isolated from positive blood cultures (BACTEC, Becton Dickinson, Heidelberg). During PCR reaction genomic DNA anneals to immobilized primers. The subsequent primer extension and incorporation of fluorescence label into the captured PCR product is used to detect the presence of a specific DNA sample. The PCR process was performed in an in situ block cycler. The slides were analyzed via a confocal fluorescence scanner.
Results and conclusions: After careful evaluation of many parameters effecting specificity and sensitivity (chemistry support, design of primers, PCR conditions, sample preparation, etc.), we developed an accurate multiplex PCR-based microchip assay which allows precise, specific enzymatic amplification and species detection for all investigated strains within 3 h after sample preparation from positive blood cultures. Due to its high discriminatory power the assay has the potential to address the demands in molecular diagnostics for rapid and sensitive bacterial differentiation today.

P1329 Automated and generic nucleic acid extraction using magnetic silica particles

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Objectives: The bioMérieux is developing a new system for nucleic acid extraction in the clinical laboratory. The instrument uses generic Boom chemistry in combination with magnetic silica particles. In contrast to probe-based methods, it does not use target specific reagents. The objective of this study is to validate the instrument in combination with five NAD assays.
Methods: The following clinical applications were validated: (i) HIV-1 RNA, (ii) HBV DNA, (iii) CMV mRNA, (iv) Enterovirus RNA, (v) *Mycobacterium tuberculosis* rRNA. These targets were recovered from plasma, serum, whole blood, stool and sputum samples, respectively. Detection of the isolated nucleic acid was performed with NucliSens EasyQ HIV-1, NucliSens EasyQ HBV, NucliSens CMV pp67, and NucliSens Basic Kit.
Results: All targets were recovered very efficiently from their relevant clinical specimen, which illustrated the system's potential as a universal sample preparation module for NAD testing. Furthermore, the automated magnetic silica method resulted in an improved time to result compared to alternative isolation methods. Typically, 48 sample preparations were completed within 90 min.
Conclusions: This study demonstrates that the new nucleic acid extraction instrument can be used as a flexible, fast and general front-end module for a variety of NAD assays.

P1330 DNA microarray as a diagnostic tool for viral CNS infections

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Objectives: To develop a DNA microarray for rapid diagnosis and specific pathogen identification in patients with meningitis and encephalitis. Initially developed for viral pathogens, once the utility has been assessed, the array can be extended to nonviral pathogens causing CNS infections.
Methods: A DNA microarray was constructed comprising 39 unique gene sequences for 14 viral causes of meningitis and encephalitis, namely, cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), human herpesviruses 6A, 6B and 7 (HHV-6 and HHV-7), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), papovaviruses BK and JC, measles, mumps, Coxsackie B and ECHO viruses. PCR-amplified and purified viral probes representing a mean of three (range 1–5) different regions of the viral genomes were printed on glass slides and arranged to create an easily recognizable virus-specific pattern. CSF samples from patients with viral CNS syndromes, diagnosed previously by nested PCR and gel electrophoresis detection, were subjected to DNA and RNA extraction followed by single round of multiplex DNA PCR or RT-PCR, respectively. Amplified DNA was purified and enzymatically labeled using random primers and the Cy3 fluorescent dye. As an internal control, in every reaction the mixture of original DNA probes was labeled with the Cy5 fluorescent dye. A copurified two-dye target mixture was hybridized to the array and the fluorescence emitted by bound labeled DNA was measured in the control and sample channels. By superimposing the two alternatively

labeled images the pattern of spots appeared characteristic for a specific virus found in the specimen. All positive targets were validated by direct sequencing.
Results: To date, all CSF samples diagnosed previously as CMV, HSV-2, HHV-6A and 6B, HHV-7, VZV, EBV, JC, Coxsackie and ECHO viruses were confirmed as such by the microarray. Although not every target within the viral genomes was amplified, at least one target per virus yielded a specific pattern.
Conclusions: This pilot DNA microarray is capable of detecting at least 14 different viruses in a single test and is as sensitive as nested PCR.

P1331 Sequencing and comparative analysis of the partial structural protein gene of two human parvovirus B19 Chinese isolates

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Objectives: To determine the partial structural protein gene of two human parvovirus B19 (PVB19) Chinese isolates AF221904, AF221905 and to compare them with other PVB19 strains as well as to analyze the relationships between strain's genetic diversity and the clinical manifestation.
Methods: The partial structural protein region gene (2809–3806nt) of two PVB19 strains isolated, respectively, from a child with acute leukemia (AF221904) and an adult with active hepatocirrhosis and aplastic crisis (AF221905) were amplified by re-polymerase chain reaction (re-PCR). Then cloned into pGEM-T vector and sequenced. The nucleotide variability and phylogenetic trees were analyzed by DNASTAR and CLUSTAL W.
Results: Compared with standard strains, the nucleotide changes of the two isolates were 10 (AF221904) and 32 (AF221905), and the amino acid changes were 1 and 6, respectively. On the phylogenetic tree, AF221905, U38506 (isolated from a child with aplastic anemia in Xian, China in 1993), U38507 (isolated from a child with erythema infectiosum in Xian, China in 1992), U38509 (isolated from blood donor in Japan in 1983) assembled in one cluster. The strain (AF221904) and some isolates from Europe, America and Korea assembled in another cluster. The similarities both in nucleotide and amino acid levels between the two clusters were lower than those within one cluster.
Conclusions: The genetic diversity of virus strains can be used to examine whether a virus strain causes a specific clinical manifestation. Our studies suggested that PVB19 strains with different molecular characteristics disseminated widely in China, and that no particular genotype of PVB19 was associate with the distinct clinical symptoms.

P1332 The development of multiplex PCR assay for investigation of 'high-risk' HPV type prevalence

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Objectives: The 'high-risk' HPVs (14 types) is associated with greater than 95% cervical cancers. The PCR with consensus primers that amplify a region of the L1 gene (MY11/MY09 and GP5+/GP6+ primers) allows to detect more than 40 anogenital types, but it requires the additional strategies for type identification. The aim of this study was to develop a technique for the detection of 'high-risk' types of HPV, to compare the developed technique with other PCR-assays and to estimate the prevalence of 10 'high-risk' HPV types among women in Moscow.
Methods: The cervical swabs from 397 women were investigated in this study. The presence of 10 'high-risk' HPVs DNA were tested in two multiplex PCRs with genotype-specific primers. The various HPV genotypes gave amplification products of different size. In the first PCR-test the DNA of 18, 39, 45 and 59 types were amplified giving amplification products of 240, 340, 475 and 455 bp, respectively. In the second PCR-test, the DNA of 16, 31, 33, 35, 52, 58 types were amplified giving amplification products of 325, 520, 227, 280, 360 and 242 bp, respectively. The results were compared with those obtained by 'nested' PCR with MY11/MY09 as outer primers and GP5+/GP6+ as inner primers.
Results: Among 397 samples 210 where negative in both MY-GP and multiplex PCR-tests. 187 where positive, 182 (45.85%)—in MY-GP PCR and 107 (26.96%)—in multiplex PCR. Five samples (1.26%) where positive only in multiplex PCR-test (three of them contained HPV 39 type, one sample contained HPV 45 type and one sample contained HPV 52 type). The HPV 16 detected in 40 (10.08%) samples, HPV 18—in 10 (2.52%), HPV 31—in 18 (4.54%), HPV 33—in 20 (5.04%), HPV 35—in 5 (1.26%), HPV 45—in 7 (1.77%), HPV 39—in 6 (1.52%), HPV 59—in 18 (4.29%), HPV 52—in 10 (2.52%), HPV 58—in 2 (0.51%). Single types were detected in 83 cases, double types—in 19 cases and triple types—in 5 cases.
Conclusions: We propose one-step procedure for simultaneous detection and typing 10 'high-risk' HPV types. The developed method is simple, rapid and sensitive. Next modification of this approach will allow to detect all of the 14 'high-risk' HPV types. The prevalence of 10 'high-risk' HPV types among Moscow women, estimated by the developed technique was 26.96%.

P1333 Genetic heterogeneity of calicivirus infection in Moscow, Russia

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Objectives: Calicivirus is recognized as one of the most important viral cause of acute gastroenteritis and outbreaks. The aim of the present study was to investigate the role and heterogeneity of CV in structure of acute gastroenteritis in Russia using in-house RT-PCR method for detection of these viruses in stool samples.
Methods: We designed a set of primers for separate detection of NLV1, NLV2, SLV. Conservative regions of polymerase (ORF1) and capsid (ORF2) genes were selected for two sets of primers for each of three types of caliciviruses. For nucleic acids purification from feces, we used the silica-guanidiniumthiocyanate method described previously by Boom with our minor overpatches. To investigate the variability of the strains of caliciviruses detected in the study, the amplified with primers to the capsid region 322 bp products were sequenced and aligned with known sequences, published in the GeneBank. All the nucleotide sequence data had been submitted to the GeneBank.
Results: A total of 123 cases of acute sporadic gastroenteritis in children were tested for the presence of viral and bacterial agents. Caliciviruses were detected in 8/123 (6.5%) cases. All viruses isolated during 2-year period were shown to belong to the NLV2 and none of the eight isolated caliciviruses were related to the NLV1 and SLV. All isolates showed high rate of variability between each other (73–100%). The maximum rate of homology (98–99%) was detected between four isolates which were closely related to the NLV2 strains isolated in Japan (homologs to the reference strain Hu/NLV2/Hilingdon/1990/UK). The rest three isolates were more variable, one of them was related to the Hu/NLV2/Grimbsy/1995UK and two others (homology 96%) were related to the Japan strain—Hu/NLV2/Chita/1996/JP. In one case primers to the capsid gene did not amplify cDNA of NLV2, and the positive RT-PCR result was achieved with primers to the polymerase gene. The 190 bp PCR product was sequenced and aligned to the known sequences. This isolate was related to the reference strain NLV2 Hu/NLV2/Melksham/1994/UK.
Conclusion: This study presents the first experience of detection and genetic analyses of human caliciviruses in Moscow, Russia. The rate of sporadic cases of calicivirus diarrhea was 6.5%. The clear predominance of NLV2 confirms the prevalence data of the NLV2, demonstrated in numerous studies. The sequence data shows heterogeneity of NLV2 circulated during two winter periods in Moscow.

P1334 Real-time detection and quantification of HBV DNA using a sensitive DNA NASBA assay covering a dynamic range of at least 6 log 10

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Objectives: Worldwide, over 340 million people are persistently infected with hepatitis B virus (HBV). A significant minority develops chronic hepatic insufficiency, cirrhosis or hepatocellular carcinoma, resulting in about 1 million deaths per year. Viral loads up to 10^{11} copies/mL have been registered. The aim is to develop a sensitive assay for the quantification of HBV DNA covering a broad dynamic range.
Methods: A nucleic acid sequence-based amplification (NASBA) assay was developed for the quantification of HBV DNA. NASBA is originally designed for detection of RNA targets. However, with some important adaptations, NASBA was shown also to be a functional tool for the amplification of HBV DNA. The most important adaptation is the inclusion of a restriction enzyme digestion. To assure detection of all different genotypes, the conserved restriction sites *Xba*I and *Bss*SI, both located in the S gene of HBV DNA, have been selected. Both restriction enzymes are included in the test. Primers and a molecular beacon probe were selected in the region adjacent to the restriction sites. HBV DNA is isolated from serum or plasma samples using the NucliSens Extractor (Organon Teknika/bioMérieux). The minus strand of HBV DNA is used as template. Amplification takes place during 1 h. The amplification product is detected real-time with molecular beacon technology. An internal calibrator is included for quantification.
Results: It is shown that a 100–1000-fold increase in sensitivity is obtained due to the restriction enzymes included in the test. Using a two-step approach, a dynamic range from $\sim 10^2$ to $>10^8$ WHO copies/mL can be detected without the need to dilute samples. Data from analytical performance testing will be presented. Correlation with other HBV tests that are commercial available will be shown.
Conclusions: A sensitive quantitative HBV DNA test covering a dynamic range of at least 6 log 10 is developed based on NASBA and molecular beacon technology.

P1335 Evaluation of 'end point' and 'real-time' molecular-based assays for amplification and detection of influenza viruses

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Objectives: Molecular assays provide some advantages over culture and antigen methods for diagnosis of RNA respiratory tract infections, particularly where acellular samples are taken for surveillance or where a sensitive, rapid diagnosis will greatly assist patient management. The aim of this study was to develop and evaluate NASBA and 'real-time' RT-PCR for diagnostic detection of influenza A and B viruses in clinical samples.
Methods: Respiratory samples previously tested for influenza A, influenza B and other respiratory infections by culture and antigen-based procedures, together with in vitro synthesized RNAs and titrated reference viruses, were used for development and evaluation of the molecular-based assays. Primers and probes for the assays were designed within the influenza nucleoprotein region. Influenza A and B were targeted separately and the assay for influenza A was designed to detect all subtypes. NASBA was undertaken using the NucliSens™ Basic Kit (bioMérieux) with detection of amplicons using 'end-point' and 'real-time' procedures. Reverse transcription was undertaken in a separate step prior to cDNA PCR amplification using the LightCycler (Idaho Technologies). 'In tube' detection of amplified PCR products utilized Cy5 labeled probes.
Results: The feasibility of using rapid molecular-based assays for detection of influenza A or B genomic RNA in respiratory samples was confirmed. The NASBA procedures picked up a lower RNA copy/virus control input than the RT-PCR but both methods correctly identified clinical samples positive by conventional procedures.
Conclusion: The influenza A and B molecular assays will provide important, rapid diagnostic information allowing consideration of the use of antiviral therapies in those at most risk from severe influenza infection and avoiding inappropriate antibiotic use.

P1336 Diagnosis of parainfluenza virus infections using NucliSens™ Basic Kit NASBA with 'end point' and 'real-time' detection

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Objectives: Outbreaks of human parainfluenza virus (HPIV) infections have been reported with potentially severe disease in the very young and immunocompromised. The aims of this study were to develop assays based on NucliSens™ Basic Kit NASBA for diagnosis of HPIV infection and to assess the possible advantages of using such molecular approaches in a hospital environment.
Methods: Kit-based reagents were utilized for extraction, amplification and detection of amplified products (NucliSens™ Basic Kit, bioMérieux). Primers and probes were based on the HN protein (separate assays for HPIVs 1–3) or P protein (HPIV 4a and 4b combined assay). Detection of amplified products was by electrochemiluminescence (ECL, 'end-point' detection) or by use of molecular beacons ('real-time' detection). Titrated reference viruses, synthetic RNA (prepared by cloning of the target and in vitro transcription) and a range of diagnostic respiratory specimens ($n = 50$ to date) were utilized to evaluate the assays. Feasibility of multiplexing primers and probes in a single reaction was assessed using a combined HPIV1 and HPIV3 assay as a model with 'real-time' and 'end point' detection.
Results: The assays using ECL detection proved to be very sensitive and specific. Typically, less than or equal to 100 RNA copies or 1 $TCID_{50}$ input was detectable with no cross-reaction with other respiratory viruses. Results for clinical samples were concordant with those obtained by 'conventional' procedures. 'Real time' detection utilized probes specific for either HPIV1 or HPIV3 with similar performance characteristics to the assays with 'end point' detection. The combined HPIV1 and HPIV3 multiplex assay gave good results for ECL and molecular beacon detection on control material and clinical samples.
Conclusion: Basic Kit NASBA is a suitable diagnostic molecular amplification method for rapid detection of HPIVs in a range of specimen types. This assay will allow a specific diagnosis to be made in a time-frame relevant to patient management and to hospital infection control.

P1337 Development and evaluation of 'real-time' molecular amplification assays for diagnosis of respiratory syncytial virus infection

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Objectives: Respiratory syncytial virus (RSV) has been associated with severe respiratory tract symptomology and complications in the very young, the elderly and immunocompromised individuals. The aims of this study were to develop and evaluate molecular assays based on NucliSens™ Basic Kit NASBA and 'real-time' PCR for diagnosis of RSV infection and to assess the possible advantages of using such molecular approaches in a hospital and community settings.
Methods: Primers and probes were designed within the RSV fusion protein region which allowed amplification and detection of both RSV A and B strains by NASBA and RT-PCR. NASBA was undertaken using the NucliSens™ Basic Kit (bioMérieux) with detection of amplified products by electrochemiluminescence (ECL) or 'real-time' using a molecular beacon. Reverse transcription was undertaken in a separate step prior to cDNA PCR amplification using the LightCycler (Idaho Technologies). Detection of amplified PCR products utilized Cy5 labeled probes. Sensitivity and specificity of the molecular assays were assessed using respiratory samples ($n = 112$ to date) and titrated reference viruses.
Results: The molecular assays had a considerable advantage above culture-based procedures because of the rapid turn-around time for results. Direct antigen detection was relatively insensitive on swab material. The NASBA assays with ECL or molecular beacon detection gave equivalent results and proved to be very sensitive and specific. Typically, less than or equal to 0.01 $TCID_{50}$ RSV input was detectable by NASBA with no cross-reaction with other respiratory viruses. The sensitivity of the NASBA assay was found to be 100-fold higher than the RT-PCR on cultured material and the latter procedure missed some culture and or direct antigen positive samples.
Conclusion: Molecular amplification methods will impact on surveillance of RSV infections and patient management. Basic Kit NASBA is a suitable

diagnostic method for rapid detection of RSV in a range of respiratory samples. The assay will allow a specific diagnosis to be made in a time-frame relevant to patient management and to hospital infection control.

P1338 Molecular analysis of human parechovirus type 2 (formerly echovirus 23)

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Picornaviruses have been divided into five genera until recently, when a sixth genus, Parechovirus, was defined. Human parechovirus type 1 (HpeV1; formerly echovirus 22) was the first recognized member of this genus and preliminary sequence analysis of echovirus 23 [now renamed human parechovirus type 2 (HPeV2)] suggested that it is also a parechovirus. Here we describe the complete nucleotide and predicted amino acid sequences of HpeV2, which indicate a close relationship to HpeV1 throughout the genome. Sequence covariance in the five untranslated region allows a prediction of the secondary structure, which indicates that these parechoviruses have a type 2 internal ribosome entry site, most closely related to that of cardioviruses. Overall, HpeV2 has 87.9% amino acid identity with HpeV1, most divergence being seen in regions of the capsid proteins that probably define antigenic sites. The N-terminal sequence extension to VP3, seen only in parechoviruses, is highly basic in both viruses, but has a variable sequence, suggesting that it dose not have a sequence-specific role. There is an RGD motif near the C terminus of VP1, in an analogous location to that in HpeV1which is believed to be functionally significant. The results confirm that both viruses are parechoviruses and give insights into the molecular features of this genus.

P1339 Multiplex RT-PCR for diagnosis of human picornavirus infections

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The best known picornaviruses are enteroviruses which cause meningitis, encephalitis, poliomyelitis, myocarditis, respiratory infections and rashes. Rhinoviruses are the major cause of common cold while hepatitis A virus (hepatovirus) infection affects liver. Of the two recently established picornavirus genera, parechoviruses appear to cause mainly gastroenteritis and respiratory infections, and Aichi virus (a kobuvirus) has been detected in oyster-associated gastroenteritis cases in Japan. The aim of our work was to develop an RT-PCR test which would detect all human picornaviruses in a single assay. For the purpose, we designed primers from the 5′ untranslated region of the viral genome which give rise to 116–260 nt long amplicons and combined the reactions as multiplex RT-PCR. Furthermore, we developed a liquid hybridization assay where one of the primers in each reaction is biotinylated to mediate binding of the amplicons to streptavidin-coated wells. The product was then detected with digoxigenin-labeled oligonucleotide probes and the results measured by chemiluminescence. We have further optimized the amplification and liquid hybridization reactions which currently allow detection approximately 25 cDNA copies of the viral genome. The assay enables simultaneous identification of enteroviruses, rhinoviruses, parechoviruses, hepatitis A virus and Aichi virus. The method is currently used for analysis of clinical samples including CSF, serum, stool and nasopharyngeal specimens. In particular, we are interested in the clinical outcome of infections caused by the currently poorly known human picornavirus groups, parechoviruses and Aichi virus.

P1340 Nuclisens® Basic Kit NASBA including internal control and molecular beacons for reliable 'real-time' diagnosis of enterovirus infections

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Objective: Enteroviruses are among the most common viral pathogens of humans and are responsible for a wide range of clinical symptoms. The aim of this study was to enhance diagnostic detection of enteroviruses in a range of clinical specimens.

Methods: Based on the 5′ noncoding region of the enterovirus genome primers and an enterovirus specific internal control (IC) to monitor assay fidelity at the individual sample level were developed. Standard reagents from the Nuclisens® Basic Kit were used for automated nucleic acid extraction and subsequent RNA amplification and simultaneous detection. Differently labeled molecular beacons were used for detection of enterovirus and IC RNA amplicons during the amplification process. Reference viruses, clinical samples and quality control specimens were analyzed.
Results: Introduction of molecular beacons enabled the detection of both enterovirus RNA and IC RNA amplification simultaneously over time. In this way, performance validity could easily be assessed for each individual sample while detecting the presence of enterovirus RNA. The analytical sensitivity of this 'real-time' NASBA assay was approximately 1000 copies of synthetic RNA in the isolation-amplification reaction for a wide range of enteroviruses. Analysis of reference strains showed a specificity of almost 100% (with the exception of HRV 45) and a sensitivity of 0.1–1.0 $TCID_{50}$. Specificity and sensitivity of the 'real-time' NASBA assay were confirmed by the results obtained from the EUCA panel that were similar to those of three reference laboratories. The newly developed 'real-time' NASBA appears to be user-friendly and results are available within one working day.
Conclusion: The internally controlled 'real-time' pan-enterovirus NASBA in Nuclisens® Basic Kit format is a very reliable assay and applicable for rapid clinical diagnosis of a wide range of viruses. The approach of a closed tube system by using molecular beacons enlarges the utility of the assay in a routine diagnostic setting.

P1341 Molecular diagnosis of periodontitis

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Objective: It is generally accepted that periodontitits is initiated by the establishment of a specific subgingival bacterial flora. A number of marker organisms, namely *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus* and *Treponema denticola* have attracted particular attention because exotoxins produced by these organisms were associated with continuous inflammation and tissue degradation. About 10–15% of all periodontitis cases exhibit an aggressive progress indicating microbiological testing, for example forms of early onset periodontitis, as the preadolescent, the juvenile and rapidly progressive periodontitis. In addition, severe forms of the adult generalized periodontitis, the refractory and the therapy-resistant periodontitis as well as infection of osteointegrated implants represent indications for microbiological testing.
Materials and methods: It's very difficult to use traditional methods of culture because the organisms responsible for periodontitis are anaerobic and their vitality is reduced in the subgingival sample. Than we have utilized a commercial molecular test that allows the fast and easy identification of five periodontopathogenic bacterial species. DNA isolated from subgingival samples is used to amplify a specific gene fragment from each species. Biotinylated amplicons are subsequently identified by a reverse hybridization assay. We have used a case–control study and examined 25 patients affected by periodontitis and 50 healthy controls.
Results: The results evidenced that in all the patients with periodontitis are present alone or in association the five periodontophatogenic bacteria investigated. The first detected is *T. denticola* (20%) and the following *B. forsythus* (12%); frequently the association of *T. denticola* with *B. forsythus* (24%). The bacterial concentration present in a healthy sulcus lead to a negative result in all the controls.
Conclusions: The specific and sensitive detection of periodontopathogenic bacteria from subgingival pockets allows for the quick identification of risk patients and gives valuable information when choosing an effective therapy.

P1342 Detection of community-acquired pneumonia pathogens in lower respiratory specimens using the BDProbeTec™ ET System

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Objectives: (1) To develop strand displacement amplification (SDA)-based assays for use on the BDProbeTec™ ET System to detect three of the leading

causes of community-acquired pneumonia (*Legionella pneumophila, Mycoplasma pneumoniae* and the Chlamydiaceae family) from a single processed lower respiratory sample. (2) To assess the efficacy of a universal sample processing method for lower respiratory specimens including sputum and bronchial washings: (a) Evaluate the effect on the internal amplification control (IAC), (b) determine analytical sensitivity in spiked processed specimens.

Methods: Performance of the IAC for each of the three assays was evaluated using 119 lower respiratory specimens (97 sputum samples and 22 bronchial washings). All samples were processed according to a customized Qiagen DNA extraction protocol and assayed using a universal buffer system and dried amplification reagents. To determine the analytical sensitivity for each assay, serial dilutions of the three organisms were seeded all together into a negative lower respiratory specimen pool, then processed and assayed.

Results: For the 119 lower respiratory samples examined, the IAC, an indicator of SDA reaction inhibition, showed an indeterminate rate for the *L. pneumophila* and *C. pneumoniae* assays of 1.8 and 5.4%, respectively. All of the inhibited samples were sputum specimens. The analytical sensitivity using a processed lower respiratory specimen pool was determined to be 1092 colony forming units/mL for *L. pneumophila* and 1092 elementary bodies/mL for *C. pneumoniae*. Performance of the BDProbeTec^{TM} ET *M. pneumoniae* Assay is currently being investigated.

Conclusions: The BDProbeTec^{TM} ET community-acquired pneumonia assays offer the potential for highly sensitive detection of *L. pneumophila, M. pneumoniae* and the Chlamydiaceae family from lower respiratory specimens. All three assays employ a common, rapid and straight-forward sample processing protocol that provides for efficient recovery of DNA and removal of potential assay inhibitors.

P1343 Evaluation of PreservCyt® solution (Cytyc®) for the detection of *Chlamydia trachomatis* by PCR on COBAS® Amplicor

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Objectives: The aim of this study was to evaluate the convenience of cervical samples in Cytyc® medium for the detection of *Chlamydia trachomatis* by PCR on COBAS® Amplicor.

Methods: Cervical specimens collected in Cytyc medium were obtained from women for a cervical cytological screening. A total of 110 samples were randomly chosen, 55 in a group of specimens without particular cytological findings and 55 among specimens presenting atypical squamous cells of undetermined significance (ASCUS) or low grade cervical lesion (LSIL). Samples were prepared by centrifugation and a single washing step in Phosphate–sucrose buffer prior to standard procedures for PCR preparation on cervical swabs. In addition, five women with a *C. trachomatis* infection detected by PCR or direct immunofluorescence and for whom a Cytyc sample was available, were also included in this study.

Results: Out of the 110 Cytyc specimens, 2 (1.8%) were found positive for *C. trachomatis* and the five Cytyc samples from infected women were also found positive. Five out of these seven samples were classified in the ASCUS-LSIL group. Cytyc solution of specimens positive for *C. trachomatis* conserved at room temperature were still found positive by PCR on COBAS Amplicor 6–12 months later. Negative specimens were negative. No inhibition was observed with the procedure used for preparation of the samples from Cytyc medium.

Conclusion: PreservCyt solution (Cytyc) is a highly stable medium for PCR detection of *C. trachomatis* on COBAS Amplicor. It appears to be particularly well adapted for cytological examination, as well as for detection of pathogens like Human papillomavirus, *C. trachomatis*.... Detection of ASCUS-LSIL may help for the selection of women at risk for *C. trachomatis* infections. Samples in Cytyc medium are well adapted for retrospective epidemiological study on *C. trachomatis*.

P1344 PCR diagnosis of invasive disease caused by *Neisseria meningitidis* and direct multilocus sequence typing of PCR products

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Objectives: The polymerase chain reaction (PCR) for nonculture diagnosis of invasive meningococcal disease was introduced to increase the percentage of laboratory confirmed cases. The multilocus sequence typing (MLST) of PCR products was introduced with the aim to reveal sequence types (STs) from patients with suspected invasive meningococcal disease.

Methods: The PCR method was optimized on *N. meningitidis* and sensitivity and specificity of the PCR detection of *N. meningitidis* from cerebrospinal fluid (CSF) were assessed in 195 samples from patients with the following diagnoses: invasive meningococcal disease, bacterial nonmeningococcal meningitis and viral meningitis. DNA was extracted from the CSF by IsoQuick kits (ORCA Research Inc., USA). MLST was made according to the method developed at the University of Oxford.

Results: The following parameters of the PCR detection of *N. meningitidis* from the CSF were calculated: sensitivity = 0.85, specificity = 0.95, positive predictive value = 0.88, negative predictive value = 0.93. Under our conditions, PCR gave laboratory confirmation in nearly 50% (47.4%) of patients with invasive meningococcal disease where the etiology was not confirmed by classical methods. MLST performed directly from cerebrospinal fluid revealed STs in all PCR products.

Conclusion: The PCR method for nonculture diagnosis of invasive meningococcal disease and MLST typing of *N. meningitidis* directly from clinical material were introduced in the National Reference Laboratory for Meningococcal Infections in Prague. These molecular methods improve laboratory diagnosis of invasive meningococcal disease.

Acknowledgments: This study was supported by research grant NI/6882-3 of the Internal Grant Agency of the Ministry of Health of the Czech Republic and made use of the Multi Locus Sequence Typing website (http://neisseria.mlst.net) developed by Dr. Man-Suen Chan, cited at the University of Oxford and funded by the Wellcome Trust. We thank Dr. K. Jolley (University of Oxford, UK) for kind editing of the text.

P1345 Development and comparison of two PCR-based methods for the detection of serogroup A *Neisseria meningitidis*

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Objectives: The diagnosis of meningococcal disease (MD) is usually based on clinical presentation, and is ideally confirmed with the isolation of *Neisseria meningitidis* from a patient source. With the introduction of the meningococcal serogroup C vaccine (MenC), there is a need for enhanced surveillance of other serogroups within the population. The gene cassette, required for the biosynthesis of the nonsialic acid capsular polysaccharide of *N. meningitidis* serogroup A, has been characterized. The cluster contains four genes: *mynA*, *mynB*, *mynC* and *mynD* which are specific to *N. meningitidis* serogroup A. The aim of this study was to develop, and evaluate both a conventional gel-based PCR detection system and a new dual-labeled end-point fluorescence PCR system (DEF-PCR) for the detection of *mynA*.

Methods: A conventional PCR for serogroup A detection was developed and evaluated for specificity and sensitivity using a total of 88 isolates (comprising 13 serogroup A meningococci; 26 'other' meningococcal serogroups and a variety of 49 nonmeningococcal isolates). A novel PCR method was developed for serogroup A detection, using primers labeled with reporter and quencher dyes. The chemistry results in the production of fluorescent emissions, which can be detected on a standard fluorescent reader.

Results: The sensitivity of the DEF-PCR method was greater, compared to the conventional method. The DEF-PCR also allowed a high throughput (by giving a digital fluorescence value), eliminating the subjective visual reading of gels. An appropriate cut-off value could be calculated to ensure straightforward interpretation. Both PCR methods were found to be highly specific, for the detection of *N. meningitidis* serogroup A. However, a small percentage of false positives could not be eliminated from the cut-off values created with the DEF-PCR. This could be attributed to the very high sensitivity, highlighting small amounts of contamination within some samples.
Conclusion: It appears that the DEF-PCR method can provide an excellent efficient screening method for the detection of *N. meningitidis* serogroup A. Advantages include its high throughput capacity, documented specificity and high sensitivity. However, further evaluation studies are required before this method is used routinely as a detection system.

P1346 Comparison of detectability of *Mycoplasma pneumoniae* infections in children, using PCR-test and serological methods: indirect immunofluorescence and immunoenzymatic assay

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Objectives: *Mycoplasma pneumoniae* is an important cause of community acquired infections of upper and lower respiratory tracts in children, mainly atypical pneumonia. The aim of our study was the comparison of the serological procedures used in routine diagnostic (enzymatic- and fluorescence assays) with the polymerase chain reaction (PCR) which is currently the most reliable and sensitive method applied in *M. pneumoniae* diagnostic.
Materials and methods: The materials from 100 children hospitalized in Pediatric and Alergology Department of Medical University of Wroclaw were analyzed. The PCR assay using *M. pneumoniae* Diagnostic Kit Venor Mp (Minerva, Germany) was performed to amplify the gene encoding the P1 adherent protein. PCR was used to examine the presence of *M. pneumoniae* in throat swab specimens. The level of IgM and IgG antibodies were evaluated in sera samples using two immunoenzymatic tests: with membrane P1 protein (ELISA DiaSorin, USA) and with whole cell mycoplasma antigen (ELISA Euroimmun, Germany) and indirect immunofluorescence test with Biochip technique—fibroblastic cells infected by *M. pneumoniae* (Euroimmun, Germany). Swabs and sera were taken from children in acute infection.
Results: Fifty-two percent throat swabs from children investigated by PCR were positive. Depending on serological test used, different percentages of positive results (IgM or both IgM and IgG antibodies) were detected in sera samples—30% using ELISA from DiaSorin, 27% in fluorescence test and 10% in ELISA from Euroimmun. Additional in fluorescence test 10% of samples were considered as the positive on the basis of the high titer (>200) of IgG antibodies. In 15% of cases, positive results in PCR were negative in all serological methods.
Conclusions: Detectability of *M. pneumoniae* was highest with PCR test applied. In serological test used in routine diagnostic, the false negative results could be obtained. The indirect fluorescence test was considered as the most reliable and sensitive in comparison with PCR assay.

P1347 Detection of *Legionella pneumophila, Mycoplasma pneumoniae,* and the Chlamydiaceae family from a single throat swab using the BDProbeTec™ ET System

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Objectives:
1 To develop a panel of strand displacement amplification assays for the detection of community-acquired pneumonia pathogens (*Legionella pneumophila, Mycoplasma pneumoniae,* and Chlamydiaceae family) from a single dry throat swab (stored without transport media) using the BDProbeTec™ ET System.
2 To determine analytical sensitivity, specificity, and specimen stability for all three pathogens.
3 To evaluate asymptomatic individuals to rule out the potential of a carrier state in otherwise healthy individuals.

Methods: Analytical sensitivity was determined for each assay by testing serial dilutions of organisms and a cloned target nucleic acid sequence. Specificity was evaluated by challenging the assays with a variety of potential cross reactants, including genetically related bacteria. To determine whether a carrier state exists for the target organisms, approximately 100 throat swab specimens were collected from asymptomatic volunteers. The stability of dry throat swab specimens was determined by testing a second panel of swabs obtained from asymptomatic individuals that were seeded with organisms and stored at various temperatures. All specimens were processed and assayed using a universal buffer system and dried amplification reagents.
Results: The analytical sensitivity of the three assays using cloned target nucleic acid was 160, 131, and 110 copies per test for *L. pneumophila, M. pneumoniae,* and *C. pneumoniae* DNA, respectively. The analytical sensitivity using seeded organisms was 44 and 10 organisms per test for *L. pneumophila* and *M. pneumoniae,* respectively, and 38 elementary bodies per test for *C. pneumoniae.* No cross-reactivity was observed. Dry throat swab specimens were found to be stable for 3 days at 2–8 °C and at least 7 days at −20 °C. Evaluation of ambient temperature stability is ongoing. No false-positive results were observed in testing of throat swab specimens from asymptomatic individuals.
Conclusion: The BDProbeTec™ ET *L. pneumophila, M. pneumoniae,* and Chlamydiaceae family assays offer the potential for highly sensitive and specific detection of these pathogens. All three assays may be performed from a single swab specimen using a streamlined workflow.

P1348 Development of a multiplex real-time quantitative TaqMan assay to detect *Chlamydia pneumoniae, Legionella pneumophila* and *Mycoplasma pneumoniae* in respiratory tract secretions

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Objective: Atypical pathogens such as *Chlamydia pneumoniae, Legionella pneumophila* and *Mycoplasma pneumoniae* are an important cause of community-acquired pneumoniae. The available detection methods either lack sensitivity or give only a retrospective diagnosis. In order to improve detection and identification of these pathogens, we developed a quantitative real time multiplex PCR for the simultaneously diagnosis of *C. pneumoniae, L. pneumophila* and *M. pneumoniae* in respiratory samples.
Methods: Real-time PCR using the ABI Prism 7700 Sequence System (Applied Biosystem) was performed. Primers and probe were selected from highly conserved sequence of the PST-1 fragment of *C. pneumoniae,* the MIP gene of *L. pneumophila* and the P1 protein gene of *M. pneumoniae).* Three plasmids containing the respective target genes were constructed to allow quantification and used as positive controls.
Results: This multiplex PCR was able to detect simultaneously one copy of *C. pneumoniae, L. pneumophila* and *M. pneumoniae* genome per 10 µL extracted DNA. Coamplification of two target genes without loss of sensitivity was demonstrated. The specificity was assessed using 35 reference or clinical strains of bacteria and respiratory viruses. The comparison of multiplex real-time PCR and single conventional PCR assay for the 73 respiratory specimens analyzed so far showed an overall agreement of 98.3% corresponding to 95.8, 100 and 100% for *C. pneumoniae, L. pneumophila* and *M. pneumoniae,* respectively. Clinical application of this multiplex PCR was performed up to now on 38 respiratory samples from 36 patients with respiratory tract infections. Of 15 patients with positive serology, 12 were confirmed to be positive with the multiplex PCR for one of the three pathogens. All patients with negative serology were negative with multiplex PCR. In addition, the multiplex real-time PCR was found to be more sensitive than *L. pneumophila* culture.
Conclusion: The multiplex PCR is a rapid, sensitive and specific method for the detection of *C. pneumoniae, L. pneumophila* and *M. pneumoniae* and can thus, contribute to adequate clinical management.

Pharmacological issues

P1349 Cellular accumulation and membrane-binding properties of ester prodrugs of ampicillin (pivaloylampicillin [PIVA], phthalimidomethylampicillin [PIMA])

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Objectives: The β-lactams do not accumulate in eucaryotic cells which contributes to their poor activity against intracellular infection. This feature is due to the presence of a free carboxylic function on the molecule. PIVA and PIMA, in which the carboxylic function is masked and are therefore weak organic bases, accumulate in cells (Biorg. Med. Chem. 2001; 9: 493). The aim of this work is to study the mechanisms governing their cellular accumulation.
Methods: All experiments were performed on J774 mouse macrophages. We measured the kinetics of accumulation and efflux at 37 and 4 °C as well as the influence of a variation of the extracellular concentration (5–200 μM) or of the pH of the medium (6–8). Binding to phospholipids was determined by equilibrium dialysis against liposomes. Chloroquine [CQ] and azithromycin [AZ], two weak bases known to be sequestrated into acidic compartment by proton-trapping, were examined in parallel.
Results: Both prodrugs accumulated to a high level (cellular to extracellular concentration ratio [Cc/Ce] = 40 and 35 for PIVA and PIMA, respectively) at 37 °C as well as at 4 °C, and were rapidly released ($t_{1/2} < 5$ min) at both temperatures. Accumulation was not affected by variation of the extracellular pH, but was saturable at concentration of >5 μM. Equilibrium dialysis experiments demonstrated a high binding to phospholipids ($K_d = 45$ μM). This behavior was in sharp contrast to that of CQ and AZ (accumulation at 37 °C with marked decrease at acid pH, no accumulation at 4 °C; slow release at 37 °C ($t_{1/2}$ 2 h and 30 min) and no release at 4 °C ($t_{1/2} > 24$ h); lack of saturation at concentration of <80 μM; low binding to phospholipids).
Conclusion: The mechanism by which PIVA and PIMA accumulate in cells is very different from that described for CQ and AZ. We may suggest that the prodrugs mainly bind to the pericellular membrane, perhaps because of their property of cationic amphiphile.

P1350 Pharmacokinetics of cefepime in experimental acute necrotizing pancreatitis

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Objective: To determine tissue penetration of cefepime since it may be a candidate of prophylaxis in acute necrotizing pancreatitis.
Methods: Acute necrotizing pancreatitis was induced 6 days after the intraperitoneal injection of DL-ethionine when three doses of 50 mg/kg of cefepime were administered intramuscularly at 8 h time intervals. Thirty-five rabbits with acute necrotizing pancreatitis and 33 controls were sacrificed and concentrations of cefepime were determined in serum and pancreas at regular time intervals by a microbiological assay.
Results: Respective mean (±SD) pancreatic concentrations in rabbits with acute necrotizing pancreatitis and in controls were 22.3 ± 6.9 and 12.7 ± 5.4 μg/g at 60 min, 9.3 ± 1.2 and 8.5 ± 0.8 μg/g at 90 min, 5.7 ± 1.3 and 5.5 ± 0.4 μg/g at 120 min and 2.3 ± 0.4 and 2.8 ± 0.6 μg/g at 180 min after the last dose of cefepime. Respective mean (±SD) serum concentrations in rabbits with acute necrotizing pancreatitis and in controls were 46.1 ± 21.5 and 45.2 ± 16.6 μg/mL at 60 min, 40.1 ± 9.4 and 48.5 ± 21.5 μg/mL at 90 min, 38.7 ± 9.7 and 33.9 ± 4.3 μg/mL at 120 min and 24.8 ± 2.7 and 17.3 ± 21.5 μg/mL at 180 min after the last dose of cefepime. Tissue/serum ratio of cefepime was found to be 0.48, 0.23, 0.15 and 0.09 at 60, 90, 120 and 180 min, respectively, after the last dose of cefepime in rabbits with acute necrotizing pancreatitis and 0.28, 0.18, 0.16 and 0.16, respectively, at 60, 90, 120 and 180 min in controls.
Conclusions: Tissue penetration of cefepime in acute necrotizing pancreatitis is adequate over the first 120 min after the administration of the last dose of cefepime, resulting in tissue levels much higher than the MICs of the common pathogens of pancreatic superinfection.

P1351 Cefepime and nonconvulsive status epilepticus (NCSE) in renal failure (RF) patients

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Objectives: A retrospective analysis of seven cases of NCSE in patients (pat.) with renal failure (RF) treated with the fourth generation cephalosporin cefepime.
Methods: As a result of an increased incidence of NCSE in our end-stage renal disease (ESRD) pat., the suspicion of an association with the use of cefepime and the recently published data on NCSE and cephalosporins, we reviewed the records of the pat. with RF and NCSE treated with cefepime between March 2000 and May 2001.
Results: NCSE developed in seven pat. Six pat. were known with ESRD, requiring dialysis. One patient had acute renal failure (ARF). Creatinine clearance varied from 5.5 to 24.5 mL/min (mean 12.7 ± 7.2 mL/min) on the day NCSE was diagnosed. The daily administered dose of cefepime was 2 g in five dialysis pat., one patient developed NCSE after receiving only one dose of 2 g, and the patient with ARF received 6 g after 24 h. All pat. presented with change in mental status with confusion, apathy or agitation, without convulsions. In all, EEG recordings were compatible with status epilepticus. The interval between the start of cefepime and the diagnosis of NCSE varied from 2 to 9 days (mean 4.3 ± 3 days). NCSE persisted despite the use of anticonvulsants in four pat. where cefepime was continued. They all died. In three pat., cefepime was stopped and NCSE disappeared within 24–48 h. They survived. Serum cefepime levels were available in three patients and were high, ranging from 14.4 to 78.1 μg/mL (mean 53.5 ± 34.2 μg/mL). We also looked for other causes of NCSE. One patient was HIV positive and had a viral meningitis, another had a ventriculoperitoneal drain. However, the serum cefepime levels in these two pat. were very high (68 and 78.1 μg/mL). The causality between the presence of NCSE and the use of cefepime is considered as probable (WHO criteria) because of the temporal relationship, the lack of other causes in five out of seven pat. and the positive dechallenge in three pat.
Conclusion: Treatment with cefepime, in pat. with RF, can result in NCSE. When changes in mental status or encephalopathy occur during treatment with cefepime, NCSE must be considered. In EEG-confirmed NCSE, cefepime should be stopped. Prognosis appears to be good after discontinuation. Although, the mechanism is unknown and the relationship with the dose unclear, dosage adjustment according to RF should be rigorously applied.

P1352 Lack of interaction between faropenem daloxate and warfarin in healthy volunteers

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Objectives: Faropenem daloxate (FD) is a new oral antibiotic with a penem structure unique from carbapenems and other available β-lactams. FD is rapidly hydrolyzed in vivo to faropenem (F), the active moiety. In vitro, F has a targeted spectrum of antimicrobial activity against Gram-positive and -negative bacteria. FD is being clinically investigated for the oral treatment of community-acquired infections. Concomitant administration of FD with warfarin (W) could potentially lead to an interaction by protein displacement of W or by Vitamin K deficiency due to changes in the normal gastrointestinal flow as a result of antibiotic treatment. Thus, the potential influence of the coadministration on pharmacokinetics and blood clotting was investigated in this study.
Methods: The study was conducted in a randomized, double-blinded, two-way cross over design. Twenty-four healthy male subjects, 18–45 years of age, were included. After a priming dose of 25 mg W sodium (16 days before treatment start), FD (corresponding to 300 mg F) or placebo were given bid for 8 days, with a concomitant single dose of 25 mg W sodium on day 5. Safety and tolerability were evaluated by monitoring of adverse events, vital signs, ECG and clinical laboratory parameters. Pharmacokinetic parameters were determined through analysis of plasma profiles for R-/S-warfarin and for F. In

addition, prothrombin time (PT) and clotting factors II, VII and X were determined for evaluation of a pharmacodynamic interaction.
Results: No changes in AUC (0–96 h) for PT and the clotting factors II, VII and X were seen, and all 90% confidence intervals were within the predefined equivalence ranges of 95–105% (for PT) and 90–110% (for factors II, VII and X). The study treatments were safe and well tolerated, without any clinically relevant changes in vital signs, ECG, and laboratory parameters. The 90% confidence intervals for C_{max} and AUC for R-/S-warfarin and F were all within the conventional equivalence range of 80–125%.
Conclusions: No pharmacokinetic or pharmacodynamic interaction between FD and warfarin was observed in this study. The coadministration of FD and W was safe and well tolerated in healthy volunteers.

P1353 Postantibiotic effect of high dose AmBisome (AmBis) 3 weeks after treatment

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Objectives: Previous preclinical reports of amphotericin B in tissues of AmBis-treated animals (5–20 mg/kg), 1 week after termination of treatment, suggested that AmBis may have some postantibiotic effects. Since, fungal infections are difficult to eradicate from tissues, prolonged activity of the drug would probably help to produce fungal clearance at these sites.
Methods: To study this, we injected C57Bl/6 mice with phosphate buffer (control) or 20 mg/kg AmBis i.v., three times per week, for 1 or 3 weeks. Kidney homogenates were prepared (three mice per time point) 48 h after the first week and third week of treatments, and again 3 weeks later. Homogenates were mixed with 1.0, 2.0 or 3.0 $\log_{10}$ cfu of *Candida albicans* and plated immediately or incubated overnight at 30 °C prior to plating onto Sabouraud dextrose agar. For comparison, exogenous AmBis or fungizone (Fz) (10 µg/mL) was added to some homogenates from buffer-treated mice.
Results: When the homogenates from drug-treated or control mice were plated immediately, there was no inhibition of fungal growth (e.g. 2.0 $\log_{10}$ cfu of yeast incubated with homogenates yielded 2.0 $\log_{10}$ cfu on plates). However, overnight incubation of untreated control homogenates with 1.0, 2.0 or 3.0 $\log_{10}$ cfu yielded 5.0, 6.4 and 7.3 $\log_{10}$ cfu, respectively. Homogenates from mice treated with drug for 1 week showed marked inhibition of yeast growth (two- to five-fold log reduction) after overnight incubation compared to growth in homogenates from untreated mice. Inhibition was 100% when yeast were incubated overnight with homogenates from mice given 3 weeks before treatment and mice tested 3 weeks post-treatment. Exogenous addition of Fz or AmBis to control homogenates also showed 100% inhibition, but only after overnight incubation.
Conclusions: A carry-over effect from tissue containing AmBis was not observed if the tissue was plated soon after homogenization, although antifungal activity of the drug in the kidneys, even 3 weeks after treatment, was observed when the homogenized tissue was incubated overnight with a yeast challenge. Thus, a postantibiotic effect probably occurs with high-dose AmBis treatment of mice.

P1354 Ex vivo synergy of arachidonate-enriched serum with ceftazidime and amikacin on multidrug-resistant *Pseudomonas aeruginosa*

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Objective: Multidrug-resistant *Pseudomonas aeruginosa* (MDPA) may become in vitro susceptible to ceftazidime and amikacin in the presence of arachidonic acid (AA) [1]. The present study aimed to the ex vivo effect of arachidonate-supplemented serum on MDPA isolates.
Methods: An emulsion of AA was administered i.v. at a dose of 25 mg/kg over 10 min by the left jugular vein of 10 rabbits and blood was sampled at 15-min intervals by catheters inserted in the left carotid and in the hepatic veins. Blood was centrifuged and 1 mL of serum separately was added in 9 mL of Mueller broth containing a 5.6 log-phase inoculum of each of the two MDPA blood isolates. Isolates were resistant to ceftazidime, imipenem, amikacin and ciprofloxacin. Ceftazidime and amikacin were already added in broth at 16 µg/mL each, i.e. their mean serum level. Bacterial growth was counted after 3, 5 and 24 h of incubation at 37 °C. Synergy was defined as more than 2 $\log_{10}$ decrease of viable counts compared to the effect of serum sampled before infusion of AA and to controls grown with antimicrobials.
Results: Synergy was achieved for both isolates only after 5 h of incubation. At that time, mean decreases of viable cells compared to the effect of serum sampled before the infusion of AA were 1.39, 2.09, 2.51 and 1.50 after incubation in the presence of antimicrobials and sera sampled from the hepatic veins 15, 30, 45 and 60 min after infusion of AA, respectively. Mean decreases were 3.71, 2.28, 2.57 and 1.64 after incubation in the presence of antimicrobials and sera sampled from carotid artery 15, 30, 45 and 60 min after infusion of AA, respectively.
Conclusions: Ex vivo incubation of sera from rabbits intravenously administered AA may act in synergy with ceftazidime and amikacin on MDPA. The presented data may be applied for the therapeutic management of infections by multidrug-resistant nosocomial isolates in animal models.

Reference

1 Giamarellos-Bourboulis et al. *AAC* 2000, 44: 2187.

P1355 Pharmacodynamics of moxifloxacin in fibrin clots

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Background: Fibrin has pleiotropic functions: it plays a major role in tissue repair; it is one of the main constituents of the inflammatory response associated with most infectious processes; it is an integral component of intravascular thrombi, and it provides a protective environment for bacteria as fibrin clots serve as diffusion barriers for antibiotics that permit bacteria to develop resistance. Therefore, the penetration of moxifloxacin into fibrin clots, its antibacterial action and its propensity to permit resistance development were studied.
Methods: A modified method according to Bergeron et al. was used. Fibrin clots were infected with *S. pneumoniae*, *S. aureus* (MSSA), MRSA/β-lactamase negative, MRSA bla pos. and *E. coli*. Infected fibrin clots were placed in dialysis tubings and placed either into a one-compartment in vitro model simulating human serum kinetics of moxifloxacin following doses of 200 and 400 mg or were implanted subcutaneously into NMRI mice; animals were treated with 100 mg/kg i.v. every 6 h. Bacterial counts and moxifloxacin concentration within the fibrin clots were quantitated and compared to viable counts of nonentrapped bacteria exposed to moxifloxacin under the same experimental conditions.
Results: Penetration of moxifloxacin into the clots was rapid and was not different from the surrounding medium or serum. Viable counts of all four test organisms declined dose-dependently. In vitro *E. coli* was eradicated within 2 h, *S. pneumoniae* in 3 h, MSSA in 7 h, and both MRSA strains in 12 h from the infected clots. Analogous results were obtained in vivo. MICs of pre- and postexposure isolates were not different. Nonentrapped controls were eliminated from the test systems within the same time periods.
Conclusions: Fibrin clots did not constitute a penetration barrier for moxifloxacin. The antibacterial action was not impaired due to the protective environment of a fibrin clot, and resistance did not emerge. These data suggest that moxifloxacin would provide efficient tissue penetration and clinical efficacy in serious infections.

P1356 Ornidazole-induced liver damage

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Introduction: Ornidazole is used in the treatment of infections caused by anaerobic bacteria and protozoa. It is generally well tolerated. Only two cases of hepatotoxicity have been reported. We here describe the three patients who developed hepatitis after ornidazole use.
Case 1: A 38-year-old woman was initiated fluconazole and ornidazole for her vaginitis. Nine days later, she was admitted with fever and diarrhea. The stool revealed *Entamoeba histolytica* cysts, and ornidazole 1 g/day was used for 4 days,

but she felt worse and was readmitted the third day. She had jaundiced scleras and hepatomegaly (HM) (3 cm). ALT was 977 U/L, AST 1288 U/L, t. bilirubin 32 mg/dL (d. 19), ALP 1062 U/L (64–306), HBsAg, anti-HBc IgM, anti-HCV, ASMA, and IgM Ab. against HAV, EBV-VCA, CMV, and HSV were negative. MR cholangiography was normal. Biopsy: prominent cholestasis, acute cholangitis, moderate fibrosis. Ornidazole was discontinued and in 1 month, laboratory returned to normal. For the past 1 year, she is doing well.

Case 2: A 50-year-old woman presented with jaundice, dark urine and weakness for 7 days. Three weeks ago, she was prescribed ornidazole (1 g/day for 3 days) for vaginitis and used it for 3 days. Four days after discontinuation, the symptoms began. She reported an acute hepatitis after ornidazole use of 7 days, 10 years ago. She had jaundiced skin and scleras and HM (2 cm). ALT was 3042 U/L, AST 1665 U/L, t. bilirubin 47.9 mg/dL (d. 19.3), AP 422 U/L, HBsAg, anti-HBc IgM, anti-HAV IgM, anti-HCV, HCV-RNA, anti-HEV, anti-LKM, ASMA, FANA, and IgM Ab. against CMV and EBV VCA were negative. An abdominal USG was normal. Liver biopsy: focal and bridging necrosis, 52 days after laboratory returned to normal. For 8 months, she is healthy.

Case 3: A 25-year-old woman was initiated ornidazole (1 g/day) for vaginitis. Three days later, was readmitted with nausea and the drug was discontinued. Fifteen days later, she presented with jaundice, dark urine, and vomiting. She had jaundiced scleras. ALT was 1160 U/L, AST 790 U/L, t. bilirubin 10.1 mg/dL (d. 8.7), AP 431 U/L. Anti-HAV IgM, HBsAg, anti-HBc IgM, Anti-HCV, HCV-RNA, anti-HEV, and IgM Ab. against CMV and EBV-VCA were negative. MR cholangiography was normal. Biopsy: prominent cholestasis, acute cholangitis, moderate fibrosis. Laboratory returned to normal in 1 month. For the past 1 year, she is doing well. Ornidazole is commonly used and may lead to hepatic toxicity. Cholestasis, cholangitis, fibrosis or necrosis may be seen. Clinicians should be aware of the hepatotoxicity of the drug.

P1357 Inhibitors of P-glycoprotein increase the accumulation of azithromycin and other macrolides in macrophages

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Objectives: Macrolides (M) accumulate in J774 macrophages. These cells express P-glycoprotein (Pgp), an efflux pump transporting basic drugs. We have examined the influence of Pgp inhibitors on accumulation (C_i/C_e) of M and intracellular activity of AZM.

Methods: Wild J774 macrophages were exposed simultaneously to M and Pgp inhibitors. Cell-associated M was measured by microbiological assay. AZM activity towards intracellular *Staphylococcus aureus* (ATCC25923; MIC 0.5 μg/mL) was evaluated by cfu counting after 20-h incubation with AZM at C_e ranging from 0.05 to 10 μg/mL.

Results: Accumulation data is shown in the table.

Drugs[a] (time)	C_i/C_e control	% of control: Cyclosporin (20 μM)	Verapamil (20 μM)	GF120918 (250 nM)
AZM (3 h)	21 ± 4	352 ± 77**	339 ± 52**	218 ± 46**
ERY (2 h)	3 ± 0	451 ± 19***	336 ± 11***	238 ± 7**
TEL (1 h)	25 ± 1	172 ± 6***	139 ± 5***	146 ± 6***
CLR (1 h)	46 ± 4	125 ± 15[c]	81 ± 6*	112 ± 9[c]
RO[b] (1 h)	26 ± 3	150 ± 18*	106 ± 11[c]	131 ± 14*

[a]5 μg/mL for all but ERY (50 μg/mL); [b]roxithromycin; [c]not significant vs. control; ***$P < 0.01$; **$P < 0.01$; *$P < 0.05$.

We tested the effect of verapamil on AZM intracellular activity. Verapamil enhanced the activity of AZM (0.4 log cfu decrease over controls) only at suboptimal AZM C_e (0.5–1 μg/mL).

Conclusion: Pgp may play a key role in regulating the accumulation of AZM and ERY, and, to a lower extent, of other M. Increased C_i/C_e of AZM appears however, of limited chemotherapeutic value, probably because its intracellular activity is largely concentration-independent.

P1358 Effect of voriconazole on the pharmacokinetics of sirolimus

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Objectives: Voriconazole is a second-generation triazole agent for the treatment of severe fungal infections and has a potential inhibitory effect on CYP3A4-mediated metabolism. Sirolimus is an immunosuppressive agent largely metabolized by CYP3A4. Immunosuppressed patients are susceptible to fungal infections and therefore coadministration of sirolimus and voriconazole may be clinically important. The objectives of this study were to determine the effect of steady-state voriconazole on the pharmacokinetics (PK) of single-dose sirolimus, and to investigate the safety and toleration of a single dose of sirolimus during coadministration with voriconazole.

Methods: This was a single-blind, randomized, placebo-controlled, two-period crossover study in 16 healthy men aged 19–43 years. All subjects received voriconazole orally on days 1–9 (400 mg/day/12 h on day 1; 200 mg/day/12 h on days 2–9) or placebo (per day/12 h on days 1–9). Single-dose sirolimus (2 mg, p.o.) was administered on the morning of day 4. Log-transformed maximum plasma concentrations (C_{max}), area under the plasma concentration–time curve to infinity (AUC) and untransformed time to C_{max} (T_{max}) for sirolimus were subject to an analysis of variance (ANOVA) that allowed for variation due to sequence, subject, period and treatment.

Results: Mean C_{max} and AUC of sirolimus were increased by concomitant administration of voriconazole. The means for C_{max} and AUC were approximately 11.1- and 6.6-fold higher, respectively (see table).

Parameter	Sirolimus + voriconazole	Sirolimus + placebo	Comparison	90% CI
AUG (ng h/mL)[a]	1242	112	1114%	987, 1258
C_{max} (ng/mL)[a]	53.6	8.2	656%	573, 752
C_{max} (h)[b]	1.26	0.85	0.41	0.22, 0.59

[a]Geometric mean; comparison is ratio (%) of means.
[b]Arithmetic mean; comparison is difference between means.

Treatment-related adverse events (AEs) were reported by eight subjects (13 events) during voriconazole treatment, 10 subjects (15 events) during sirolimus + voriconazole treatment, three subjects (six events) during placebo treatment and six subjects (nine events) during sirolimus + placebo treatment. The majority of AEs were mild or moderate. One subject discontinued due to an AE which was not regarded as treatment related.

Conclusions: Exposure to sirolimus is increased significantly when coadministered with voriconazole. Coadministration of voriconazole and sirolimus was well tolerated. Mean PK parameters of sirolimus were obtained on day 4 sirolimus (S), voriconazole (V), placebo (P).

P1359 In vitro antimicrobial activity of the essential oils of Greek propolis

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Objectives: Propolis (bee-glue) is a complex mixture of beeswax, small amounts of sugar and plant exudates collected by honey-bees (*Apis mellifera*) from various trees, shrubs and herbs. It has been known as a remedy since ancient times and is still used in folk medicine, in 'bio-cosmetics,' 'health foods' and beverages intended to maintain or improve health. As a part of a systematic research on the chemical composition of propolis, we report in this study the antimicrobial activities of the essential oils and of pure isolated compounds of Greek propolis, which, to our knowledge, have never been studied before.

Methods: The antimicrobial activities of the essential oils and of pure isolated compounds of Greek propolis were determined, using the dilution technique,

by measuring their MIC against two Gram-positive bacteria: *S. aureus*, and *S. epidermidis*, four Gram-negative bacteria: *E. coli*, *E. cloacae*, *K. pneumoniae* and *P. aeruginosa*, and three pathogenic fungi, *Candida albicans*, *C. tropicalis* and *C. glabrata*, all are strains of ATCC. Standard antibiotics were used in order to control the sensitivity of the test organisms.

Results: Through the antimicrobial screening, the oils proved to be active against all six tested bacteria as well as the tested fungi (MIC values 0.5–8.75 mg/mL). The chemical composition of the oils have been studied by GC–MS and from the 105 identified constituents, the major compounds (α-pinene, junipene, α-eudesmol, α-cedrol and manoyl oxide) have been isolated and were tested on the same cultures, under identical conditions, to compare their activities with those of the oil.

Conclusions: The results of the assays suggest that the activity of the oils can be attributed, to a considerable degree, to the existence of α-pinene and manoyl oxide which appeared to possess strong activities against all tested microbia.

P1360 A model using *Staphylococcus aureus* and HEp-2 cells for evaluation of the intracellular pharmacodynamics of antibiotics

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Objectives: Some bacteria, although not obligatory intracellular (IC) pathogens, can have an IC habitat. Such facultative IC distribution might reflect a pathogenic mechanism that may have to be taken into account when treating patients. IC location of *Staphylococcus aureus* (SA) could, e.g. in endocarditis cause treatment failure and relapse after antibiotic regimes. Pharmacodynamic studies of antibiotics in vitro are mostly performed in acellular systems. Few models exist for studies of nonprofessional phagocytes and the effect of antibiotics on facultative IC bacteria. HEp-2 cells can harbor IC pathogens such as *Helicobacter pylori*, *Streptococcus pyogenes* and staphylococcal species. We have adapted a HEp-2 cell model to look at IC killing of SA with IC active antibiotics.

Methods: *S. aureus* strain type 42 D was used; MIC was 0.03125 mg/L to rifampicin (Rifa) and 0.5 mg/L to levofloxacin (Levo). HEp-2 cells (ATCC CCL 23) were cultured to a confluent cell layer and inoculated with challenge doses of SA. Different exposure times were tested. Electron microscopy (EM) photographs were taken. Methods to remove remaining extracellular (EC) bacteria were studied. Infected cells were treated with study antibiotics (Rifa 20–200 × MIC or Levo 50 × MIC). EC antibiotics were removed. Viable count on IC bacteria was done.

Results: The number of IC bacteria increased during the first 2 h of exposure. To reach the intended IC bacterial concentration of 10^5/mL, a final concentration of $\sim 1 \times 10^7$ staphylococci/mL in the exposure medium was needed. IC location of bacteria was confirmed by EM. Gentamicin was the most efficient method for removal of EC bacteria. When treating with Rifa or Levo, the most prominent IC killing of SA occurred during the first 6–9 h. IC bacteria were less efficiently killed than bacteria in the EC medium.

Conclusion: The time for bacterial challenge and control of postexposure EC bacteria showed to be important factors for quantification of IC effects. The study antibiotics, although known to have IC accessibility, primarily affected EC bacteria. Complete IC killing was not achieved at the concentrations used. Further antibiotic studies on facultative intracellular pathogens, also including dynamic models, are warranted. Future and more efficient IC-active antibiotics could have an important clinical application in preventing treatment failures and relapses of potentially dormant IC infections.

New drugs II

P1361 The ketolide antibiotic telithromycin inhibits the in vitro secretion of IL-1α and TNF-α by human monocytes stimulated with lipopolysaccharide

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Objectives: A number of antibiotics have been shown to have significant immunomodulatory properties both in vitro and in animal models. Although it remains unclear whether such properties are clinically relevant, immunomodulation by antibiotics may have significance in the inflammation caused by infection and in septic shock. Ketolides are semisynthetic derivatives of clarithromycin, representing a new class of antibacterials within the macrolide–lincosamide–streptogramin group. Our previous demonstration that the macrolides clarithromycin and azithromycin modulate in vitro cytokine production by human monocytes prompted us to examine the new ketolide antibiotic telithromycin for similar activity.

Methods: Purified human peripheral blood monocytes, obtained from eight healthy adult volunteers, were stimulated with 100 ng/mL of *Escherichia coli* lipopolysaccharide (LPS) and exposed to either 0.5, 1, 2, 5 or 10 μg/mL of telithromycin. These concentrations of telithromycin were not toxic for the monocytes as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cytotoxicity assay (MTT assay). At 3, 6, and 24 h of incubation, the amounts of interleukin (IL)-1α, IL-1β, IL-6, IL-10 and tumor necrosis factor (TNF)-α were measured in the supernatants of the monocyte cultures using enzyme-linked immunosorbent assay.

Results: Stimulation with LPS alone significantly increased secretion of each of the cytokines examined. Treatment of LPS-stimulated monocytes with each of the concentrations of telithromycin significantly inhibited ($P < 0.01$) secretion of IL-1α and TNF-α by monocytes of each of the eight volunteers. A clear dose-response was noted with the most remarkable effect observed at 5 and 10 μg/mL of telithromycin. Secretion of IL-1β, IL-6 and IL-10 was not significantly inhibited in monocytes from any of the donors.

Conclusion: Telithromycin has immunomodulatory effects through its capacity to alter secretion of IL-1α and TNF-α by human monocytes.

P1362 Efficacy of telithromycin in the treatment of community-acquired pneumonia (CAP) caused by resistant pneumococci

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Objectives: Telithromycin, the first ketolide antibacterial to be approved for clinical use, has been specifically designed to offer the ideal spectrum of activity for upper and lower respiratory tract infections, including those caused by resistant strains, with a short, well tolerated treatment regimen. The clinical and bacteriological efficacy of telithromycin were assessed in patients with CAP caused by *Streptococcus pneumoniae* resistant to penicillin G (PEN) and/or erythromycin A (ERY).

Methods: Adult patients with a diagnosis of CAP (radiologically confirmed) received telithromycin 800 mg orally once daily for 7–10 days in one of six phase III clinical trials ($n = 1373$), or telithromycin 800 or 600 mg once daily for 7 days in a phase II study ($n = 96$). Patients with *S. pneumoniae* as the causative pathogen were identified, with particular focus on those infected with PEN- and/or ERY-resistant strains (MICs ≥ 2.0 and ≥ 1.0 mg/L, respectively). Per-protocol (PP) clinical and bacteriological outcomes were assessed at a post-therapy visit (Days 17–21 in the phase III studies or at the end of therapy (Day 7) in the phase II study.

Results: Of 191 patients with documented *S. pneumoniae* infection (single and mixed infections), 32 (16.8%) were infected with PEN- and/or ERY-resistant strains. All isolates were susceptible to ≤ 1.0 mg/L telithromycin. Patients with CAP due to PEN- and/or ERY-resistant *S. pneumoniae* showed high rates of clinical cure (87.5% [28/32]) and bacteriological eradication (90.6% [29/32]). Corresponding clinical cure and bacteriological eradication rates for all pneumococci isolates were both 94.8% (181/191). A total of 9/47 patients with pneumococcal bacteraemia had PEN- and/or ERY-resistant infections, with seven of these nine patients clinically cured with bacteriological eradication.

Conclusion: Telithromycin is a highly effective empirical treatment for patients with CAP caused by PEN- and/or ERY-resistant pneumococci.

P1363 Five-day therapy with telithromycin has good bacteriological efficacy against key respiratory pathogens in acute maxillary sinusitis (AMS)

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Objectives: Telithromycin (TEL) is the first member of a new class of antibacterials – the ketolides – to undergo clinical development and to be approved for clinical use. It has been specifically designed to offer an optimal, well balanced spectrum of coverage in the treatment of respiratory tract infections, including those caused by resistant strains, with a well tolerated, short and reliable treatment regimen. A pooled analysis of bacteriological efficacy of short-course therapy with TEL against key respiratory pathogens in patients with AMS from three phase III clinical trials is reported.
Methods: Adults (aged ≥13 years; $n = 980$) with clinical signs and symptoms of AMS (radiologically confirmed) received 5- or 10-day TEL 800 mg once daily ($n = 608$ and 372, respectively). Causative pathogens were isolated from sinus secretion cultures. PP bacteriological outcomes were assessed at a post-therapy visit (Days 16–24) and classified as satisfactory if the causative pathogen was eradicated or presumed eradicated at this visit.
Results: A total of 324 causative pathogens for 5- and 10-day TEL ($n = 225$ and 99, respectively) were isolated from 253 patients in the bacteriological PP population. The most frequently isolated pathogens were *S. pneumoniae* ($n = 91$), *Haemophilus influenzae* ($n = 64$) and *Moraxella catarrhalis* ($n = 18$). The overall bacteriological eradication rate was 196/225 (87.1%) for 5-day TEL and 90/99 (90.9%) for 10-day TEL. Bacterial eradication rates by key pathogen for 5- and 10-day TEL, respectively, were 56/61 (91.8%) and 27/30 (90%) for *S. pneumoniae*; 14/16 (87.5%) and 6/7 (85.7%) for *S. pneumoniae* with reduced susceptibility to penicillin and/or erythromycin A; 42/48 (87.5%) and 14/16 (87.5%) for *H. influenzae*; 7/9 (77.8%) and 2/2 (100%) for β-lactamase positive *H. influenzae*; 13/14 (92.9%) and 3/4 (75.0%) for *M. catarrhalis*; 19/19 (100%) and 4/4 (100%) for *Staphylococcus aureus*; 2/2 (100%) and 3/3 (100%) for *Streptococcus pyogenes*.
Conclusion: Short-duration therapy with TEL (800 mg) once daily for 5 days is effective at eradicating key respiratory pathogens in AMS, including resistant strains.

P1364 Utility of an 800-mg dose of telithromycin for CAP caused by extracellular pathogens: a preliminary assessment by pharmacodynamic modeling and Monte Carlo simulation

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Objective: Our objective was to evaluate the frequency with which an 800 mg once daily dose of telithromycin attained target AUC/MIC ratios for organism eradication and good clinical outcome.
Methods: A total of 354 patients with CAP who had one or more plasma concentrations determined for telithromycin served as the population for a pharmacokinetic analysis using BigNPAG. A two-compartment open model with a lag time and with first-order input to and clearance from the central compartment was employed. Classification and Regression Tree (CART) analysis and logistic regression were employed to link exposure to outcome in a subset of CAP patients with a defined extracellular pathogen and outcome (microbiological outcome [MO], $n = 72$; clinical outcome [CO], $n = 78$), as well as an MIC to telithromycin. A 2500-subject Monte Carlo simulation was performed with ADAPT II. MIC distributions for *H. influenzae* ($n = 276$), *S. pneumoniae* ($n = 337$), and *Moraxella catarrhalis* ($n = 102$) were obtained from the PROTEKT database.
Results: The mean, median, mode, and standard deviation values for serum clearance normalized to bioavailability were 81.7, 63.6, 43.4, and 53.7 L/h, respectively. After the MAP-Bayesian step, the observed–predicted plot demonstrated a best-fit linear regression with an r of 0.88 ($r^2 = 0.77$). CART analysis demonstrated breakpoint values for AUC/MIC of 2.93 for MO and 4.25 for CO. These exposure variables were included in the final logistic regression models for both clinical and microbiological outcome. Monte Carlo simulation with expectation over the MIC distributions for the target pathogens demonstrated target attainment rates (MO/CO) of >99.9/94.4% (*H. influenzae*), >99.9/>99.9% (*S. pneumoniae*), and >99.9/>99.9% (*M. catarrhalis*).
Conclusion: Using Monte Carlo simulation modeling, telithromycin at a dose of 800 mg once daily generates high probabilities for good microbiological and clinical outcomes for common extracellular respiratory pathogens. These results will be bolstered by having an increased number of patients added to the analysis.

P1365 Antimicrobial susceptibility of 1213 *H. influenzae* isolates against cefditoren and 10 other antimicrobials. A multicenter international study in South Europe (ARISE Project)

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Objective: A multicenter and international study to asses the in vitro susceptibility of *H. influenzae* against cefditoren, a cephem antibiotic, and 10 other antimicrobials was carried out in South Europe (ARISE Project).
Methods: A total of 1213 *H. influenzae* strains were collected: 607 from Spain, 448 from Italy, 129 from Portugal and 29 from Greece between September 2000 and March 2001 from adult patients (≥17 years) with respiratory tract infections (respiratory tract and blood samples). All the isolates were sent to a central Laboratory (Fundación Jiménez Díaz, Madrid, Spain) where susceptibility test was performed by broth microdilution (Sensititre) following NCCLS recommendations.

Antibiotic	MIC range	MIC_{50}	MIC_{90}	No. (%) susceptible strain
Penicillin	≤0.03–≥8	0.5	≥8	N.E.
Amoxicillin	≤0.12–≥32	0.5	8	977 (80.5)
Amox-clav.	≤0.06–8	0.25	1	1210 (99.8)
Cefditoren	≤0.03–0.12	≤0.03	≤0.03	N.E.
Cefadroxil	≤0.25–≥64	16	32	N.E.
Cefpodoxime	≤0.12–1	≤0.12	≤0.12	1213 (100)
Cefuroxime	≤0.12–16	0.5	2	1203 (99.2)
Cefotaxime	≤0.03–0.5	≤0.03	≤0.03	1213 (100)
Erythromycin	≤0.06–≥16	2	4	N.E.
Clarithromycin	≤0.06–64	4	8	1157 (95.4)
Levofloxacin*	≤0.06–1	≤0.06	≤0.06	1212 (100)

*Levofloxacin MIC was not correctly measured in one strain.

Results: The results are shown in the table. Data are expressed in μg/mL. Breakpoint is not established (N.E.) for penicillin, cefditoren, cefadroxil and erythromycin.
Conclusions: Cefditoren inhibited 1206 (99.4%) strains by a concentration <0.03 μg/mL and 236 (19.5%) strains were nonsusceptible to amoxicillin. Susceptibilty to clarithromycin was similar in Italy (95.8%), Greece (96.6%), Spain (95.2%) and Portugal (94.6%).

P1366 In vitro susceptibility of 709 isolates of methicillin-susceptible *S. aureus* (MSSA) against cefditoren and 10 other antimicrobials. A multicenter international study in Southern Europe (ARISE Project)

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Objetive: A multicenter and international study to asses the in vitro susceptibility of MSSA against cefditoren, a cephem antibiotic, and 10 other antimicrobials was carried out in South Europe (ARISE project).
Methods: A total of 709 SAMS strains were collected, 406 from Spain, 155 from Italy, 74 each from Portugal and Greece between September 2000 and March 2001 from adults patients (≥17 years) with respiratory tract or skin/soft tissue infections. All the isolates were sent to a central Laboratory (Fundación Jiménez Díaz, Madrid, Spain) where susceptibility test was performed by broth microdilution (Sensititre) following NCCLS recommendations.

Results: Results are shown in the table. Data are expressed in μg/mL. Breakpoint is not established (N.E.) for cefditoren and cefadroxil.

Antibiotic	Range of MIC	MIC_{50}	MIC_{90}	No. (%) susceptible strain
Penicillin	≤0.03–≥8	≥8	≥8	70 (9.9)
Amoxicillin	≤0.12–≥32	8	≥32	54 (7.6)
Amox-clav.	≤0.06–4	0.5	1	709 (100)
Cefidtoren	≤0.03–2	0.5	0.5	N.E.
Cefadroxil	≤0.25–32	4	4	N.E.
Cefpodoxime	≤0.12–8	2	2	671 (94.6)
Cefuroxime	≤0.12–≥32	1	2	708 (99.9)
Cefotaxime	≤0.03–≥8	2	2	709 (100)
Erythromycin	≤0.06–≥16	0.25	≥16	563 (79.4)
Clarithromycin	≤0.06–≥256	0.25	≥256	573 (80.8)
Levofloxacin	≤0.06–8	0.25	0.5	683 (96.3)

N.E. not established.

Conclusions: All strains were inhibited by a cefditoren concentration of 2 μg/mL. No resistance to Amox-clav. and cefotaxime was detected. Resistance to macrolide antibiotics was similar (around 20%) and resistance to levofloxacin, cefpodoxime and cefuroxime was unusual (less than 6%), however, resistance to amoxicillin and penicillin were high (more than 90%).

P1367 In vitro activity of cefditoren and 10 other antimicrobials against 877 *S. pneumoniae* strains isolated in Southern European Countries (ARISE Project)

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Objective: In order to describe the susceptibility of *S. pneumoniae* against cefditoren, a cephem antibiotic, and 10 other antimicrobials, a multicenter study in South Europe was carried out.
Methods: A total of 877 *S. pneumoniae* strains were collected, 459 from Spain, 312 from Italy, 89 from Portugal and 17 from Greece between September 2000 and March 2001 from adult patients (≥17 years) with respiratory tract infection (respiratory tract samples and blood cultures). All the isolates were sent to a central Laboratory (Fundación Jiménez Díaz, Madrid, Spain) where susceptibility test was performed by broth microdilution (Sensititre) following NCCLS recommendations.
Results: The results are shown in the table.

Antibiotic	MIC range	MIC_{50}	MIC_{90}
Penicillin	≤0.03–≥8	≤0.03	2
Amoxicillin	≤0.12–16	≤0.12	2
Amox-clav.	≤0.06–≥16	≤0.06	2
Cefidtoren	≤0.03–4	≤0.03	0.5
Cefadroxil	≤0.25–≥64	2	≥64
Cefpodoxime	≤0.12–≥32	≤0.12	2
Cefuroxime	≤0.12–≥32	≤0.12	8
Cefotaxime	≤0.03–≥8	≤0.03	1
Erythromycin	≤0.06–≥16	≤0.06	≥16
Clarithromycin	≤0.06–≥256	≤0.06	≥256
Levofloxacin	≤0.06–≥16	1	1

Data are expressed in μg/mL. Breakpoint is not established (N.E.) for cefditoren and cefadroxil.
Conclusions: Cefditoren was the most active antibiotic tested (MIC_{90} = 0.5 μg/mL), followed by levofloxacin and cefotaxime (MIC_{90} = 1 μg/mL). Penicillin susceptibility was lower in Greece (35.3%) and Spain (52.9%) than in Portugal (83.1%) and Italy (84.3%). Erythromycin susceptibility was lower in Italy (61.2%) and Spain (63.4%) than in Greece (70.6%) and Portugal (84.3%). The prevalence of levofloxacin resistance was low and occurred only in Spain (2.6%) and Italy (0.3%).

P1368 In vitro susceptibility of 590 isolates of *S. pyogenes* against cefditoren and 10 other antimicrobials. A multicenter international study in Southern Europe (ARISE Project)

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Objective: A multicenter and international study to assess the in vitro susceptibility of *S. pyogenes* against cefditoren, a cephem antibiotic, and 10 other antimicrobials was carried out in South Europe (ARISE project).
Methods: A total of 590 *S. pyogenes* strains were collected, 286 from Spain, 278 from Italy, 13 each from Portugal and Greece between September 2000 and March 2001 from adult patients (>17 years) with pharyngitis or soft tissue infections. All the isolates were sent to a central Laboratory (Fundación Jiménez Díaz, Madrid, Spain) where susceptibility test was performed by broth microdilution (Sensititre) following NCCLS recommendations.
Results: The results are shown in the table. Data are expressed in μg/mL. Breakpoint is not established (N.E.) for Amox-clav., cefditoren and cefadroxil.

Antibiotic	Range of MIC	MIC_{50}	MIC_{90}	No. (%) susceptible strain
Penicillin	≤0.03–0.06	≤0.03	≤0.03	590 (100)
Amoxicillin	≤0.12–≤0.12	≤0.12	≤0.12	590 (100)
Amox-clav.	≤0.06–≤0.06	≤0.06	≤0.06	N.E.
Cefidtoren	≤0.03–≤0.03	≤0.03	≤0.03	N.E.
Cefadroxil	≤0.25–8	≤0.25	≤0.25	N.E.
Cefpodoxime	≤0.12–≤0.12	≤0.12	≤0.12	590 (100)
Cefuroxime	≤0.12–≤0.12	≤0.12	≤0.12	590 (100)
Cefotaxime	≤0.03–0.12	≤0.03	≤0.03	590 (100)
Erythromycin	≤0.06–≥16	≤0.06	≥16	461 (78.1)
Clarithromycin	≤0.06–≥256	≤0.06	16	461 (78.1)
Levofloxacin	0.12–2	0.5	1	590 (100)

N.E. not established.

Conclusions: All strains were inhibited by a cefditoren concentration <0.03 μg/mL. No resistance to penicillin and levofloxacin was detected. Resistance to macrolide antibiotics was detected in Italy (32.0%), Greece (23.1%) and Spain (12.9%) but not in Portugal.

P1369 In vitro activity of ketolide ABT773 against *Listeria monocytogenes* and Coryneform bacteria of clinical interest

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Objectives: To evaluate the in vitro activities of ketolide ABT 773 (ABT), ampicillin (AMP), cefuroxime (CUR), ciprofloxacin (CIP), clindamycin (CLD), cotrimoxazole (SXT), erythromycin (ERY) and vancomycin (VAN) against *L. monocytogenes* and coryneform bacteria strains isolated from clinical samples.
Methods: Two hundred and eleven strains were evaluated, including: *L. monocytogenes* (15), *Corynebacterium amycolatum* (40), *C. jeikeium* (40), *C. minutissimum* (14), *C. pseudodiphteriticum* (12), *C. striatum* (40), *C. urealyticum* (40), and *Rhodococcus equi* (10). Organisms were identified using API CORYNE strips and additional phenotypic tests when necessary. A microdilution assay using cation-adjusted Mueller Hinton broth (with 0.5% Tween 80 for *C. jeikeium*

and *C. urealyticum* was used. Plates were incubated aerobically at 35 °C for 24 h or in the case of *C. jeikeium* and *C. urealyticum*) for up to 48 h.
Results: MIC_{50}/MIC_{90} (mg/L) values against all 211 tested strains were 0.25/>32 (ABT), 2/>64 (AMP), >128/>128 (ERY), >64/>64 (CLD), 4/>64 (CUR), >16/>16 (CIP), >16/>16 (SXT) and 0.5/1 (VAN). MIC_{50}/MIC_{90} against *L. monocytogenes* were 0.015/0.015 (ABT), 0.5/1 (AMP), 0.25/0.5 (ERY), 2/2 (CLD), >64/>64 (CUR), 1/4 (CIP), 0.015/0.03 (SXT) and 1/1 (VAN). For the remaining species. MIC_{50}/MIC_{90} of ABT, ERY and CLD were: 0.125/0.5, 16/128 and >64/64 (*C. amycolatum*), 1/>32, >128/>128 and >64/>64 (*C. jeikeium*), 0.03/>32, 32/>128 and >64/>64 (*C. minutissimum*), >32/>32, >128/>128 and >64/>64 (*C. pseudodiphteriticum*), 0.125/>32, 8/>128 and >64/>64 (*C. striatum*), >32/>32, >128/>128 and >64/>64 (*C. urealyticum*), and 0.03/0.5, 0.5/>128 and 4/>64 (*R. equi*).
Conclusions: All strains tested were inhibited by vancomycin at 2 mg/L. ABT 773 was the most active compound of those herein evaluated against *L. monocytogenes*. ABT was also the most active compound against *C. amycolatum* and *R. equi*. ABT was more active than erythromycin and clindamycin against *C. jeikeium*, *C. minutissimum*, and *C. striatum*.

P1370 Synthesis and studies of bacteriostatic activity of 5-aminothiazole

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Earlier we have synthesized diamides of dicarboxylic acids, amide components of which are 5-nitrothiazole on the one hand, and urea, biuret, benzolsulphamides, triazol, on the other hand. All the above compounds exhibit bacteriostatic activity towards certain microorganisms (Khimicheskii i Farmakologicheskii Zhurnal, Vestsi AN Belarusi., 1993, no. 4, s. 61–63). For further studies of bacteriostatic activity of amides and diamides of dicarboxylic acids, as well as for determination of structure–activity' relationship, we have synthesized a range of monoamides of 5-nitro- or 5-sulpho-2-aminothiazole; that of monoamides of 2- or 4-aminopyridines; as well as that of diamides of dicarboxylic acids, with their amide component being 5-nitro- or sulpho-thyazole-2-yl on the one hand, and 2-or-4-pyridyl on the other hand. Antimicrobial activity of the synthesized compounds was studied in vitro with the use of the method of double series dilution in a liquid broth. For this purpose, approximately 50 various Gram-positive and Gram-negative microorganisms: *S. aureus*, *Bacillus subtilis*, *Serratia marchescens*, *E. coli*, *Proteus morganii*, *Micrococcus lisodeicticus*, *Staphylococcus epidermidis*, *Shigella sonnei* and others. These included strains obtained from patients (*E. coli* 026, *S. aureus* 877 st, *S. epidermidis* 994). Minimum inhibitory concentration was expressed in mkg/mL. Nitazole was used as a comparison substance. The obtained data demonstrate that diamides of dicarboxylic acids containing a pyridine cycle possess at least the same bacteriostatic activity as nitazole or have a wider activity range. They inhibit growth of all microorganisms in concentrations 30–240 mkg/mL, diamides of azelainic acid turned out to be stronger bacteriostatics than nitazole 103 mkg/mL. All mono-(5-nitrothiazole-2-yl) amides of dicarboxylic acids did not exhibit any more antimicrobial activity than nitazole in relation to all the microorganisms under study with the exception of *E. coli* K-12 240 mkg/mL. At minimum inhibitory concentration of less then 1000 mkg/mL, monopyridilamides proved to be inactive. All similar mono- and diamides of 5-sulpho-2-aminothiazole proved inactive too. Analysis of the obtained results makes it obvious that heterocycles function inadditively in the studied diamides. The diamides' bacteriostatic effect is due to synergic activity of heterocycles of thiazole and pyridine. Diacyl radical, too, provides insignificant effect.

P1371 Comparison of daptomycin and vancomycin MIC results by DIN, NCCLS, SFM and SRGA methods for 297 Gram-positive organisms

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Objective: Daptomycin is a novel lipopeptide antibiotic with potent bactericidal activity against Gram-positive strains. Broth containing physiologic levels of free Ca^{2+} (50 μg/mL) is recommended for daptomycin MIC testing (a higher Ca^{2+} level than is used in most MIC methods. Validation of MICs using calcium supplemented broth or agar by various MIC methods is necessary.
Methods: Daptomycin and vancomycin susceptibility of 297 Gram-positive organisms [50 each of *S. aureus* (SA), *S. epidermidis* (SE, *Enterococcus faecium* (EM), *Enterococcus faecalis* (ES), *S. pneumoniae* (SP) and 47 viridans *Streptococcus* (SV)] was determined by DIN using Isotonic broth (instead of Isosensitest broth), by NCCLS using Mueller Hinton broth, by SFM using Mueller Hinton agar from three different manufacturers and by SRGA using PDM agar. All media was supplemented to 50 μg/mL Ca^{2+}, except for SFM1 which was supplemented to 25 μg/mL Ca^{2+}. Lysed horse blood was added to the media for all methods for Streptococcus testing. NCCLS recommended QC organisms were tested on each day of testing.
Results: All QC results were within expected NCCLS ranges. All vancomycin mean MICs were within ±1 doubling dilution of one another. Daptomycin mean MICs (μg/mL) appear in Table 1.

Table 1

	NCCLS	SFM1	SFM2	SFM3	SFM4	SRGA	DIN
SA	0.26	0.54	0.23	0.22	0.12	1.95	0.34
SE	0.28	0.76	0.35	0.27	0.22	1.13	0.33
EM	2.91	3.84	1.69	0.76	1.56	3.89	2.23
ES	1.67	1.79	0.68	0.50	0.30	2.71	1.36
SP	0.1	0.46	0.21	0.22	0.12	0.47	0.16
SV	0.35	1.08	0.44	0.42	0.29	0.78	0.43
ALL	0.49	1.06	0.45	0.40	0.27	1.44	0.54

Conclusions: Isotonic broth, used in the DIN, is a valid alternative to Isosensitest broth for daptomycin testing. Modification of inoculum preparation for enterococci testing and additional calcium supplementation of lysed horse blood containing media may be required in order to accurately assess daptomycin MICs using SFM methods. Additional calcium supplementation of PDM agar used in the SRGA method is also recommended.

P1372 Ramoplanin is active against *E. gallinarum* isolates from neutropenic patients expressing *vanA*, *vanB* and *vanC* resistance to vancomycin

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Objectives: Ramoplanin is an investigational agent for the prevention of bloodstream infection due to vancomycin resistant enterococci (VRE). This study reports on the prevalence and resistance genotypes of *E. gallinarum* from neutropenic patients, and the comparative activity of ramoplanin and glycopeptides.
Methods: In an ongoing study, patients expected to be profoundly neutropenic for at least 10 days are screened for the presence of VRE in stools prior to enrolment. We report on 16 patients from 7 study centers found to be colonised with *E. gallinarum*. The isolates from these patients were genotyped (for *vanA*, *B*, *C* and *D*) by multiplex PCR and subjected to chromosomal fingerprinting by pulsed field gel electrophoresis.
Results: Various combinations of *van* genes were found: seven isolates had both *van A* and *vanC* genes present, two had both *vanB* and *vanC*, two had *vanC* only and two had *vanA*, *vanB* and *vanC* genes. Three remain unidentified. The vancomycin MICs for these organism ranged from 4 to 128 mg/L, the highest value being recorded for one of the two isolates with the *vanB*, *vanC* genotype. All isolates of the *vanA*, *vanC* genotype had vancomycin MIC of 8 or 16 mg/L, and those with *vanA*, *vanB* and *vanC* had MIC of 8 mg/L. PFGE analysis showed patient-to-patient transmission of one genotype (*vanA*, *vanC*) at one study center. All isolates of *E. gallinarum* isolated to date were susceptible to ramoplanin and also to teicoplanin.
Conclusions: Whilst this study confirms that *E. gallinarum* can harbor and express vancomycin resistance genes other than *vanC*, the clinical relevance of this species in causing bloodstream infection in at-risk patients remains to be elucidated.

P1373 Evaluation of the pharmacokinetic/pharmacodynamic (PK/PD) features of SB-264128, a novel Pleuromutilin antibiotic, in rodent models of infection

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Objectives: SB-264128 is a novel Pleuromutilin derivative characterized by good in vitro antimicrobial activity against respiratory tract pathogens such as *S. pneumoniae* (Sp) and *H. influenzae*. The objective of this study was to investigate the pharmacodynamic activity of SB-264128 and to assess which PK/PD parameter best correlates with in vivo antimicrobial efficacy.
Methods: Single dose pharmacokinetics of SB-264128 were determined following subcutaneous (SC) dosing of 10, 50 and 200 mg/kg in immune-competent mice (IC), and 50 mg/kg in immunosuppressed mice (IS). Thigh infection in mice was induced by injecting 2×10^5 cfu of Sp ATCC 10813 into both thighs. Two hours post-infection (p.i.) SB-264128 was administered SC at dosages ranging from 10 to 400 mg/kg per day. Thigh bacterial load was determined 24 h p.i. Nonlinear regression analysis was used to identify the PK/PD parameter that best correlates with cfu/thigh at 24 h p.i. Pneumonia in IC mice was induced by intranasal instillation of 5×10^6 cfu of Sp BG1. SB-264128 was given SC over a dosage range of 10–400 mg/kg per day starting 6 h p.i. Lung bacterial load was assessed 24 h p.i.
Results: SB-264128 was highly and rapidly absorbed after SC administration in uninfected IC mice, with C_{max} values of 2486 and 12 453 ng/mL (0.25 h at 10 and 50 mg/kg, respectively, and 39018 ng/mL (0.5 h at 200 mg/kg. PK profiles appeared linear within the dose-range tested. Calculated apparent half-lives were 1.1, 1.5 and 1.4 h at 10, 50 and 200 mg/kg, respectively. No significant difference was found between the PK parameters of IC and IS mice. In an experimental thigh infection model, the antimicrobial activity of SB-264128 was found to be highly correlated with the time (free drug concentration) >MIC (T_f > MIC). Bacteriostatic effect was attained at a T_f > MIC value of 9 and 30% in IC and IS animals, respectively. SB-264128 also proved to be highly efficacious in a pneumonia model, with complete bacterial clearance at a T_f > MIC value of 25%.
Conclusion: Dosing frequency of SB-264128 was the major determinant of efficacy against *S. pneumoniae* both in thigh and pneumonia infection models in mice. Consistently, T_f > MIC was the PK/PD parameter that showed the best correlation with in vivo activity of SB-264128. Collectively these results suggest that the efficacy of SB-264128 is affected by the duration of bacterial exposure to the drug rather than its concentration.

P1374 Comparative efficacy and safety of pharmacokinetically enhanced amoxicillin/clavulanate b.d. versus amoxicillin/clavulanate 875/125 mg t.d.s. in CAP

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Objectives: The pharmacokinetically enhanced formulation of amoxicillin/clavulanate (AMX/CA 2000/125 mg) was designed to achieve high levels of amoxicillin over the dosing duration to eradicate isolates of *S. pneumoniae* with amoxicillin MICs up to and including 4 mg/L. This study compared the clinical and bacteriological efficacy and safety of AMX/CA 2000/125 mg b.d. vs. AMX/CA 875/125 mg t.d.s. in the treatment of typical CAP in adults.
Methods: This was a randomized, double-blind, double-dummy, multicenter, parallel-group study conducted in Spain and Italy. Patients received either AMX/CA 2000/125 mg b.d. or AMX/CA 875/125 mg t.d.s. orally for 7 or 10 days.
Results: A total of 319 patients aged 18–94 years received AMX/CA 2000/125 mg (158) or AMX/CA 875/125 mg (161). At test of cure (TOC), Days 18–39, clinical success in the clinical PP population (primary efficacy endpoint) for AMX/CA 2000/125 mg was 94.7% (108/114) vs. 88.8% for AMX/CA 875/125 mg (103/116) (treatment difference 5.9; 95% CI −7.1, 13.0). Clinical efficacy at end of therapy (EOT, Days 8–17) in the clinical PP population was 96.0% (121/126) for AMX/CA 2000/125 mg vs. 92.2% (118/128) for AMX/CA 875/125 mg (treatment difference 3.8; 95% CI −1.9, 9.6). Bacteriological success in the bacteriology PP population for AMX/CA 2000/125 mg was 85% (17/20) at TOC and 91.3% (21/23) at EOT vs. 77.3% (17/22) and 80.8% (21/26), respectively, for AMX/CA 875/125 mg. At TOC, three patients (including two with bacteremia) in the AMX/CA 2000/125 mg group and one patient in the AMX/CA 875/125 mg group with penicillin-resistant *S. pneumoniae* isolated at screening were clinical and bacteriological successes. Both agents were well tolerated, with the frequency of adverse events similar for AMX/CA 2000/125 mg (62.0%) and AMX/CA 875/125 mg (57.8%); only 7.0 and 6.2% of patients, respectively, had adverse events leading to withdrawal.
Conclusions: AMX/CA 2000/125 mg b.d. was at least as effective clinically as AMX/CA 875/125 mg t.d.s. in the treatment of CAP, with a safety profile similar to that of other AMX/CA formulations.

P1375 Efficacy of a pharmacokinetically enhanced formulation of amoxicillin/clavulanate against experimental respiratory tract infection in rats caused by *H. influenzae*

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Objectives: The efficacy of a novel pharmacokinetically enhanced formulation of amoxicillin/clavulanate (AMX/CA 16 : 1) was studied in experimental respiratory tract infection (RTI) in rats against *H. influenzae* (BLA + and BLNAR).
Methods: Rats were infected intrabronchially. Therapy began 24 h later to simulate in the rat serum levels following human oral dosing of AMX/CA 2000/125 mg (16 : 1 b.d.), AMX/CA 875/125 mg (7 : 1 t.d.s., b.d.), AMX/CA 1000/125 mg (8 : 1 t.d.s.), azithromycin (AZI) 1000/500 mg (o.d.) and levofloxacin (LEV) 500 mg (o.d.). Therapy continued for 3 days; 14 h after therapy the rats were killed and the lungs removed for bacterial enumeration.
Results: AMX/CA 16 : 1 was highly effective against RTI caused by *H. influenzae* H128 (BLA+, AMX/CA MIC 1.0/0.5 mg/L), reducing bacterial numbers by 4 log compared with controls (2.0 ± 0.7 vs. 6.4 ± 0.6 log[10] cfu/lungs, respectively; $P \leq 0.01$. The effect achieved with AMX/CA 16 : 1 was similar to AMX/CA 7 : 1 b.d., AMX/CA 8 : 1 t.d.s., AMX/CA 7 : 1 t.d.s., AZI and LEV (2.0 ± 0.8, 2.1 ± 1.0, 2.8 ± 1.3, 3.2 ± 1.7 and ≤ 1.7 log[10] cfu/lungs, respectively; $P \geq 0.05$. Following infection with *H. influenzae* Chesterfield (BLNAR, AMX MIC 4.0 mg/L), AMX/CA 16 : 1, AMX/CA 7 : 1 t.d.s. and AMX/CA 8 : 1 t.d.s. produced a marked effect, reducing bacterial counts by 3 log compared with controls (3.1 ± 0.9, 3.6 ± 1.1, 3.8 ± 1.3 vs. 6.7 ± 0.6 log[10] cfu/lungs, respectively; $P \leq 0.01$). AMX/CA 7 : 1 b.d. was less effective (5.1 ± 0.9; $P \leq 0.05$ vs. controls; $P \leq 0.01$ vs. AMX/CA 16 : 1). AZI produced a marginal effect with bacterial counts 1–1.5 log lower than controls, even though the AZI MIC was 2 mg/L for this strain (5.8 ± 1.1 log[10] cfu/lungs; $P \leq 0.05$). AMX/CA 16 : 1 showed significant improvements in efficacy compared with AZI ($P \leq 0.01$).
Conclusions: These data indicate the potential benefit of AMX/CA 16 : 1 b.d. compared with existing therapies and support a susceptible breakpoint for this formulation of ≥4.0 mg/L.

P1376 XOB: a novel, orally absorbed, inhibitor of Class A and C β-lactamases

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Background: β-lactamase production is the principal mechanism of resistance to penicillins and cephalosporins. Existing β-lactamase inhibitors are generally ineffective against Class C β-lactamases and/or are not orally available. Oxapenem XOB has the potential to fill this clinical niche.
Methods and results: XOB inhibited all 8 (TEM-1, TEM-10, SHV-5, P99, S2, S & A, OXA-1 & OXA-5) cell-free β-lactamases (spectrophotometric assay), with IC_{50} values of <0.001–0.003 mg/L, whereas IC_{50}s for clavulanic acid were >3 mg/L against Class C and D β-lactamases. In MIC tests (NCCLS guidelines) against 36 nonfastidious isolates many producing high levels of Class A, C or D β-lactamases. XOB enhanced ceftazidime activity against all species tested except *P. aeruginosa* and *E. faecalis*. XOB possesses little intrinsic antibacterial activity (MICs > 8 mg/L) against nonfastidious species but, at 8 mg/L, causes filamentation in *E. coli*. XOB exhibited similar DHP stability to meropenem (>95% stability over 1 h at 37 °C), and peak serum levels of 5.3 and 16.2 mg/L following oral and SC administration at 50 mg/kg in mice.

Conclusion: XOB has the potential to become the first β-lactamase inhibitor to be developed for oral and parenteral administration against organisms producing Class A, C and D β-lactamases.

P1377 Contributions to the study of cellular activity of some polyacetate carbohydrates

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Objectives: Latest 20 years are characterized by the introducing in therapy of many new molecules. Polyacetates carbohydrates such as α-D-glucose pentaacetate, D-sorbitol hexaacetate, D-mannitol hexaacetate are chemically well known, but their cellular properties are less known. We intended to verify if these substances possess bacteriostatical or bactericide properties against some microorganisms and also we intended to study if these substances possess mutagenic properties.

Material and method: In our study, we used following substances:

- α-D-glucose pentaacetate 99% from Aldrich;
- D-sorbitol hexaacetate 97% from Aldrich;
- D-mannitol hexaacetate from Aldrich;
- four series of mannitol hexaacetate we synthesized, and characterized from purity point of view.

All reagents were European Pharmacopoeia or USP grade. In order to verify microbiological properties we tested the activity of substances mentioned above on following microorganisms: *E. coli* (IP52166), *S. aureus* (ATCC 9144), *P. aeruginosa* (IP5842), *B. subtilis* (ATCC 6633), *C. albicans* (IP 4872), *S. cerevisiae* (ATCC 2601) and *Aspergillus niger* (ATCC 16604). We added the substances as microtablets and we measured the inhibition zone. Mutagenic activity was tested using Feulgen method. We colored in violet red the genetic material from *Secale* sp. with Schiff reagent. The *Secale* sp. seed were incubated with different concentrations of substances mentioned above. As a blank we used distilled water. After young plants raised, we croped 1 cm from the superior regions of leaves and first prefixed and then we fixed the coloration of genetic material. In order to assure the maximum spirallyzation of chromosomes we kept some samples at 4 °C between 12 and 24 h and some we treated with a colchicine solution 2 h. We have fixed the color 5 min with Bataglia solution (a mixture of ethanol, chloroform, glacial acetic acid and formaldehyde solution in a 5 : 1 : 1 : 1 : 1 ratio). After that we washed the samples 5 min at room temperature with a normal solution of hydrochloric acid, and we hydrolyzed the samples 10–12 min with a 50% solution of hydrochloric acid. The color of chromosomes was obtained by immersing in Schiff reagent at room temperature.

Conclusions: The tested substances have some microbiological properties and have no mutagenical properties against the DNA from *Secale* sp.

P1378 AM-112 (PFOB): chemistry and biological activity of a novel, broad spectrum, β-lactamase inhibitor

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Introduction: Current β-lactamase inhibitors are effective against β-lactam resistance mediated by Class A but not Class C β-lactamases. This report describes the chemistry and biological activity of AM-112, an oxapenem with potent activity against both Class A and Class C β-lactamases.

Methods and results: AM-112, prepared using a convergent 7-step synthesis. Its stability to hydrolysis by porcine dehydropeptidase is similar to meropenem. AM-112 inhibits cell-free Class A, C and D β-lactamases (TEM-1, TEM-10, SHV-5, P99, S2, S & A, OXA-1 & OXA-5) with IC_{50} values of <0.001–0.003 mg/L. AM-112 possesses antibacterial activity (MIC against *S. aureus* (0.5–2 mg/L), penicillin-susceptible *S. pneumoniae* (<0.5 mg/L) and *M. catarrhalis* (<0.5 mg/L). AM-112 synergises with cephalosporins, markedly enhancing their activity against Enterobacteriaceae isolates producing high levels of characterized β-lactamases and producing unexpected synergy against β-lactamase negative enterococci. Following i.v. administration at 10, 50 and 100 mg/kg, AM-112 exhibits similar pharmacokinetics to ceftazidime in mice and rats. In animal models of infection, AM-112 complements ceftazidime activity against *S. aureus* and enhances ceftazidime activity against *E. cloacae* P99 and *E. coli* SHV-5.

Conclusion: AM-112 has the potential to markedly increase ceftazidime efficacy in the treatment of serious infections (Fig. 1).

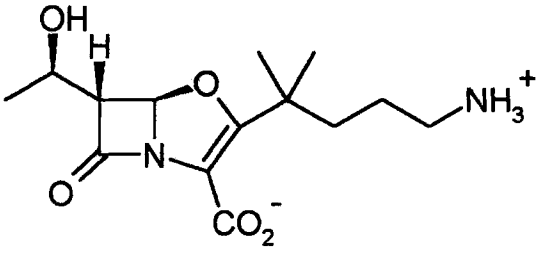

Figure 1

P1379 Clearance of moxifloxacin during continuous hemofiltration in vitro

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Background/aims: Moxifloxacin is a new 8-methoxy-fluoroquinolone with broad-spectrum Gram-positive and Gram-negative activity. Mean renal clearance was between 24 and 53 mL/min after administration of both the single oral and i.v. formulations. [1] The clearance of moxifloxacin was unaltered in the presence of renal insufficiency following single oral doses in one study, [2] suggesting lack of need for dose adjustment. However, moxifloxacin has not been studied during continuous hemofiltration. Aim of this in vitro study was to determine the clearance of moxifloxacin during continuous hemofiltration.

Methods: Continuous hemoflitration with the following conditions: 1000 mL of human blood (600 mL) washed human erythrocytes, 200 mL human albumin 20%, 100 mL Ringer solution, 100 mL $NaHCO_3$, pH 7.35, hematocrit 41 was enriched with 8 mg moxifloxacin and was hemofiltrated (blood pump: HF Edwards BM 11, post-dilution hemofiltration system with SH-Bic 35 filtration solution, hemofilter: Hospal Multiflow 100, blood flow 100 mL/min, turnover 2000 mL/h); samples before filter, after filter, filtrate (min): 0, 5, 10, 15, 20, 25, 30, 40, 50, 60; determination of moxifloxacin: HPLC; determination of clearance, sieving coefficient: Excel 7.0.

Results: Under these hemofiltration conditions in vitro we found a clearance of 20 mL/min and a sieving coefficient of 0.9.

Conclusion: During continuous hemofiltration in vitro with a blood flow of 100 mL/min and a turnover rate of 2000 mL/h we found a nearly normal clearance. This finding would suggest a lack of need for dose adjustment under these hemofiltration conditions and a normal hepatic function. The high sieving coefficient would suggest that moxifloxacin is filtered nearly as free as creatinine and therefore independently of the blood protein concentration. Following investigations with variations of hemofiltration conditions, protein concentrations and real clinical situations have to prove this hypothesis.

References

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P1380 Safety update of oral moxifloxacin: a review of worldwide post-marketing surveillance

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Introduction: Moxifloxacin (MXF), a new 8-methoxy fluoroquinolone, has been shown to be effective and safe for the treatment of respiratory tract infections in controlled clinical studies. Recently compiled safety data from oral post-marketing surveillance are reviewed.

Methods: An analysis of one large phase IV safety study and of two large post-marketing safety observational studies (PMOS) was performed for the incidence of adverse drug reactions (ADR), and to put this in perspective to the data generated during preauthorization clinical trials. Data are also reported for the >7.2 million patients as of August 2001 who have been prescribed MXF.

Results: Post-marketing safety surveillance studies and clinical trial ADRs and serious ADRs (SADRs) for oral moxifloxacin were as in the table.

Trials	No. Pts.	ADR	SADR	Most frequent ADRs
30 Clinical trails	7368	25%	0.5%	7% Nausea, 5% diarrhea, 3% dizziness
Phase IV study (USA)	18374	14.3%	0.1%	5.3% Nausea, 2.2% diarrhea, 2.0% dizziness
PMOS community (Germany)	16007	2%	0.2%	0.5% Nausea, 0.4% diarrhea, 0.2% dizziness
PMOS hospital (Germany	2188	3%	0.8%	1% Diarrhea, 0.2% rash, 0.1% nausea

The ADR frequency in the post-marketing studies conducted under 'real world' conditions was much lower than in the controlled clinical trials. There was no evidence for significant cardiac events potentially related to QT prolongation in the safety surveillance studies involving 36 569 patients. As of August 2001, Bayer has received from worldwide sources a total of 1979 spontaneous ADRs on the background of 7.2 million patients exposed. There were three confirmed cases of Torsades de Pointes (TdP) reported for these 7.2 million patients, all confounded by multiple arrhythmogenic factors (e.g. coexisting heart disease, electrolyte disturbance and concurrent medications). The incidence of 2.1 cases/100 000 patient years TdP seen with MXF treatment is similar to the background rate (four cases/100 000 patient years). Only rare (<0.001%) reports of MXF-induced tendon rupture, convulsion or severe hepatic events have been noted. No new ADRs have been detected from the post-marketing safety data unknown to the class of newer fluoroquinolones.

Conclusion: MXF is as safe and well tolerated as other commonly prescribed antibiotics.

P1381 Moxifloxacin lung penetration after repeated oral and i.v. administrations of 400 mg once daily dose

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Objectives: Pharmacokinetic of moxifloxacin in plasma and lung tissue at steady state (400 mg) o.d. was studied in 48 patients (24 i.v. and 24 oral) subject to lung surgery for bronchial carcinoma.

Methods: Mean (±SD) characteristics of the patient population were: age = 67.14 (±7.87) years, weight = 75.45 (±12.63) kg and creatinine clearance = 68.34 (±10.65) mL/min. Plasma samples (two for each patient) and lung samples (one for each patient) were obtained and analyzed by a validated HPLC assay with UV detection. To determine lung/plasma concentration ratios, one plasma sample was drawn simultaneously with a lung sample. In total, 12 groups, 6 for i.v. and 6 for oral were made according to the time of sampling after the last administration (fifth) of moxifloxacin to obtain pharmacokinetic steady-state: 1 h ($n = 4$), 2 h ($n = 8$), 3 h ($n = 8$), 8 h ($n = 4$), 9 h ($n = 4$), 12 h ($n = 4$), 24 h ($n = 8$) and 36 h ($n = 8$).

Results: Results are presented for i.v. and oral administrations, respectively. The lung/plasma concentration ratios were calculated in each group and ranged from 1.32 to 3.31 and from 2.64 to 5.96. The mean (±SD) lung/plasma ratios were 1.97 (±0.74) and 4.33 (±1.35). Minimum mean (±SD) steady state lung concentrations were 1472 (±447) ng/g and 1731 (±636) ng/g. Mean (±SD) maximum steady state lung concentrations were 7706 (±2435) ng/g and 14965 ± 2704 ng/g and were reached rapidly, 1 and 3 h after last administration. Respective mean (±SD) maximum steady state plasma concentrations were 6754 (±2416) ng/mL and 2919 (±550) ng/mL. Times to obtain these concentrations were 01.52 and 3 h. The terminal elimination half-lives in lung tissue were 10 and 12 h during the period of 12–24 h. Mean concentrations ratios are time dependent and interindividual variability is higher for oral than i.v. administration.

Conclusions: These results indicate that a dosage regimen of 400 mg moxifloxacin given once daily provides excellent lung penetration as evidenced by the minimum steady state lung concentrations and steady-state moxifloxacin concentrations (lung/plasma) ratios and moxifloxacin therefore is a good choice for the treatment of lower respiratory tract infections.

P1382 In vitro release of moxifloxacin from a new type calcium phosphate bone cement (Norian)

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Objective: Norian skeletal replacement system (NSRS) is a novel injectable calcium phosphate cement proposed for the structural augmentation of regions of cancelous bone after fractures (Elder et al. *J Orthop Trauma* 2000, 14: 386). The present study aimed to the in vitro release of moxifloxacin from NSRS in order to investigate its enrichment with moxifloxacin as a means to surgical prophylaxis.

Methods: Five grams of NSRS was mixed by 100:3 ratio to moxifloxacin at room temperature. After solidification it was placed in the bottom of a cylindrical vial. Five replicates were prepared. One mL of Mueller Hinton broth was added over the free surface of the mixture and left to 37 °C. Broth was replaced daily for 1 month (Days 1–28). Concentrations of moxifloxacin in broth were determined by a microbiological method using *B. subtilis* 66 633 as an indicator strain.

Results: Mean concentrations of moxifloxacin of five vials after 1 h was 173.5 μg/mL; after 1 day 168.2 μg/mL. The latter concentration ranged between 81.3 and 98.4 μg/mL on days 2–5, between 10.9 and 19.6 μg/mL on days 6–9, between 18.6 and 37.4 μg/mL on days 10–20 and between 38.8 and 55.8 μg/mL on days 21–28. Highest means of 98.4 and 55.8 μg/mL were detected on days 2 and 21, respectively.

Conclusions: Enrichment of NSRS with moxifloxacin is accompanied by its slow release over 28 days at concentrations adequately above the MICs of the commonest Gram-positive cocci implicated in bone infections. Supplementation of NSRS with moxifloxacin may be proposed in surgical prophylaxis.

Faropenem

P1383 Comparative activities of faropenem and 19 antimicrobials against 200 penicillin-susceptible and -resistant *Streptococcus pneumoniae* isolates recovered from two Spanish hospitals

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Objectives: (1) To compare the activities of faropenem, an oral penem, to that of 19 antimicrobials against 102 invasive and 98 non-invasive clinical isolates of *S. pneumoniae* consecutively obtained from adult patients from October 2000 to June 2001 in two Spanish hospitals. (2) To characterize the macrolide-resistant phenotypes. (3) To analyze the serotypes most frequently isolated.

Methods: The in vitro studies were performed by microdilution method (Sensititre™). The genes *ermB*, *ermTR* and *mefA* were detected by PCR.

Results: MIC_{50} and MIC_{90} (μg/mL) of 20 antimicrobials against 200 isolates and percentage of resistance (R) according to NCCLS criteria were: penicillin (PEN) <0.03/4 (15% I, 25% R), ampicillin <0.25/4 (NC = no criteria), amoxicillin–clavulanate <0.5–0.25/4–2 (5% I, 7% R), cefuroxime <0.5/8 (2% I, 30% R), cefotaxime <0.06/1 (17% I, 7% R), cefepime <0.5/>2 (12% I, 16% R), imipenem <0.12/0.5 (18% I, 9% R), faropenem <0.03/0.5 (NC), erythromycin (ERY) <0.25/>32 (29%R), azithromycin <0.5/>4 (29% R), josamycin <0.5/>2 (NC), clindamycin (CLI) <0.25/>1 (28%R), quinupristin/dalfopristin <1/<1 (0% R), tetracycline (TET) <2/>4 (2% I, 33%R), chloramphenicol (CHL) <2/>8 (14%R), cotrimoxazole (SxT)

<0.5–9.5/>2–38 (15% I, 35% R), vancomycin 0.5/0.5 (0% R), teicoplanin <0.25/<0.25 (0% R), ciprofloxacin 1/2 (NC) and levofloxacin 1/1 (0.5% I, 1% R). The percentage of antibiotic R was higher in non-invasive than in invasive pneumococci: PEN 50% versus 30%, ERY 38% versus 23%, CLI 35% versus 22%, TET 44% versus 26%, CHL 18% versus 10% and SxT 54% versus 46%. No antibiotic susceptibility differences between the two hospitals were found. Among all isolates studied, 58 (29%) were positive for *ermB* gene and resistant to all macrolides and CLI (MLS-B phenotype), and one strain (0.5%) showing M phenotype was positive only for *mefA* gene. The most frequent serotypes found among invasive strains were 14 (12%), 19 (11%), 9 V (8%), 3 (7%), 1 (7%), 4 (7%), 7 (5%), 8 (5%) and 6B (5%).

Conclusions: In recent years, 40% of strains in Spain have decreased susceptibility to penicillin and 29% have shown resistance to >3 antimicrobials. The majority of multiresistant strains were positive for *ermB* gene. Faropenem was the most active β-lactam studied, inhibiting 96.5% of strains at concentrations less than or equal to 0.5 μg/mL. This antimicrobial could be effective in treating infections caused by pneumococci including those with acquired macrolide resistance.

P1384 Comparative in vitro activity of faropenem against community-acquired respiratory pathogens

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Objectives: The increasing prevalence of antibiotic-resistance among respiratory pathogens documented worldwide, poses major therapeutic problems. The purpose of the present study was to evaluate the in vitro activity of faropenem, a new oral carbapenem, against a large collection (600) of well-characterized respiratory pathogens circulating in Italy.

Methods: A total of 360 *S. pneumoniae* (including 50 penicillin-intermediate and 40 penicillin-resistant) 100 *S. pyogenes*, 70 *H. influenzae* (20 β-lactamase-positive) and 70 *M. catarrhalis* (50 β-lactamase-negative) have been studied. In addition to faropenem, ampicillin, amoxicillin, coclavulanate, cefaclor, cefuroxime, clarithromycin, azithromycin, clindamycin, levofloxacin, tetracycline, cloramphenicol and rifampin have been tested on the appropriate pathogens. MICs have been obtained by a broth microdilution method (NCCLS, 2001).

Results: Against penicillin-susceptible *S. pneumoniae*, in term of MIC_{90}, faropenem was the most potent drug tested (MIC_{90} 0.015 mg/L). The MIC_{90} values of the other compounds ranged from 0.03 mg/L (amoxicillin) to 32 mg/L (azithromycin). Faropenem showed good activity against penicillin-intermediate (MIC_{90} 0.25 mg/L) and penicillin-resistant (MIC_{90} 1 mg/L) strains, with MIC values from 4- to >64-fold lower than amoxicillin, coclavulanate, cefuroxime, cefaclor, clarithromycin, tetracycline and chloramphenicol. Towards erythromycin-resistant *S. pyogenes*, showing MLSB, iMLSB and M phenotype, faropenem (MIC_{90} 0.03 mg/L) was the most active drug followed by ampicillin, levofloxacin, chloramphenicol, and tetracycline (MIC_{90} values ranging from 0.06 to >64 mg/L). Against β-lactamase-positive and -negative *H. influenzae*, faropenem showed MIC_{90} of 1 mg/L, whereas MIC_{90} of the other drugs ranged from 0.06 mg/L (levofloxacin) to 32 mg/L (clarithromycin). Against β-lactamase-negative and positive *M. catarrhalis*, faropenem showed MIC_{90} of 0.03 and 0.5 mg/L, respectively, the MIC_{90} of the other antibiotics varying from 0.015 mg/L (rifampin) to 16 mg/L (cefaclor).

Conclusion: Faropenem confirms a broad and potent in vitro activity against all major respiratory pathogens analyzed, irrespective of their resistance patterns, and may therefore play a role in the treatment of community-acquired infections.

P1385 The activity of faropenem and other oral antibacterial agents against biotyped and serotyped recent clinical isolates of *Haemophilus influenzae*

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Objectives: Faropenem is a new oral penem antibiotic currently being investigated for the treatment of community-acquired respiratory tract infections. In this study, we investigated the in vitro activity of faropenem and other oral antibacterial agents against *H. influenzae*.

Methods: A collection of 1122 recently circulating clinical isolates were used. These isolates were fully biotyped and serotyped and assessed for the presence of β-lactamase before MICs were determined for faropenem (FAR), ampicillin (AMP), amoxycillin/clavulanate (AMC), clarithromycin (CLA) and levofloxacin (LEV) using the NCCLS microbroth dilution method.

Results: The vast majority of the isolates (95%) were nontypeable. Of the remaining encapsulated bacteria, the majority were either serotype b (1.7%), serotype f (1.2%) or serotype a (1.1%). The predominant biotype was biotype II (46.8%), followed by biotype III (27.0%) and biotype I (14.6%). β-Lactamase was present in around 20% of the *H. influenzae*. All β-lactamase-positive isolates were resistant to AMP but susceptible to AMC. FAR's activity was unaffected by the presence of β-lactamase. The activity of each antibiotic remained fairly constant irrespective of biotype or serotype (although assessment of the less-common biotypes and serotypes was difficult because of low numbers). A summary of the activities of the test antibiotics against all 1122 isolates is shown in the table.

MIC (mg/L)	FAR	AMP	AMC	CLA
MIC_{50}	0.25	0.25	0.5	8
MIC_{90}	1	>16	1	16
MIC range	0.03–4	<0.12–>16	<0.12–8	<0.12–>64

Conclusions: FAR showed good activity against *H. influenzae* with MIC values similar to AMC. CLA activity was borderline with MIC_{50} just at the susceptible NCCLS breakpoint (8 mg/L). These data confirm the potential role of FAR in the treatment of respiratory tract infections.

P1386 In vitro activity of faropenem against Gram-negative pathogens

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Background: Treatment of Gram-negative bacterial infections with oral β-lactams may be impaired by resistance due to hydrolysis by class A and class C β-lactamases. Faropenem daloxate is a novel penem antibiotic, which is rapidly hydrolyzed after oral administration to the active drug constituent faropenem (FAR).

Objective: To determine the in vitro activity of FAR against a variety of Gram-negative pathogens including strains producing defined β-lactamases.

Material and methods: A total of 1282 clinical isolates were analyzed for their MICs by an agar dilution procedure following NCCLS standards.

Results: The organisms are grouped by their MIC_{90} (mg/L) for FAR: $MIC_{90} \leq 1$: *E. coli* AMP ≤ 8, *E. coli* AMP > 8, *K. pneumoniae* CAZ < 2, *K. oxytoca* CAZ < 2, *Shigella* spp., *Salmonella spp.*, *Y. enterocolitica*, *A. hydrophilia*, *R. pickettii*, *C. jejuni*, *H. pylori*, *B. catarrhalis* AMP > 0.25 (producing BRO-1 or BRO-2), *N. gonorrhoeae* PEN < 4, *N. gonorrhoeae* PEN > 8, *H. influenzae* AMX < 8; $MIC_{90} = 2$–4: *E. coli* CAZ > 2*, *K. pneumoniae* CAZ > 2*, *K. oxytoca* CAZ > 2* (*producing defined Extended Spectrum or AmpC type β-lactamases), *E. aerogenes*, *P. agglomerans*, *C. freundii*, *C. divs*, *H. alvei*, *P. mirabilis*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*, *M. morganii*, *H. influenzae* AMX > 8; $MIC_{90} = 8$–32: *E. cloacae*, *S. marcescens*, *S. liquefaciens*, *A. xylosoxidans*, *B. cepacia* complex; $MIC_{90} = \geq 256$: *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *S. maltophilia*. FAR is active against pathogens producing broad spectrum, extended broad-spectrum and Amp C type β-lactamases.

Conclusion: Given the in vitro activity of FAR against Gram-negative pathogens and its marked β-lactamase stability, we conclude that FAR warrants further investigation for the treatment of community-acquired infections.

P1387 In vitro activity of faropenem against Gram-positive pathogens

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Background: Therapy of infections caused by Gram-positive organisms with oral β-lactams is impaired by low affinity to penicillin binding proteins or by

penicillinases. Faropenem daloxate is an oral penem, which rapidly hydrolyzes in vivo to the active drug constituent faropenem (FAR).

Objective: To explore the potential spectrum of activity of FAR against Gram-positive pathogens including penicillin and oxacillin-resistant strains.

Material and methods: A total of 676 clinical isolates were analyzed for their MICs by an agar dilution procedure following NCCLS standards. Drugs studied: FAR, penicillin (PEN), oxacillin (OXA).

Results: The table above contains organisms (no. of strains tested), strain characteristics and concentrations of FAR at which at least 90% of the strains were inhibited. The majority of organisms were susceptible at MIC_{90} of 0.25 mg/L or below; MIC_{90} of 0.5 or 1 mg/L were found only for *Listeria* spp. or PEN-resistant *S. pneumoniae*. FAR resistance was detected only for OXA-resistant staphylococci and enterococci.

FAR MIC_{90} (mg/L)					
≤0.13		**0.5**		**64**	
S. pneumoniae (30)	PEN ≤ 0.06	*Listeria* spp. (14)		*S. epidermidis* (20)	OXA > 4
S. Pyogenes (47)					
S. Agalactiae (38)					
Steptoc. Gr. C (8)		**1**		**256**	
Steptoc. Gr. C (22)		*S. pneumoniae* (15)	PEN > 0.5	*S. aureus* (35)	OXA > 8
S. milleri (22)				*S. haemolyticus* (18)	OXA > 8
0.25					
S. aureus (90)	OXA ≤ 2	**4**		**>256**	
S. epidermidis (35)	OXA ≤ 2	*E. faecalis* (100)		*E. faecium* (40)	
S. haemolyticus (20)	OXA ≤ 2				
S. saprophyticus (20)	OXA ≤ 2				
S. cohnii (20)	OXA ≤ 2	**8**			
S. simulans (20)	OXA ≤ 2	*S. saprophyticus* (17)	OXA > 2		
S. hominis (25)	OXA ≤ 2				
S. pneumoniae (20)	PEN 0.13–0.5				

Conclusion: The in vitro activity of FAR against Gram-positive pathogens suggests that FAR warrants clinical investigation as an oral treatment for community-acquired infections.

P1388 Comparative in vitro activity of faropenem, a new oral penem, against recent respiratory tract isolates recovered from outpatients in Germany

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Objectives: Faropenem (FAR) is a novel oral antibiotic with a penem structure, unique from carbapenems and other available β-lactams. It is characterized by potent antimicrobial activity against both Gram-positive and Gram-negative bacteria, including β-lactamase-producing strains. We have evaluated the current susceptibilities of respiratory-tract isolates from outpatients to FAR in comparison to 11 other commonly used antimicrobial agents.

Methods: Organisms were recovered in five geographic areas in Germany during October 2000–April 2001. Minimal inhibitory concentrations were determined using the broth microdilution method according to NCCLS in a central laboratory. The antimicrobials studied were: FAR, telithromycin (TEL), penicillin (PEN), amoxicillin (AMX), coamoxiclav (AMC), cefuroxime (CXM), cefaclor (CEC), moxifloxacin (MOX), levofloxacin (LEV), azithromycin (AZM), clarithromycin (CLR), and roxithromycin (ROX).

Results: A total of 369 isolates were tested. The table below shows MIC_{90} values (mg/L) and percentages of susceptible isolates (%S) for *S. pneumoniae* (SP), *S. pyogenes* (GAS), *H. influenzae* (HI), *M. catarrhalis* (MC), and *S. aureus* (SA). FAR had potent activity against all species tested. The highest MIC observed was 0.125 mg/L.

	SP ($n=100$)		GAS ($n=63$)		HI ($n=92$)		MC[a] ($n=80$)		SA ($n=34$)	
	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S
FAR	≤0.06	n.a.	≤0.06	n.a.	0.125	n.a.	0.125	n.a	≤0.06	n.a.
TEL	≤0.06	100	≤0.06	100	2	17.4	≤0.06	100	4	67.6
PEN	≤0.06	100	≤0.06	100	0.25	n.a.	≥16	2.5	4	14.7
AMX	≤0.06	100	≤0.06	100[b]	0.5	100[b]	4	11.3[b]	4	0[b]
AMC	≤0.06	100	≤0.06	n.a.	0.25	100	0.125	100	1	100
CXM	≤0.25	100	≤0.25	n.a.	0.5	100	1	100	2	97.1
CEC	≤0.5	100	≤0.5	n.a.	4	100	1	100	2	100
MOX	0.125	100	0.125	n.a.	0.06	100	0.06	n.a.	0.06	n.a.
LEV	0.5	100	0.5	100	≤0.06	100	≤0.06	100	0.125	100
AXM	≤0.125	95	0.25	92	1	100	≤0.125	100	≥16	58.8
CLR	≤0.25	95	0.5	90.5	8	97.8	≤0.25	100	≥64	58.8
ROX	≤0.25	n.a.	≤0.25	n.a.	16	n.a.	≤0.25	n.a.	≥64	n.a.

[a]Break points (bp) for *S. aureus* were applied. [b]bp of ampicillin; n.a., no NCCLS by available.

Conclusion: FAR possesses significant potency and antimicrobial profile compared to marketed antimicrobials suggesting that FAR may be a valuable future option for the treatment of community-acquired respiratory tract infections.

P1389 Analyses of in vitro activities of faropenem against Gram-positive bacterial isolates resistant to levofloxacin

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Objective: Faropenem daloxate (FD) is a novel penem antimicrobial agent intended for oral administration. FD is rapidly hydrolyzed in vivo to faropenem, the active moiety. The purpose of the presented study is to evaluate the in vitro activity of faropenem against genetically characterized Gram-positive isolates resistant to levofloxacin.

Methods: The strains tested have either been collected from clinical specimens or derived from resistance selection experiments with different quinolones. The following isolates were tested: 97 methicillin-susceptible levofloxacin-resistant *S. aureus* isolates, 93 levofloxacin-resistant *S. pneumoniae* isolates, 48 levofloxacin-resistant *S. pyogenes* isolates, and 44 levofloxacin-resistant viridans streptococcal isolates. All isolates have been characterized for alterations within the gyrase and topoisomerase enzymes using PCR and sequencing of the target genes, *gyrA*, *gyrB*, *grlA*, *grlB* or *parC* and *parE*, respectively. MIC values were determined using NCCLS criteria for levofloxacin and faropenem.

Results: Faropenem demonstrated good activity with MIC_{90} values of 1 mg/L for methicillin-susceptible, levofloxacin-resistant staphylococci, levofloxacin-resistant *S. pyogenes* and viridans streptococci tested. The MIC_{90} value for levofloxacin-resistant *S. pneumoniae* isolates was 0.5 mg/L. In total, only 7 out of 282 (2.5%) isolates displayed MICs for faropenem of ≥2 mg/L.

Conclusions: Based on its in vitro activity against levofloxacin-resistant Gram-positive cocci and given its oral bioavailability, faropenem daloxate appears to be a promising new antimicrobial agent for the treatment of a variety of infections due to Gram-positive cocci and warrants further clinical investigation.

P1390 The influence of protein binding on the effect of faropenem against *H. influenzae*

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Objectives: Faropenem daloxate (FD) is a novel ester pro-drug of faropenem sodium. Serum protein binding (PB) of 80–95% has been reported. We

studied the influence of human albumin and serum on the pharmacodynamics of faropenem (F) against 14 clinical strains of *H. influenzae*.
Methods: PB was determined by centrifugation-filtration at 757 g for 1 h in the following media: 50% human serum in broth, and 20 g/L albumin in broth. The concentration of F was 2 mg/L, as determined by a standardized biological method. The killing effect of F was studied in broth, broth with 20 g/L albumin and broth with 50% active human serum and inactivated serum (56, 30 min). The killing was determined by measuring viable count for 24 h.
Results: PB of F in both media was 91–92%. A clinical strain (MIC = 0.125 mg/L) was exposed to F at a concentration of 2 × MIC. A reduction of 4 log 10 cfu/mL was seen in broth (mean of three experiments). To obtain similar results in broth supplemented with 20 g/L albumin, the concentration had to be 50× MIC. Two other strains, MIC 0.5 and 2 mg/L, were exposed to F at a total concentration of 12 mg/L, corresponding approximately to the C_{max} obtained in patients following 300 mg of FD. The medium consisted of 50% inactivated human serum. Thus, the free fraction of F was approximately 1.2 mg/L. At 24 h the strain with the MIC of 0.5 mg/L was reduced by 4.3 log 10 cfu/mL. For the strain with a MIC of 2 mg/L, re-growth occurred by 1.2 log 10 cfu/mL. Similar exposure were performed in 50% active serum, which showed a fast bactericidal effect for both strains. A further 12 clinical strains (MICs from 0.125 to 0.5 mg/L) were investigated for the ability to grow in the presence of active serum. Half of the strains grew, and they were further studied by exposure to F at the total concentrations of 3, 6 and 12 mg/L. A reduction of 5 log 10 cfu/mL was noted for all strains at the concentration of 6 and 12 mg/L. At 3 mg/L, three strains were not completely killed at 24 h. The findings were expected, because the free concentration was approximately 0.3 mg/L, and the concentration was not sufficient to exceed the MIC.
Conclusions: Our study showed that it is the free fraction of faropenem in serum that has antibacterial effect against *H. influenzae*. Active human serum had bactericidal effect for 7/14 investigated strains. Further studies are needed to clarify the potential synergistic effects of F and human serum in systems mimicking human kinetics.

P1391 Faropenem daloxate vs. cefuroxime axetil in the treatment of acute sinusitis

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Objectives: To compare the efficacy and safety of faropenem daloxate (FAR), 300 mg twice daily for 7 days, with cefuroxime-axetil (CEF), 250 mg twice daily for 7 days, in the treatment of acute bacterial sinusitis.
Methods: A total of 561 patients with acute bacterial sinusitis (ABS) diagnosed by the presence of three or more clinical signs or symptoms of acute sinusitis and confirmed by sinus X-ray were enrolled in this multinational, randomized, double-blind study. A sample was obtained for culture by either sinus puncture or middle-meatus swab. A total of 452 patients (80.6%) were valid for the per protocol efficacy (PP) analysis, 228 FAR and 224 CEF; 136 (30.1%) were microbiologically valid, 71 FAR and 65 CEF.
Results: Clinical cure rates at 7–16 days post-therapy (test-of-cure) were equivalent: FAR 89.0%, CEF 88.4% (95% CI: −5.2%, 6.4%). The most frequently isolated organisms were: *S. pneumoniae* (47.1%), *H. influenzae* (30.1%), *S. aureus* (14.7%) and *M. catarrhalis* (8.8%). Bacteriological success at test-of-cure was observed in 91.5 and 90.8% of FAR- and CEF-treated patients, respectively (95% CI: −9.2%, 9.5%). Eradication/presumed eradication was detected for 97.3 and 96.3% of *S. pneumoniae*, 85.0 and 90.5% of *H. influenzae*, 88.9 and 90.9% of *S. aureus*, 100.0 and 83.3% of *M. catarrhalis* in FAR and CEF recipients, respectively. At 28–35 days post-therapy, continued clinical cure rates were 92.6 and 95.0% of FAR- and CEF-treated patients, respectively. Both drugs were well tolerated; the most frequently reported drug-related adverse events were diarrhea (2.2% vs. 2.9%), nausea/vomiting (1.5% vs. 0.7%), abdominal pain (0.7% vs. 1.5%) and skin reactions (1.5% vs. 1.1%) in FAR and CEP recipients, respectively.
Conclusions: In this study, FAR was an effective and well-tolerated therapy for ABS with high clinical and bacteriological success rates.

P1392 Bactericidal activity and post-antibiotic effect of faropenem against anaerobic pathogens

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Objectives: Periodontal anaerobic bacteria can be involved in systemic infections such as bacteremia, endocarditis, brain abscesses, urogenital infections, skin and soft tissue infections. Faropenem is a unique oral penem antibiotic with a broad spectrum of activity against Gram-negative, Gram-positive and anaerobic bacteria. This study tested the antibacterial activity of faropenem, in comparison with that of amoxicillin/clavulanate, by time-kill kinetics and post-antibiotic effect against various anaerobic bacteria supposed to cause systemic infections.
Methods: One strain of *Porphyromonas gingivalis*, one strain of *Bacteroides ureolyticus* and one strain of *Actinomyces* sp. were tested to evaluate the time-kill kinetics and the post-antibiotic effect of both faropenem and amoxicillin/clavulanate.
Time-kill kinetics: A closed system using incubation of bacteria in the presence of MIC, 4 × MIC and 10 × MIC concentrations of faropenem, and MIC and 4 × MIC concentrations of amoxicillin/clavulanate was used; viability counts were performed at 0, 8, 12 and 24 h. Killing curves were performed according to the time-kill protocol described for anaerobic bacteria by Rosenblatt in 'Antibiotics in Laboratory Medicine' (edited by Lorian, 1986).
Post-antibiotic effect (PAE): The PAE of faropenem was determined by the viable plate count method of Craig and Gudmundsson, against all the three isolates of anaerobic bacteria. The PAE was calculated with the following equation: PAE (time) = $T - C$, where T is the time required to increase by 1 log above the count (cfu/mL) observed immediately after completion of the same procedure on the culture after drug removal.
Results: Time-kill kinetics: killing curves of faropenem against *P. gingivalis* showed that 4 × MIC and 10 × MIC concentrations caused a bactericidal effect with a 99.9% reduction of bacterial inoculum (three log decrease) in 12 h against *B. ureolyticus* a three log reduction was obtained in 24 h at 10 × MIC concentration; whereas for *Actinomyces* sp., faropenem showed a bactericidal effect only at 10 × MIC in 12 h. The PAE was 02.60 h for *P. gingivalis*, 01.50 h for *B. ureolyticus* and 1 h for *Actinomyces* sp.

P1393 Bactericidal activity of faropenem (FAR) and amoxycillin (AMX) investigated in the presence and absence of serum

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Objectives: The effect of 70% heat inactivated human serum on FAR and AMX time kill kinetics (protein binding 95 and 20%, respectively) was compared for a total of 12 isolates, 4 *M. catarrhalis* (2 β-lactamase-producing strains), 4 *H. influenzae* (2 β-lactamase-producing strains), 2 Group A streptococci and 2 *S. pneumoniae*.
Methods: Concentrations of FAR and AMX equivalent to 2, 5 and 10 × MIC were added to logarithmic cultures (~105 and 107 cfu/mL) in Iso-Sensitest broth (ISB) and 70% ISB/human serum (v/v) both supplemented with 5% lacked horse blood, 20 mg/L NAD and 20 mg/L hemin. Antibiotic-free controls were included at both inocula. Viable counts were determined at 0, 2, 4, 6 and 24 h on supplemented Columbia agar following appropriate serial dilution in phosphate-buffered saline. Bacteria were enumerated after 48 h incubation at 35–37 °C in air enriched with 4–6% CO_2.
Results: See Table 1.

Table 1 Range of bactericidal rates (change in log10 cfu/mL from 0 to 6 h) at an inoculum of 10^5 cfu/mL at 10 times MIC

Organism		Absence of serum		Presence of serum	
	No.	FAR	AMX	FAR	AMX
M. catarrhalis	4	(2) ↓↓ (2) ↓↓↓	(1) ↓↓ (3) ↓↓↓	(1) ↑ (3) ↓↓↓	(4) ↓↓↓
H. influenzae	4	(2) ↓ (2) ↓↓	(1) ↑ (2) ↓↓ (1) ↓↓↓	(1) ↓↓ (3) ↓↓↓	(1) ↑↑ (3) ↓↓↓
Group A Sterp	2	(1) ↓↓ (1) ↓↓↓	(2) ↓↓	(1) ↓ (1) ↓↓	(2) ↓↓
S. pneumoniae	2	(2) ↓↓↓	(2) ↓↓↓	(2) ↓↓↓	(1) ↓↓↓ (1) ↑↑↑

Number strains: ↑ increase or ↓ decrease in viable counts by up to 1 log ↓, 1–2 logs ↓↓, 2 to ≥3 logs ↓↓↓.

Conclusions: FAR and AMX exhibited similar time-dependent kinetics. In ISA, in the absence of serum FAR and AMX were bacteriostatic (i.e. less than 99.9% kill) after 4 h with ×5 and 10 MICs on *M. catarrhalis*, *H. influenzae* and Group A streptococci and bactericidal for *S. pneumoniae*. In 70% serum FAR was bactericidal against *M. catarrhalis*, *H. influenzae* and one strain of *S. pneumoniae*. Despite significant differences in protein binding both FAR and AMX were more active in serum. The higher protein binding of FAR did not have a significant effect on its pharmacodynamic properties.

P1394 The bactericidal activity of faropenem and other β-lactam antibiotics at peak serum concentration against *Streptococcus pneumoniae* with reduced susceptibility to penicillin G

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Objectives: To assess the effect of penicillin G (PEN) resistance on the bactericidal activity of antibacterial against *S. pneumoniae* (SP).
Methods: Five recent clinical isolates of SP were incubated with antibacterial agents at peak serum concentration (value after standard oral dosing except ceftriaxone (CEF) where iv data was used) at 35 °C in brain heart infusion broth supplemented with lacked horse blood (5%, v/v) and in the same medium either with 20% (v/v) complement active or with 20% (v/v) heat inactivated human serum added. Viable counts were made after 1, 2, 3, 6 and 8 h. Reduction in log 10 viable count was plotted against time and the area under the curve (AUC) calculated.
Results: No difference in kill was observed in the three media, indicating that the SP were not serum sensitive. To compare the test agents, average results for the three media were used to calculate time to reduce viability by 99.9% ($T_{99.9}$) and AUC. These results are shown in the table (AMO = amoxycillin; FAR = faropenem; NA = not achieved).

Isolate (PEN MIC)	PEN (6 mg/L)		AMO (5 mg/L)		CEF (150 mg/L)		FAR (12 mg/L)	
	$T_{99.9}$	AUC	$T_{99.9}$	AUC	$T_{99.9}$	AUC	$T_{99.9}$	AUC
SP24 (1)	3.3 h	24.7	3.3 h	24.6	5.3 h	18.2	2.0 h	27.2
SP06 (2)	5.6 h	17.2	5.2 h	18.7	8.0 h	12.2	3.0 h	23.3
SP40 (4)	5.4 h	18.1	5.0 h	19.9	8.0 h	13.9	2.4 h	25.2
SP75 (6)	6.6 h	15.7	NA	5.4	NA	12.7	4.6 h	21.0
SP56 (8)	NA	6.3	NA	0	NA	8.9	5.4 h	18.1

Conclusions: FAR was more bactericidal than the other β-lactams tested. In addition, FAR was less affected by reduced PEN susceptibility than PEN, AMO or CEF.

P1395 The impact of protein binding of faropenem on its antibiotic activity

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Objectives: The aim of this study was to compare the antibiotic activity of the new oral penem Faropenem (F) in presence and in absence of 40 g/L human serum albumin. Faropenem is bound to plasma proteins to 96%. To study the effect of protein binding on the antibiotic activity, we determined kill curves of three *S. pneumoniae* strains and *H. influenzae*.
Methods: *H. influenzae* [MIC F: 0.5 mg/L] and three strains of *S. pneumoniae* (penicillin resistant [MIC F: 0.25 mg/L], penicillin intermediate [MIC F: 0.0625 mg/L] and penicillin susceptible [MIC F: 0.0625 mg/L]) were used. We simulated concentration–time curves by using different modified in vitro models. The human pharmacokinetic of a 300-mg dose of F with and without 40 g/L of human serum albumin was simulated during a time period of 24 h. For *H. influenzae* we determined the kill kinetics also in a batch culture for 6 h. Bacterial counts were performed by preparing 10-fold dilutions of the samples with 0.9% saline, then plating a 0.05-mL aliquot on an appropriate agar. Colony forming units (cfu) were read after 24–48 h incubation of 37 °C in an CO_2 incubator.
Results: For *S. pneumoniae* we did not find any difference between the antimicrobial activity of F with or without the addition of albumin in the in vitro model (reduction of the bacterial count was at least 5–6 orders of magnitude). The simulation of a 300-mg F dose plus albumin for *H. influenzae* results in an bacteriostatic effect over 24 h. The same concentration without albumin shows a decrease of 2 log cfu for *H. influenzae*. This difference of the antibiotic effect is concentration dependent. To elucidate the impact of the protein binding of F on the antimicrobial effect of *H. influenzae* we studied the kill kinetics with increasing constant concentrations. The data (Table 1) demonstrate that the difference between the effect with and without protein decreases with increasing concentration of F.

Table 1

Concentration of faropenem	log Dif. cfu (after 6 h) Without albumin	With albumin	Δ log Dif. cfu
2 × MIC	−1.72	2.14	3.86
4 × MIC	−1.7	0.42	2.12
8 × MIC	−1.65	−0.03	1.62
16 × MIC	−1.9	−0.33	1.57

Conclusion: As the protein binding of F is 96% the actual concentration available at C_{max} (11.8 mg/L) for a 300-mg dose is ~0.5 mg/L. For strains with MIC below this concentration the antimicrobial effect is not at all impaired, whereas for bacteria for which the MIC is in the range of the concentration of F in the free fraction, the protein binding has to be taken into consideration.

P1396 The effect of protein on the bactericidal activity of faropenem against *S. pneumoniae* and *H. influenzae* using serum bactericidal tests

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Objectives: Faropenem is an oral penem with in vitro activity against a wide range of pathogens including *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Protein binding of faropenem is approximately 95%. This series of experiments was designed to investigate the effect of protein on the activity of faropenem against *S. pneumoniae* and *H. influenzae* using simulated serum cidal titers.
Methods: Media was spiked with faropenem concentrations of 5.5, 11.0 and 20 mg/L with 0, 35 and 70% added human serum. Serum was either heat treated or not and one strain each of *S. pneumoniae* and *H. influenzae* (both MIC 0.12 mg/L) were employed.

Results: The serum cidal titers are shown in the table.

		Cidal titer [faropenem concentration (mg/L)]		
	% Serum	5.5	11.0	20.0
S. pneumoniae	0	16	64	64
(serum not heated)	35	4	4–8	8–16
	70	2	4–8	4–16
H. influenzae	0	2	16	64
(serum not heated)	35	4	8	8
	70	>1024*	>1024*	>1024*
H. influenzae	0	8	16	64
(serum heated)	35	2	4	4
	70	<2	2	2

*Also no growth in antibiotic-free control.

The effect of serum on faropenem activity against *S. pneumoniae* and *H. influenzae* differed. Addition of serum had the effect of reducing the in vitro activity of faropenem against *S. pneumoniae* at all ratios and concentrations and heating the serum had no effect on the activity of faropenem. In contrast against *H. influenzae* serum increased faropenem activity at some ratios and this effect was absent with heat treated serum. Although serum at higher ratios was bactericidal against *H. influenzae* on its own, almost certainly related to the presence of complement as this effect was eliminated by heat treatment at low ratios a positive interaction of faropenem and serum appears to have occurred.
Conclusions: The results indicate a potentially positive interaction of faropenem with serum and *H. influenzae*. However, due to variability in performing serum cidal tests and the use of doubling dilutions to define endpoints, it is difficult to precisely define the roles of the antibacterial effects of complement and faropenem against *H. influenzae* under these experimental conditions. Further studies are required to elucidate the interaction in more detail.

β-Lactamases

P1397 Targeted recombinant β-lactamase in prevention of antibiotic-induced changes in gut microflora

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Objectives: Broad-spectrum β-lactam drugs cause selective pressure on colonic microflora. Disturbed colonic ecology may lead to decreased colonization resistance and increased formation of resistant bacteria. The present concept was developed to overcome these untoward effects. The efficacy of the new treatment modality was investigated on intravenous ampicillin-induced changes in gut flora.
Methods: β-Lactamase of *Bacillus licheniformis* was overproduced in *Bacillus subtilis* using a bacillar secretion vector. The purified enzyme was released in the small bowel from a controlled-release formulation with a pH-dependent release. Fistula-operated beagle dogs ($n = 6$) were treated BID with either 20 mg/kg ampicillin (i.v.) + placebo (p.o.), 20 mg/kg ampicillin (i.v.) + TRBL (p.o.) or only placebo (i.v. + p.o.). Stool was collected at days 4 and 10. Fecal samples were quantitatively cultured for total and main groups of aerobic and anaerobic bacteria and yeast. Temperature gradient gel electrophoresis (TGGE) was used to separate the ribosomal RNA genes. Computer analysis of the TGGE profiles of the samples generated similarity percentages between the individual samples.
Results: Ampicillin + placebo group had clearly decreased counts of both aerobic and anaerobic bacteria d10 and d14, whereas ampicillin + TRBL group had only minor overall changes and some occasional changes by single species. Intravenous ampicillin decreased the fecal similarity percentage to 60% when 16 S rRNA fingerprints taken before treatment were compared to values taken during treatment. The similarity percentage during treatment with ampicillin + TRBL did not differ from that of placebo (86 vs. 81%).
Conclusions: According to our preliminary results the effects of TRBL are reflected in a virtually unchanged microflora of the gut. This stability was detected by both 16S rRNA fingerprints and conventional culturing. These results indicate that TRBL is a promising novel approach for overcoming ecological damage to gut flora caused by β-lactam antibiotic agents.

P1398 High prevalence of nosocomial *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M-type extended spectrum β-lactamases (ESBLs) in Russian hospitals

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Background and objectives: ESBLs of a CTX-M-type represent rapidly emerging group of β-lactamases. On the basis of sequence similarity all enzymes of this group can be distributed into four subtypes epitomized by CTX-M-1, CTX-M-2, CTX-M-8 and CTX-M-9. The aim of our study was to investigate the prevalence of different subtypes of CTX-M ESBLs among nosocomial *E. coli* and *K. pneumoniae* strains in Russian hospitals.
Methods: Consecutive nosocomial isolates of *E. coli* ($n = 494$) and *K. pneumoniae* ($n = 410$) were collected in 28 Russian hospitals during 1997–1998. ESBL production was detected by double-disc synergy test. All ESBL-producing strains were screened for CTX-M β-lactamases by PCR with primers specific to the conserved regions of the coding genes. A PCR was followed by RFLP analysis with PstI and PvuII restriction enzymes permitting the differentiation of the CTX-M subtypes. The *E. coli* strains producing the known CTX-M β-lactamases were used for quality control.
Results: The ESBL phenotype was observed in 71 (14.4%) and 239 (58.3%) *E. coli* and *K. pneumoniae* isolates, respectively. Among the ESBL producers 24 (33.8%) *E. coli* and 77 (32.2%) *K. pneumoniae* were found to possess genes for CTX-M β-lactamases. The majority of these strains (92.1%) produced the CTX-M-1-related ESBLs. The CTX-M-2-related enzymes were detected in eight *E. coli* strains from a single hospital only. Neither CTX-M-8- nor CTX-M-9-related enzymes were found in this study. The CTX-M-producing strains were detected in 17 of 28 surveyed hospitals and predominated over the strains expressing all other types of ESBLs in the medical centers located in the areas of Ural and Siberia. In two hospitals the relative frequency of CTX-M-type ESBLs ranged up to 93 and 100%, respectively.
Conclusions: We conclude that the CTX-M-type β-lactamases represent a significant and rapidly spreading group of ESBLs in Russia.

P1399 ESBLs producing *K. pneumoniae* and *E. coli* isolated in 2001

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Objective: To evaluate the prevalence of ESBL in 211 *K. pneumoniae* and 658 *E. coli* isolated in 2001 from inpatients and outpatients.
Methods: Susceptibility tests were done by disk diffusion method according with NCCLS guidelines. Screening for ESBL production was made by (1) disk diffusion test with ceftazidime (CAZ), cefotaxime (CTX), aztreonam (AZT); (2) double disk synergy test; (3) E-test ESBL screen strips (CT/CTL and TZ/TZL); and (4) ATB Expression System BioMerieux.
Results: Sixty *K. pneumoniae* (57 isolated from inpatients and 3 from outpatients) and 30 *E. coli* (27 inpatients and 3 outpatients) produce ESBL. Between ESBL-producing *K. pneumoniae* there were 27 from urine (24 from inpatients, 3 from outpatients), 21 from lower respiratory tract (all from inpatients), 6 from blood and 6 from another specimens. ESBL-producing *K. pneumoniae* were resistant 66.6% to gentamicin, 48.3% to netilmicin and 40% to ciprofloxacin. From ESBL-producing strains *E. coli* 90% were resistant to ciprofloxacin, 86.6% to gentamicin and 60% to netilmicin. ESBL-producing

E. coli were isolated 18 from urine (14 from inpatients and 4 from outpatients), 4 from lower respiratory tract, 3 from blood and 5 from another clinical specimens (all inpatients).
Conclusions: The overall prevalence of ESBL-producing strains was 28.4% for *K. pneumoniae* and 4.5% for *E. coli*. The frequency of ESBL was higher for strains isolated from inpatients, 30.4% in *K. pneumoniae* and 5.4% in *E. coli* versus 12.5% in *K. pneumoniae* and 2.5% in *E. coli* for the strains from outpatients. Resistance to quinolones and aminoglycosides is often associated with ESBL production.

P1400 Concentration-dependent selection of cefotaxime resistant *E. coli* in an in vitro kinetic model

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Objectives: Different antibiotic dosing regimens may vary in their capacity to select pre-existing mutants. In previous static experiments by Negri et al. (2000), a concentration-dependent selection of low level cefotaxime resistant genetic variants was described. Selection occurred primarily at low concentrations between MIC values of the strains, called a selective window (SW). Our aim was to extend these experiments using an in vitro kinetic model.
Methods: Isogenic *E. coli* were used, REL606 expressing the TEM-1 β-lactamase enzyme, and REL607 expressing the higher-level resistant TEM-12 enzyme. The strains differ in their ability to use L-arabinose, and form different colors on tetrazolium-arabinose (TA) indicator plates. A competition assay was performed in an in vitro kinetic model where the bacteria were kept in a closed system and the elimination rate of the antibiotic was varied. The populations were mixed 99:1 of TEM-1/TEM-12. The bacterial mixture was exposed to cefotaxime for 24 h. The initial concentrations of cefotaxime and the elimination rate in the kinetic model were adjusted to give SWs of 1, 2, 4 and 8 h. The time that concentrations exceeded MIC ($T>$ MIC) was also varied. Samples were withdrawn at different time points, and treated with β-lactamase before appropriate dilutions were plated on TA indicator plates. The grade of selection after 24 h, the selection coefficient, was estimated from $R24–R0$ h, where R is the natural logarithm of the ratio of TEM-12 and TEM-1 densities.
Results: The MIC of TEM-1 and TEM-12 producing *E. coli* was 0.0156 and 0.0625 mg/L, respectively. When a SW of 1 h was used with $T>$ MIC of 2/3 h for TEM-12 and TEM-1, respectively, the selection coefficient was 7.0. A SW of 2 h with $T>$ MIC of 2/4 h gave a selection coefficient of 7.6, and a SW of 4 h at $T>$ MIC of 2/6 h gave a coefficient of 9.0. A SW of 8 h was also tested where $T>$ MIC was 4/12 h, the selection coefficient then was 11.9. By increasing $T>$ MIC, the selection coefficient was changed. When $T>$ MIC for TEM-12 was 12 h, selection was highly variable and unpredictable. In some experiments TEM-12 was completely eliminated.
Conclusions: The selection coefficient in the in vitro kinetic model was dependent on $T>$ MIC for the two strains as well as on the length of the SW. Selection was less likely to occur when $T>$ MIC approached 12 h for TEM-12. Pharmacodynamic studies of antibiotics should include the capacity of different dosage regimens to select resistant subpopulations.

P1401 Role of permeability in carbapenem resistance of porin-deficient *E. coli* producing metallo-β-lactamases

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To assess the influence of different permeability defects on carbapenem susceptibility, we used *E. coli* K-12 derivatives in which the OmpF and/or the OmpC structural genes had been inactivated by the insertion mutation element Tn5, or in which the regulatory locus ompB had been mutated. The parent strain and its porin-deficient mutants were transformed with two different constructed vectors (namely pAx22 and pPAM) carrying the VIM-1 and IMP-1 metallo-β-lactamase genes. Both B-lactamases caused a two- to four-fold increase in the MICs of imipenem and a four- to eight-fold increase in the MICs of meropenem for the nonporin-deficient strains. Only minor increases were observed in the OmpC-deficient strains when the lower inoculum was used (10^4 cfu per spot), whilst when the higher inoculum was used (10^6 cfu per spot) both β-lactamases caused a two- to four-fold increase in the MIC of imipenem but a 32-fold increase in that of meropenem. In the OmpF-deficient strain all the MICs increased by 8 times with the lower inoculum and by 32 times with the higher, with the only exception of the meropenem MIC in the strain transformed with IMP-1 which increased by 64 and by 128 times with the low and high inoculum, respectively. The MICs of both imipenem and meropenem were even lower in the OmpC–OmpF mutant-transformed with VIM-1, but not in that transformed with IMP-1. As opposed to the results previously obtained with the CphA enzyme of *Aeromonas hydrophila*, the parent strains transformed with either VIM-1 or IMP-1 had carbapenem MICs noticeably higher than the nontransformed strains, mostly as regards meropenem and IMP-1. However, the results obtained with all the porin-deficient mutants did not significantly differ from those previously obtained with CphA.

P1402 Effect of siliconized latex urinary catheters (SLUC) on the activity of carbapenems against isogenic strains of *P. aeruginosa* with different expression of AmpC-β-lactamase, OprD and MexA–MexB–OprM

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Objective: We have described a decreased activity of carbapanems against *P. aeruginosa* (Pae) in the presence of SLUC, related to the loss of an OprD-like protein and the expression of a new outer membrane protein. In order to understand the underlying mechanism of this phenomenon we have studied the effect of SLUC on the activity of imipenem (IP) and meropenem (MP) against isogenic strains of Pae.
Methods: Isogenic strains producing or not AmpC-β-lactamase (BL), OprD porin and MexA–MexB–OprM system were studied. The activity of IP and MP was determined in cation-adjusted Mueller–Hinton broth (MHB) and in eluate from SLUC incubated in MHB (four segments of 1 cm/mL; 37 °C; 24 h). The activity of several quinolones was also studied. OMP profiles and BL activity of the strains grown in MHB or eluate were determined by SDS-PAGE and spectrophotometric assay, respectively.
Results: The activities of IP and MP in eluate from SLUC decreased by 8–16 and 4–8 times, respectively, against strains of Pae expressing all OprD, BL (either inducible or constitutive) and normal multidrug efflux system MexA–MexB–OprM. IP, but not MP, had the same activity in MHB as in eluate against all but one strains lacking BL activity but expressing OprD. MICs of IP and MP against OprD deficient strains were the same in both MHB and eluate. MICs of MP, but not of IP, were consistently one dilution lower in MHB than in eluate against strains with defective MexA–MexB–OprM. Activities of quinolones against all the strains studied were similar when tested in either MHB or eluate. Strains expressing OprD in MHB lost it when grown in eluate, and expressed a new OMP of about 50 kDa when growing in eluate. No differences in BL activity were detected when strains were grown in either MHB or eluate.
Conclusions: Resistance to IP in *P. aeruginosa* grown in eluate from SLUC is mainly due to both loss of OprD and expression of BL. For MP this phenomenon is related to both loss of OprD and integrity of the MexA–MexB–OprM system. The role of the 50 kDa OMP expressed by Pae grown in eluate is unknown, but seems not to be related to any of the currently described Pae efflux systems, since its expression is not associated with decreased quinolone activity.

P1403 Antimicrobial susceptibility to β-lactams and β-lactamases production among clinical isolates of Gram-negative nonfermentative rods

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Objectives: To study the antimicrobial susceptibility to β-lactam antibiotics, as well as the production of extended-spectrum β-lactamases (ESBLs) and inducible β-lactamases (IBLs) in clinical isolates of Gram-negative non-fermentative bacteria.
Methods: Fifty-three recent clinical isolates including *Pseudomonas* spp. ($n=23$), *Acinetobacter baumannii* ($n=20$) and *Stenotrophomonas maltophilia* ($n=10$) were examined. The strains were isolated from ICU and identified by the automatic ATB system (BioMerieux). ESBL-producing strains were detected using the double disk (DD) test according to Jarlier and the oxoid combination disk method. IBL activity was detected by the two-disk test according to Sanders. The MICs of piperacillin (PIP), aztreonam (ATM),

ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), imipenem (IPM) and meropenem (MEM) were determined by an agar dilution method according to NCCLS criteria. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were included as quality control strains.
Results: DD test results were positive for eight strains (two for *Pseudomonas* spp., and six for *S. maltophilia*). However, none of the investigated strains displayed an ESBL activity in the oxoid combination disk method. The ability to produce IBLs was detected in 24/53 strains. ESBL-positive isolates showed high resistance to typical oxyimino-β-lactams (third-generation cephalosporins and aztreonam). In addition, ESBL-producing *Pseudomonas* spp. strains were resistant to imipenem (MIC 512–1024 mg/L) and meropenem (MIC 64–256 mg/L). *A. baumannii* and *S. maltophilia* isolates were uniformly resistant to PIP (MIC 128–1024 mg/mL), ATM (MIC 64–1024 mg/mL) and CRO (MIC 128–1024 mg/mL).
Conclusions: Our results confirm the need of reliable methods for the detection of ESBL producers among Gram-negative nonfermentative strains. The DD test was more sensitive to ESBL screening than the oxoid combination disk method. The majority of the strains exhibited multiresistance to β-lactam antibiotics, including carbapenems.

P1404 Resistance mechanisms to aminoglycosides and β-lactams of *Stenotrophomonas maltophilia* strains from Greek hospitals

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Objectives: *Stenotrophomonas maltophilia* is an important emerging pathogen causing a variety of nosocomial infections. Being inherently resistant to many antibiotics, this species poses a therapeutic challenge to the clinician. The main objective of this study was to elucidate the mechanisms of *S. maltophilia* rendering resistance to aminoglycosides and α-lactams.
Methods: A total of 28 nosocomial isolates from five Greek hospitals, collected during the period April 1998 to May 1999 were studied. Susceptibility testing was performed by the agar diffusion (Kirby-Bauer) test and E-test, using the National Committee for Clinical Laboratory Standards guidelines (M7-A5, Vol. 20, No. 2, 2000). Epidemiologic relatedness between the isolates was assessed by pulse field gel electrophoresis (PFGE) of SpeI-restricted genomic DNA. Aminoglycoside resistance mechanisms were determined using the aminoglycoside resistance patterns method (AGRP) and the DNA probe analysis. β-Lactamases were determined by isoelectric focusing (IEF).
Results: All strains showed resistance to the carbapenems such as imipenem and meropenem. Ceftazidime was active against 60% of the isolates tested. Relatively few isolates were susceptible to the combination of piperacillin/tazobactam (25%) and only one was susceptible to aztreonam. Amikacin showed the lowest level of resistance of all aminoglycosides tested inhibiting 46% of *S. maltophilia* strains of our study. PFGE revealed 27 different genotypes. All isolates possessed several different α-lactamases with alkaline pI values greater than 8.2 that hydrolyzed imipenem. The majority of the strains harbor also other enzymes with pIs of 5.4–7.9 but only four of the isolates were able to hydrolyze imipenem with one of these enzymes. None of the isolates possessed any aminoglycoside modifying enzymes so that resistance due to impermeability was concluded according also to AGRP method.
Conclusions: No clear correlation appears between MIC of α-lactams, pI of extracted α-lactamases and genotype of the clinical isolates of *S. maltophilia* tested. The resistance of *S. maltophilia* is extremely complex and further work is required to clarify the mechanisms behind the wide phenotypic variations observed.

P1405 Resistance to β-lactam antibiotics in *Aeromonas caviae*

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Objectives: The contribution of β-lactamase production to β-lactam antibiotics resistance was examined in an *A. caviae* mutant strain (AC7m), selectioned in vitro by cefotaxime derived from its wildtype strain (AC7) isolated in our laboratory from crude sewage.
Methods: Antibiotics susceptibility test were performed by serial two-fold dilution in Mueller–Hinton Broth. Nitrocefin was used for β-lactamase detection on intact cells and crude enzyme preparations. A modification of a spectrophotometer acidimetric method was used for studies of substrate and inhibitor profiles. IEF was performed in PAGE pH: 3.5–9.5. Cefoxitin (0.5 μg/mL) was used for β-lactamase induction.
Results: Both strains produced β-lactamase. AC7m, in contrast to AC7 that was inducible by cefoxitin, produced β-lactamases constitutively. AC7m was regarded as a derepressed mutant from AC7, which overexpressed β-lactamase. AC7 was susceptible to most of β-lactam antibiotics tested, being resistant to aminopenicillins, first generation cephalosporins and amoxicillin plus potassium clavulanate. AC7m was resistant to aminopenicillin and its combinations with β-lactamase inhibitors, carboxipenicillins, ureidopenicillins, and cephalosporins. This strain remained susceptible to ceftazidime, imipinem and aztreonam. A common major β-lactamase band at pI: 6.5 shared AC7 and AC7m.
Conclusion: β-lactamase production by AC7 and AC7m was involved in β-lactam antibiotics resistance. Based on substrate and inhibitor profiles determined in sonic extracts for AC7 and AC7m, the enzymes diplayed on IEF at pI: 6.5, were assigned to chromosomal group 1 β-lactamases. Imipenem may be the appropiate choice for therapy of infections caused by stable derepressed mutants of *A. caviae* producing group 1 β-lactamases.

P1406 First isolation of a carbapenem-hydrolyzing β-lactamase in a *Pseudomonas aeruginosa* in Spain

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Objectives: A retrospective analysis of susceptibility to β-lactams was conducted in the *Pseudomonas aeruginosa* isolates from a Barcelona hospital to search for carbapenemase producers. Indeed, carbapenemases (of the IMP and VIM series) have been already reported from Italy, Greece, and France. One of the strains was analyzed in details for its β-lactamase content.
Methods: Preliminary β-lactam susceptibility testing was performed with 52 imipem- and ceftazidime-resistant *P. aeruginosa* strains that had been isolated at San Pau hospital in Barcelona from January 1996 to June 2001. A β-lactamase extract of culture of one of the strain (Ka.209) was prepared for detection of carbapenem hydrolysis. Using genomic DNA of Ka.209 as template, PCR experiment were performed with primers specific for detection of IMP and VIM genes and class 1 integrons. Cloning of a PCR product in pPCRScript in *E. coli* was followed by sequencing of the insert. Plasmid extraction, conjugation and electrotransformation in *E. coli* and *P. aeruginosa* reference strains, and hybridization with an internal probe for blaVIM-2 were then performed.
Results: Out of 52 *P. aeruginosa* strains, one of them, Ka. 209. was resistant to imipenem, to ceftazidime and susceptible to aztreonam. This antibiotic resistance phenotype agreed with the presence of an Ambler class B carbapenemase (metallo-enzyme). β-Lactamase extracts of culture of Ka. 209 hydrolyzed carbapenems. PCR and sequencing experiments identified the blaVIM-2 gene. It was located onto a class 1 integron. The blaVIM-2-positive plasmid of 50 kb was not conjugative but was able to replicate in *E. coli*.
Conclusion: Currently, VIM-2 is the carbapenemase most often isolated in Europe. This work constitutes a forewarning of the probable dissemination of plasmid-encoded carbapenemases at least in Southern Europe.

P1407 Molecular characterization of *E. coli* HB101 harboring *blaTEM*-1 gene after exposure to ceftazidime and ceftibuten

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Objectives: Exposure to expanded spectrum β-lactam antibiotics in Enterobacteriaceae harboring *blaTEM*-1 or *blaSHV*-1 genes results in the appearance of new enzymes with extended spectrum of activity and resistance to the above compounds. The aim of the present study was to characterize the emergence of resistence and the mechanisms involved in *E. coli* HB101.
Methods: *E. coli* HB101 containing the *blaTEM*-1 gene (pBR322) was exposed to increasing concentrations of ceftazidime (range 1–256 μg/mL) and ceftibuten (range 0.25–16 μg/mL). The grown colonies were screened for the presence of TEM-1 gene by PCR and direct sequencing. Outer

membrane protein (OMP) profiles were studied according to the procedure previously reported [1].
Results: *E. coli* HB101 with plasmid containing the TEM-1-encoding gene was exposed to different concentrations of ceftazidime and ceftibuten. *E. coli* HB101 carrying a mutated allele encoding the TEM-12 ESBL was obtained after exposure to the following ceftazidime concentrations: 32, 64 and 128 µg/mL, while exposure to similar concentrations of ceftibuten did not result in selection of TEM-1 ESBL variants. The low level of resistance in both ceftazidime and ceftibuten selected mutants was associated with the loss of an OMP with an electrophoretic mobility similar to *E. coli* OmpF.
Conclusions: In the present study, ceftazidime and ceftibuten were able to select resistant mutants of *E. coli* HB101 containing the *blaTEM-1* gene at different levels and by means of different mechanisms. Ceftazidime was able to select mutants either producing the TEM-12 ESBL or to repress OmpF expression whereas ceftibuten only selected mutants that were deficient in OmpF. These results show a different effect of ceftazidime and ceftibuten in the selection of resistant mutants.

Reference

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P1408 Activity of β-lactam antibiotics against Enterobacteriaceae with different β-lactamases patterns, including extended-spectrum enzymes

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Objectives: Extended-spectrum β-lactamases (ESBLs) are widely diffused in nosocomial isolates of Enterobacteriaceae, where they can be found alone or in various combinations with different enzymes (e.g. AmpC-like). In the latter case, the complex β-lactamase pattern may result in a peculiar resistance phenotype. The aim of this study was to evaluate the activity of several antimicrobial agents against Enterobacteriaceae with different β-lactamase patterns including various TEM- and SHV-type ESBLs.
Methods: The study was carried out on 103 ESBL-producing clinical isolates of Enterobacteriaceae. The β-lactamase pattern was investigated by analytical IEF, and the nature of the ESBLs by direct sequencing of the *blaTEM* and *blaSHV* alleles. The ESBLs repertoire included TEM-12, TEM-15, TEM-19, TEM-20, TEM-24, TEM-26, TEM-43, TEM-52, TEM-60, TEM-72, TEM-87, SHV-2a, SHV-5, SHV-11 and SHV-12. An AmpC-like enzyme was present in 26 cases. MICs were determined by an agar-dilution procedure using two different inocula (5×10^5 and 5×10^8 cfu per spot).
Results: Susceptibility rates were as follows: meropenem, 100%; imipenem, 98%; cefotetan, 94%; piperacillin-tazobactam, 93%; amikacin, 85%; levofloxacin, 66%. A significant inoculum size effect was observed with several compounds, including imipenem, piperacillin-tazobactam, cefotetan, and cefepime (susceptibility rates decreased to 83, 68, 88 and 31%, respectively). A relationship of susceptibility patterns with bacterial species and with production of different TEM or SHV ESBLs was also observed.
Conclusion: Carbapenems, and meropenem in particular, were the most active against ESBL-producing Enterobacteriaceae, and retained full activity also in the presence of complex β-lactamase patterns including AmpC-like enzymes. However, in the presence of high bacterial inocula, cefotetan and amikacin also appeared to be a good alternative for chemotherapy of infections caused by ESBL-producing Enterobacteriaceae.

P1409 Occurrence of extended spectrum β-lactamase in clinical isolates of *K. pneumoniae*

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Objectives: To determine the occurrence and antimicrobial susceptibility of extended-spectrum β-lactamase (ESBL) producing *K. pneumoniae* in patients attending Siriraj hospital in Bangkok during August 2000 to January 2001.
Methods: ESBL-producing isolates were screening with disk diffusion according to NCCLS guidelines. Confirmation of suspected ESBL-producing isolates was performed with E-test (CT/CTL or TZ/TZL). Antimicrobial susceptibility testing were determined by a microdilution automatic method (VITEX system, BioMerieux).
Results: Out of 22 178 clinical specimens, 400 (1.8%) were culture positive for *K. pneumoniae*; 26% (104/400) of all isolates were ESBL-producing strains. Rates of detection of ESBL-producing *K. pneumoniae* were 18.7, 30 and 25.9% for blood, sputum and urine samples, respectively. Susceptibility testing has revealed that all 70 tested strains including 53 isolates from blood and sputum and 17 isolates from urine samples were sensitive to imipenem (MIC 4 mg/L). None of the tested isolates were sensitive to piperacillin/tazobactam and amoxicillin/clavulanic acid or aztreonam. Rate of resistance to ciprofoxacin, levofloxacin, gentamicin, tobramycin and trimethoprim/sulfamethoxazole were 60, 64, 28, 9 and 51%, respectively, for isolates from blood and sputum; 71, 71, 18, 6, and 35% for urinary isolates.
Conclusions: A high rate (26%) of the clinical isolates of *K. pneumoniae* were ESBL-producing strains. Imipenem was highly active against ESBL-producing *K. pneumonia*. In contrast, reduced activity was observed with fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole. Further identification of ESBL-producing strains requires molecular techniques for specific control interventions.

P1410 The antimicrobial susceptibility pattern and ESBL production of common Gram-negative bacteria isolated from nosocomial infections in a Turkish hospital

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Objectives: To determine the antimicrobial susceptibility pattern and frequency of production of extended-spectrum β-lactamases (ESBL) in certain Gram-negative bacteria isolated from nosocomial infections seen in medical and surgical wards and intensive care units of an University adult hospital against commonly used antimicrobials were evaluated.
Methods: The isolates collected between January 2000 and July 2001 and included in the study were causes of nosocomial infections. The antimicrobials tested were meropenem, imipenem, ciprofloxacin, piperacillin + tazobactam, tobramicin, cefotaxime and cefepime. The susceptibilities were determined by E-test (Biodisk, Sweden) which was applied according to the manufacturer's instructions and the results were evaluated according to the NCCLS 2001 recommendations. The ESBL production was tested by E-test and accepted to be present if the ratio of ceftazidime/ceftazidime + clavulanic asid is >8. The bacteria consisted of *Pseudomonas aeruginosa* ($n = 69$), *E. coli* ($n = 71$), *Klebsiella* sp. ($n = 37$), *Acinetobacter* sp. ($n = 53$).
Results: The susceptibility pattern of the bacteria to the agents were summarized in the table.

	MP	IP	CI	P + T	TOB	CT	CE	ESBL production
P. Aeruginosa $n = 69$	43%	34%	38%	59%	26%	17%	40%	15.9%
Acinetobacter sp $n = 53$	43%	45%	19%	26%	66%	10%	32%	5.6%
E. coli $n = 71$	100%	99%	61%	77%	86%	80%	89%	15.5%
Klebsiella sp $n = 37$	100%	97%	78%	60%	73%	46%	78%	37.8%

MP: Meropenem, IP: Imipenem, CI: Ciprofloxacin, P + T: Piperacillin + tazobactam, TOB: Tobramicin, CT: Cefotaxime, CE: Cefepime.

Conclusion: Among the most common Gram-negative pathogens isolated from nosocomial infections, the ESBL production was found to be the highest for *Klebsiella* sp.The most effective antimicrobial for *P. aeruginosa* was piperacillin + tazobactam and for *Acinetobacter* sp. tobramicin.

For the rest of the pathogens tested, carbapenems were found to be highly effective.

P1411 Nalidixic acid and ciprofloxacin susceptibility of ESBL-producing and nonESBL-producing Enterobacteriaceae

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Objective: To determine and analyze nalidixic acid (NA) and ciprofloxacine (CIP) susceptibility among Enterobacteriaceae ESBL-positive and ESBL-negative.
Materials and methods: The disc diffusion for zone diameters and agar dilution for MIC determination to NA and CIP methods were used for 200 ESBL positive and 200 ESBL negative isolates. All isolates was collected over 2 years from 1999 to 2000.
Results: The overall sensitivity to NA and CIP was 68 and 80%, respectively. Three patterns of sensitivity were obtained: 272 CIP and NA-S (68%), 79 CIP and NA-R (20%) and 48 NA-R, CIP-S (12%). Of the latter group 105% were ESBL positive and 135% were ESBL negative. Only 45% of ESBL positive isolates were CIP-R and 7% NA-R, comparing to 14 and 24% of ESBL negative, respectively. There was a good correlation between MICs of NA and CIP and inhibition zone diemeters; 29 ESBL negative and 91 ESBL positive isolates obtained from children were more sensitive to NA and CIP than the isolates from adults ($P < 0.005$).
Conclusions: The reason for the decreased NA and CIP resitance among ESBL positive isolates can be linked to the high percentage of ESBLs in pediatric wards. Fluoroquinolones are rarely used to treat infections in children; 12% of tested isolates were NA-R being still susceptible to CIP which can lead to increased risk of resistance development to CIP. We found NA susceptibility testing by disc diffusion as good as agar dilution method for assesing the risk of ciprofloxacin resistance in Enterobacteriaceae.

P1412 Antimicrobial agent susceptibility of ESBL-producing *E. coli* and K. pneumoniae isolated in a regional hospital, Poland

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Objectives: Extended-spectrum β-lactamase (ESBL) producing clinical strains have became a major problem in hospitals, causing severe, difficult to treat infections. ESBL enzymes occur predominantly in *K. pneumoniae* and *E. coli*. Detection of ESBL expression has proved to be difficult in routine susceptibility testing, because using in vitro tests of resistance to cephalosporines and aztreonam at the NCCLS breakpoint for susceptibility may not be determinate. The occurrence of prevalence of ESBL among *E. coli* and *K. pneumoniae* strains isolated from inpatients during 2 months time-period this year, was noticed.
Methods: The bacterial isolates were collected at the Microbiology Department in 'Brodnowski' regional hospital in Warsaw. Species identification was performed by ID32 GN ATB System (BioMerieux) following the manufacturers recommendations. A double-disk diffusion method (DDT) was applied to detect extended-spectrum β-lactamases with amoxicilline/clavulanate (20/10 μg), ceftazidime (30 μg), cefotaxime (30 μg) and additionally aztreonam (30 μg) discs. Minimum inhibitory concentrations (MICs) were determined by an agar dilution technique using Mueller–Hinton agar according to NCCLS.
Results: Thirty-one ESBLs positive nonreplicated isolates of *E. coli* (4) and *K. pneumoniae* (27) were collected. The most of isolates were obtained from respiratory tract (intratracheal tubes, tracheal swabs and sputum) and urine. The majority of isolates showed reduced sensitivity to ceftazidime, while were highly resistant to cefotaxime due to the expression of cefotaximaze-type enzyme. The susceptibility to cefoxitin (cephamycin) was common. All but one isolates were resistant to amoxycilline/clavulanic acid when tested by DDT. One-third isolates were resistant to aminoglycosides, almost 30% to ciprofloxacin and only 25% to tetracycline. The increase of ciprofloxacin resistance among *K. pneumoniae* is observed in comparison to respective study, performed by us 2 years ago. All *E. coli* strains were resistant to mentioned antimicrobial agents. The MICs of carbapenems not exceed 0.12 mg/L value, except seven isolates with MICs range of imipenem 1–4 mg/L. Further investigations would be necessary to characterize type of ESBLs and the epidemiology of strains.

P1413 In vitro activity of cefoperazone/sulbactam vs. amoxicillin/clavulanic acid and piperacillin/tazobactam against extended-spectrum β-lactamase (ESBL)-producing strains of *E. coli* and *K. pneumoniae*

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Objectives: To compare in vitro activity of the most commonly used penicillin-inhibitor combinations (amoxicillin/clavulanic acid AMC), piperacillin/tazobactam (PTZ)) and cefoperazone/sulbactam (CPS) against nosocomial ESBL-producing isolates of *E. coli* and *K. pneumoniae*.
Methods: A total of 209 ESBL-producing isolates of *E. coli* ($n = 49$) and *K. pneumoniae* ($n = 160$) collected in 21 Russian hospitals during 1997–1998 were included in this study. The presence of an ESBL was detected by E-test ESBL strips. Susceptibility to AMC (2/1), PTZ (4 mg/L fixed tazobactam concentration) and CPS (1/1) was determined using E-test and results were interpreted according to the current NCCLS guidelines. The susceptibility to CPS was determined on the basis of cefoperazone MIC breakpoints.
Results: The results of susceptibility testing are summarized in the table. The penicillin-inhibitor combinations were not active in vitro against a significant proportion of ESBL producers and the MIC_{90} values of both AMC and PTZ exceeded the resistance levels advocated by NCCLS. At the same time, CPS was active against the majority of strains with the exception of single *K. pneumoniae* isolate (0.5%) expressing a high level of resistance (MIC 96 mg/L) and 12 (5.7%) intermediately resistant strains.

	AMC		PTZ		CPS	
	% I+R	MIC_{90}	% I+R	MIC_{90}	% I+R	MIC_{90}
E. coli	85.7	48	36.7	256	2	12
K. pneumoniae	81.9	64	48.1	256	6.9	16
All strains	82.8	64	45.5	256	5.7	16

Conclusion: Among all β-lactam-inhibitor combinations tested CPS revealed the highest activity against ESBL-producing organisms. Its superior activity is probably attributed to the improved stability of cefoperazone and to the high concentration of the inhibitor component (sulbactam).

Resistance in nonfermenting Gram-negative bacteria

P1414 Carbapenem-resistant *Pseudomonas aeruginosa* in patients with and without carbapenem treatment

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Objectives: To study carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) incidence in patients with concurrent imipenem or meropenem therapy.
Methods: We analyzed clinical and microbiological data of patients with meropenem (MEM) and imipenem (IPM) resistant *P. aeruginosa* isolates over 10 months (January–October 2001).
Results: During the study, 1092 *P. aeruginosa* isolates were obtained from 412 patients, CRPA being isolated 224 times from 43 patients. Majority of CRPA were from respiratory tract specimens 27.5%, wounds 25% and blood 23%. Patients were from ICU (50%), hematology (18%), surgery (14.5%) and general medicine (6.5%). Eighteen patients received IPM and 10 MEM during or just before CRPA isolation. They suffered from polymicrobial infections. Different phenotypes IPM-S, MEM-R and IPM-R, MEM-S were recovered from other 23 and 6 patients, respectively. However, seven and three of them also possessed IPM-R, MEM-R isolates.
Conclusions: CRPA are isolated from 10% of patients with *P. aeruginosa* infection/colonization in our hospital. A total of 28 patients (65%) received carbapenems during or before CRPA isolation. Phenotypes with different IPM, MEM sensitivity can be cultured from the same patient. We have not suspected cross-infections among CRPA positive patients. Careful administration and patients selection should decrease the risk of carbapenem resistance during treatment in *P. aeruginosa*.

P1415 Epidemiology and antibiotic susceptibility of imipenem-resistant *Pseudomonas aeruginosa*

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Objectives: To study the frequency and antibiotic susceptibility of imipenem-resistant *Pseudomonas aeruginosa* isolates from clinically significant samples.
Methods: All isolates were from clinically significant samples and only one isolate per patient was included. Strains were isolated from bronchial secretions (25.2%), urine (20%), blood (11.8%), ear secretion (8.7%), sputum (8.3%), pus (7%), central catheter (5.1%), drainage (3.6%) and others (4.4%). Antimicrobial susceptibility testing was performed by the disk diffusion method according to NCCLS.
Results: During the study period (January 1999–June 2001), 771 strains of *Pseudomonas aeruginosa* were isolated from clinical samples in our microbiology laboratory. Imipenem-resistant strains were collected from 213 (27.6%) patients. Of them, 114 were from the intensive care units, 50 general surgery units, 25 pathology clinics, 8 urology, 7 other surgery units, 5 cardiology, 4 outpatients. The antibiotic susceptibility rates for imipenem-resistant and imipenem-susceptible strains were, respectively: ticarcillin (18–77%), ticarcillin/clavulanate (21–80%), piperacillin (24–80%), aztreonam (20–65%), amikacin (21–72%), netilmicin (22–72%), gentamicin (16–51%), tobramycin (23–76%), piperacillin/tazobactam (27–85%), cefepime (22–82%), ciprofloxacin (29–85%), ceftazidime (26–89%), colistin (97–97%). We distinguised more than 30 resistant phenotypes and the most strains were multiresistant. The prevalence of multiresistant isolates differed greatly between different wards.
Conclusions: Imipenem-resistant *Pseudomonas aeruginosa* is becoming an increasing therapeutic problem in our hospital and especially in intensive care units. Many isolates exhibit multiresistant phenotype. Of the antibiotics tested, the most effective proved to be colistin, ciprofloxacin, piperacillin/tazobactam and cefepime.

P1416 Analysis of prognostic factors in *Pseudomonas aeruginosa* bacteremia

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Background: *Pseudomonas aeruginosa* causes infections with a high mortality rate, despite the availability of antimicrobials active against the microorganism.
Objective: To identify prognostic factors in *P. aeruginosa* bacteremia (PAB).
Methods: We prospectively analyzed 211 consecutive cases of PAB during a 7-year period. Cultures, isolation, and antimicrobial sensibility were performed by standard methods. Death was considered related with the infection when it was active at this moment. The variables analyzed were: sex, age (>60, ≤ 60), acquisition (nosocomial, community), baseline diseases (McCabe I, II, III), severity at first evaluation (according to Winston low, medium, high, very high), with the blood cell (WBC) count (>12 000, 5–12 000, <5000/μL), origin, presence of shock, administration of active antimicrobials. For the statistical analysis bivariant and logistic regression tests were used.
Results: Overall mortality was 28% (59/211). Death was more frequent in patients with McCabe I (21/27, 78%), and II (34/156, 28%) than in subjects classified as McCabe I (4/27, 15%) $P < 0.02$. Critical initial clinical situation at the first evaluation also was significantly related with the mortality (medium 4/31–13%, high 34/147–25%, very high 21/33–64%). The presence of shock also was related with mortality (58/72, 81% vs. 1/139 < 1% $P < 0.0001$). The poorest prognosis was detected in patients with a PAB of pulmonary origin (28/62, 45%), followed by those with unknown origin of the bacteremia (24/81, 30%), compared with other sources (7/68, 10%) $P < 0.01$. Finally, patients who did not receive an adequate treatment also had a higher mortality rate (14/21, 67% vs. 45/190, 24%) $P < 0.01$.
Conclusions: PAB has an overall high mortality rate. The prognosis of PAB is worse in patients with severe baseline diseases, critical condition at presentation, development of shock, in bacteremias of pulmonary origin and in those not adequately treated.

P1417 Comparative in vitro activity of carbapenems against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates from the intensive care unit

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Objectives: The aim of this study was to compare the in vitro activity of imipenem and meropenem against *Acinetobacter baumannii* and *P. aeruginosa* isolates recovered from patients hospitalized in the intensive care unit (ICU).
Methods: A total of 158 nonduplicate isolates of *A. baumannii* ($n = 93$) and *P. aeruginosa* ($n = 65$) were isolated from variety of specimens obtained from patients hospitalized in ICU during the period 2000–2001. The isolates were identified by the automatic ATB system (bioMerieux). The minimal inhibitory concentrations (MICs) of imipenem and meropenem were determined by the agar dilution method according to NCCLS recommendations.
Results: In vitro activity of imipenem against *P. aeruginosa* strains, expressed as MIC_{50}/MIC_{90}, ranged from 4/32 mg/L in 2000–4/64 mg/L in 2001. The MIC_{50}/MIC_{90} values of meropenem were 4/32 mg/L in 2000 and decreased in 2000 (2/16 mg/L). Imipenem activity against *A. baumannii* strains isolated in 2000 and 2001 were 4/16 and 4/32 mg/L, respectively. In contrast to *P. aeruginosa*, meropenem was less active against *A. baumannii* strains isolated in 2001 ($MIC_{50}/MIC_{90} = 16/64$ mg/L) in comparison with those isolated in 2000 ($MIC_{50}/MIC_{90} = 8/32$ mg/L).

Conclusions: The MIC_{50}/MIC_{90} values of imipenem increased moderately among *P. aeruginosa* and *A. baumannii* isolates during the period 2000–2001. It was found out that meropenem was more active against *P. aeruginosa* isolates.

P1418 Risk factors in *Pseudomonas aeruginosa* bacteremia in a medical department of a tertiary-care hospital

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Objective: Our aim was to define the factors associated with increased mortality in bacteremias from *Pseudomonas aeruginosa.*
Methods: We studied 50 episodes of bacteremia due to *P. aeruginosa.* Logistic regression was used for the identification of possible clinical and laboratory risk factors for fatal outcome. The subjects studied were 49 patients, 26 males and 22 females, of a mean age of 64.6 ± 8.2 years, all hospitalized in the medical department of a tertiary hospital.
Results: Eight of the 50 cases of *P. aeruginosa* bacteremia were polymicrobial (16%). Crude mortality was 30%. The variables significantly and independently associated with an increased risk of death, were: (1) temperature <38.5 °C (OR 5.3, $P<0.01$); (2) chemotherapy (OR 6.4, $P<0.001$); (3) pneumonia (OR 3.8, $P<0.05$); (4) shock (OR 8.6, $P<0.001$); (5) polymicrobial bacteremia (OR 6.8, $P<0.001$); and (6) severe underlying disease (OR 7.4, $P<0.001$).
Conclusion: *Pseudomonas aeruginosa* bacteremias represent 13% of the total number of bacteremias in our medical department. Clinical data easily assessed at bedside and few laboratory data can distinguish patients into high or low risk of death from *P. aeruginosa* bacteremia and therefore lead to prompt installation of proper antibiotic therapy.

P1419 Effect of extracellular lipase activity of three *Pseudomonas aeruginosa* strains isolated from human infections on chemiluminescence reaction of human peripheral blood neutrophils and monocytes

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Effect of extracellular lipase activity of *Pseudomonas aeruginsa* EF2, ATCC9027 and ATCC19660 strains isolated from human infections on chemiluminescence reaction of the peripheral blood neutrophils and monocytes investigated. Human peripheral blood neutrophils and monocytes were preincubated with various concentration of lipases at 37 °C for 60 min After preincubation, the cells were stimulated with chemotactic peptide (F-Met-Leu-Phe 10–5 M). A Beckman L 8000 scintillation counter placed under air-conditioned thermostat-controlled 21 ± 1 °C conditions was used. Chemiluminescence function of the cells was assayed in vitro and performed in duplicates. Appropriate controls were included in each experiment. Inactivation of the lipases were performed at 100 °C for 10 min. Results were given as percentage of control cells response preincubated with buffer. Heat treatment of lipase at 100 °C for 10 min almost abolished the inhibitory effect on neutrophil and monocyte chemiluminescence, while lipases at concentrations of 8 UI/mL inhibited neutrophil and monocyte chemiluminescence responses by approximately 22 and 100%, respectively. Also, preincubation of neutrophils with the lipases at concentration of 4, 2 and 1 UI/mL resulted in approximately 12, 5 and 4%, respectively, while preincubated of monocytes with the same concentrations resulted in 95, 48 and 32%, respectively, of chemiluminescence response of control cells (preincubated with buffer). The results showed that monocytes chemiluminescence response is considerably more sensitive to the lipase than the same response of the neutrophils. Also the results showed the inhibitory effect of the extracellular lipase from EF2 strains was higher than the two other strains used. Since monocytes are one of the most important cells of the host defense system, lipase activity of *Pseudomonas aeruginosa* may contribute to the pathogenesis of infections caused by this Gram-negative bacterium.

P1420 Significance of *Pseudomonas aeruginosa* colonization of the gastrointestinal tract

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Objectives: This study was conducted to determine both the association between gastrointestinal (GI) colonization and the development of invasive *Pseudomonas aeruginosa* infection, and risk factors for acquisition of *P. aeruginosa* colonization of the GI tract.
Methods: A prospective cohort study was undertaken. All stool specimen sent to microbiological examination were cultured for *P. aeruginosa* for 3 years. Colonization was defined as the isolation of *P. aeruginosa* from two consecutive cultures in the absence of infection.
Results: Stool specimens of 207 patients over 3 years were investigated. Of the 207 patients, 87 were identified as *P. aeruginosa*-colonized. Forty-five patients had undergone previous invasive procedures and 83 had been prescribed antibiotics within 1 month prior to the isolation of *P. aeruginosa.* The most common organisms isolated from stool with *P. aeruginosa* were *Staphylococcus aureus*, coagulase-negative staphylococci, and *Candida.* In colonized patients, *P. aeruginosa* were isolated from the following body sites in addition to the GI tract: respiratory tract (39%), skin (16%), urinary tract (15%), throat (13%), and nose (10%). Fourteen distinctive *P. aeruginosa* infections developed in 13 patients. Infection associated with GI colonization included four cases of pneumonia, four of urinary tract infection, three of skin infection, and three of bacteremia. No statistically significant correlation were observed between the acquisition of infection and age, gender, underlying disease, previous invasive procedure, or duration of hospitalization. Of the 87 patients, 12 were diagnosed with ileus and three underwent gastrostomy when colonized.
Conclusions: Approximately 15% of GI-colonized patients develop invasive infection. Age, gender, underlying disease, previous invasive procedure, and duration of hospitalization are not risk factors for the development of invasive *P. aeruginosa* infections in colonized patients. Gastrointestinal disorders, and obstruction and surgical interventions in particular, are important factors in GI tract colonization by *P. aeruginosa.*

P1421 Levofloxacin in the treatment of *Pseudomonas* aeruginosa in patients with nosocomial pneumonia

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Objective: A recently completed trial comparing levofloxacin (L) 750 mg i.v./p.o. and imipenem/cilastatin (IC) 500–1000 mg i.v. per every 6 h followed by oral ciprofloxacin 750 mg bid in patients with nosocomial pneumonia (NP) demonstrated no statistically significant differences in clinical efficacy (L 58.1% and IC 60.6%) or microbiological eradication (L 66.7% and IC 60.6%) between the two arms. A more detailed, pathogen-specific, analysis has recently been undertaken and features interesting differences in how the regimens compared in terms of their likelihood of eradicating *P. aeruginosa* and being associated with superinfections due to those organisms.
Methods: Patient populations in the two groups were evaluated with a variety of potential severity stratification including length of hospitalization prior to onset of NP, requirement for intubation, initial oxygenation, number of co-morbid factors, and overall APACHE II scoring. As per protocol, adjunctive therapy was encouraged when *P. aeruginosa* was proven or suspected: ceftazadime or other antipseudomonas β-lactam or an aminoglycoside were to be added to the (L) or (IC) arm, respectively.
Results: Of a total of 438 patients enrolled, 187 were microbiologically evaluable: 93 in the (L) arm and 94 in the (IC) arm. Microbiological eradication rates for select pathogens of interest are displayed in the table.

Pathogen	*N*	No. eradicated	*N*	No. eradicated
S. aureus	21	14 (66.7)	19	13 (68.4)
P. aeruginosa	17	10 (58.8)	17	5 (29.4)
S. marcescens	11	9 (81.8)	7	2 (28.6)
E. coli	12	10 (83.3)	11	7 (63.6)
K. pneumoniae	11	9 (81.8)	7	6 (85.7)

An analysis of superinfections showed differences between (L) and (IC). *S. aureus* superinfections were seen in roughly the same in number and distribution of infection types; there were five *S. faecalis* infections on (L) but only 1 on (IC). On the other hand, there were 16 cases of infections caused by pseudomonads (nine *P. aeruginosa*, seven *S. maltophilia*) on (IC) and only 4 (two *P. aeruginosa*, two *S. maltophilia*) on (L).
Conclusions: It seems likely, given the overall comparability of risk factors in the two arms, that these differences are accounted for on the basis of microbiologic properties of the two agents and strengthen the argument that serious infections like NP should be treated with at least two antimicrobial agents from different classes.

P1422 β-Lactam, aminoglycoside and fluoroquinolone resistance in *Pseudomonas aeruginosa* clinical isolates obtained from a hospital in Bratislava, Slovakia

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Objectives: The set of 35 *Pseudomonas aeruginosa* isolates selected as resistant to clinically used β-lactam antibiotics were collected in different wards of Hospital Ruzinov, Bratislava. The aim was to study the occurrence of resistance to selected β-lactams: ampicillin (AMPI), cefoxitine (CFOX), cefotaxime (CTAX), ceftazidime (CTAZ), ceftriaxone (CIAX), cefepime (CEPI), azthreonam (AZTR), meropenem (MERP); aminoglycosides: gentamicin (GEN), tobramycin (TOB), netilmicin (NET), amikacin (AMI), isepamicin (ISE); and fluoroquinolones: ofloxacin (OFL), ciprofloxacin (CIP). Transferability of aminoglycoside and β-lactam resistance, production of β-lactamases and ESBL and the presence of plasmid DNA in clinical isolates were studied, too.
Methods: Resistance was tested by standard agar dilution method (NCCLS 2000). Nitrocephin method was used for the detection of clinical isolates that produce β-lactamases. Production of ESBL was detected by double diffusion test. Transferability of resistance was tested by bacterial conjugation using *Pseudomonas aeruginosa* 1008 as a recipient strain. The plasmid DNA was isolated by alkaline lysis and its molecular weight was determined by agarose gel electrophoresis.
Results: The occurrence of resistance was: 97.1% to AMPI; 100.0% to CFOX; 77.1% to CTAX; 48.6% to CTAZ; 54.3% to CIAX; 28.6% to CEPI; 54.3% to AZTR; 11.4% to MERP; 82.9% to GEN; 80.0% to TOB; 80.0% to NET; 0.0% to AMI; 80.0% to ISE; 82.9% to OFL and 85.7% to CIP. 85.7% of clinical isolates were identified as β-lactamase producers, but none of the isolates produced ESBL. Resistance to all β-lactams and aminoglycosides was transferable by bacterial conjugation. The frequency of transfer was from 1.4×10^{-6}–2.3×10^{-1}. From the selected isolates and their transconjugants the plasmid DNA with molecular weight ranging from 60 to 73 MDa was isolated.
Conclusions: The frequent occurrence of resistance was determined in the case of all antimicrobial agents tested, with exception of aminoglycoside AMI to which all isolates were susceptible and carbapenem MERP to which only four isolates were moderately resistant. Transferability of β-lactam together with aminoglycoside resistance by conjugative plasmids may contribute to spread of multiresistant *Pseudomonas aeruginosa* clinical isolates in the hospital. Study of plasmid DNA have pointed to the occurrence of nosocomial plasmids with the same molecular weight about 65 MDa in many clinical isolates.

P1423 Evaluation of Sensi-disc diffusion method for synergy qualitative screening of Gram-negative nonfermentative (GNF) clinical isolates

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The antibiotic combination therapy is often required in the treatment of GNF nosocomial infections, to increase antibacterial activity and broaden the bacterial spectrum. Nevertheless, synergistic effect of an antibiotic combination is strain dependent and the checkerboard and killing curves methods are not easily performed in routine laboratory work because labor-intensive and time-consuming. For this reason, an easier and cheaper method for qualitative screening of synergism is desirable. In this study, we compared the checkerboard in broth and the disc diffusion method to determine synergistic effect of levofloxacin (LVX) and ceftazidime (CAZ) in combination, against 31 *P. aeruginosa* isolates, 13 *S. maltophilia* and 12 *Acinetobacter* spp. Disc diffusion method (M1) was performed placing the discs containing each antibiotic at three fixed distances among them, established on the basis of the susceptibility breakpoints. Combination was considered synergistic if an enhancement or bridging was observed at or near the junction of the two zones of inhibition. In addition, the effect of LVX/CAZ combination against *P. aeruginosa* isolates was investigated placing the discs one close to each other (M2). In this case, combination was considered synergistic if an enhancement of inhibition zone was observed compared with those of the single drugs. Results obtained by checkerboard and M1 were in agreement for 64, 69 and 50% against *P. aeruginosa*, *S. maltophilia* and *Acinetobacter* spp., respectively. Most of the disagreement cases concerned the combinations that were declared additive with checkerboard method but gave profiles closer to synergistic or indifferent effect with the disc diffusion method. The results of combination in M2 against *P. aeruginosa* isolates were in agreement for 58 and 80% with those obtained with checkerboard and M1 method, respectively. In conclusion, both disc diffusion methods here reported look promising for qualitative screening of in vitro synergy test, even though a larger number of strains and different antibiotic combinations have to be investigated.

P1424 In vitro development of resistance to β-lactam antibiotics in *P. aeruginosa*

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Objectives: To investigate whether stepwise selection of resistance in *Pseudomonas aeruginosa* reflects exposure to antibiotics in the clinical setting.
Methods: Five isolates of *Pseudomonas aeruginosa* were obtained from the culture collection of the National Reference Centre (NRC) study (Eur J Clin Microbiol Infect. Dis 2000; 19: 888–91) and five isolates from patients suffering from cystic fibrosis. The β-lactam antibiotics tested were piperacillin, ceftazidime, cefepime, imipenem and meropenem. In addition the aminoglycoside amikacin was investigated. The minimal inhibitory concentrations (MIC) were determined by microdilution technique according to NCCLS-guidelines. Development of resistance was investigated by up to 30 serial passages on blood agar plates containing subinhibitory concentrations of the respective antibiotic. Antimicrobial resistance was defined as a four-fold increase in the MIC with stability after 10 serial passages on antibiotic-free media.
Results: After 10 passages 74.5% of the *P. aeruginosa* isolates isolates had already acquired phenotypic resistance against the β-lactam antibiotics. Upon termination of the test series a significant increase in β-lactam resistance had developed in 110 of 120 (91.7%) of the isolates and resistance proved stable in 95 (86.4%). Of the NRC study isolates 86.7% (52 of 60) acquired stable β-lactam resistance, whereas only 66.7% (33 of 50) of the mucoid *P. aeruginosa* isolates from cystic fibrosis patients. For the aminoglycoside antibiotic amikacin stable resistance was acquired by 60% of the organisms.
Conclusions: From our in vitro study, we can conclude that the rate of development of resistance in *P. aeruginosa* against β-lactam antibiotics appears to be lower in mucoid *P. aeruginosa* strains from cystic fibrosis patients.

P1425 Assessment of incubation times for testing antibiotic combinations against multiply resistant isolates from cystsic fibrosis patients by E-test

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Objectives: It is not clear why E-test methods state incubation times of 48 h for both MIC and synergy testing of non lactose fermentors. Standard NCCLS MIC methods and checkerboard synergy testing recommend 24 h incubation. The objective of this study was to compare 24 and 48 h incubation times for E-test MIC and synergy testing with standard methods.
Methods: A total of 114 MICs were carried out by NCCLS broth microdilution (24 h) and E-tests (24/48 h). 100 synergy tests were carried out by microdilution checkerboard (24 h) and E-test (24/48 h) methods. Eleven isolates of *Pseudomonas aeruginosa*, 10 *Burkholderia cepacia*, five *Stenotrophomonas maltophilia* and two *Alcaligenes xylosoxidans* were tested and eight antibiotics were used. Fractional inhibitory concentration indices (FICIs) were

calculated and antibiotic combinations interpreted as follows: synergy (FICI < 0.5), additive effect (FICI > 0.5–1.0), indifference (FICI > 1.0–2.0) and antagonism (FICI > 2.0).
Results: MICs by E-test (24 h) gave better agreement (95%) with NCCLS methodology than E-test 48 h (93%) although there were marginally more major errors (15%) at 24 h than 48 h (13%). E-test (24 h) compared with E-test (48 h) gave 92% agreement with 8% major errors. E-test synergy testing at both 24 and 48 h gave 43% agreement with the standard checkerboard method. E-test (24 h) however, resulted in fewer major errors (10%) than E-test (48 h) (13%). Poorest agreement of MIC results was found for the isolates of *S. maltophilia* [NCCLS vs. E-test 24 h (70%) and 48 h (54%)] and the best agreement was found with the *A. xylosoxidans* isolates (89% NCCLS vs. E-test 24 and 48 h). Poorest agreement of synergy results was found for the Pseudomonas species [NCCLS vs. E-test 24 h (31%) and 48 h (34%)] and best agreement between synergy methods was found for the *S. maltophilia* [checkerboard vs. E-test 24 h (78%) and 48 h (89%)].
Conclusions: Our results provide no evidence to support manufacturer's recommendations that E-test MICs and synergy testing be carried out with 48 h incubation of nonlactose fermentors. 24 h incubation of E-test MIC and synergy tests was found to compare as well as 48 h incubation when compared with standard NCCLS MIC and checkerboard methods. Some inter-species variation in results was found.

P1426 Resistance development of *P. aeruginosa* to fluoroquinolones and aminoglycosides

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Objective: To explore the development of stable resistance of 10 *P. aeruginosa* strains in vitro to fluoroquinolones and aminoglycosides.
Methods: Five isolates of *P. aeruginosa* were obtained from the culture collection of the National Reference Centre study (Eur J Clin Microbiol Infect Dis 2000; 19: 888–91) and five isolates from patients suffering from cystic fibrosis. The fluoroquinolones tested were ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin and the aminoglycosides were tobramycin and netilmicin. Minimal inhibitory concentrations (MIC) were determined by microdilution technique according to NCCLS guidelines. Development of resistance was investigated by up to 30 serial passages on blood agar plates containing subinhibitory concentrations of the respective antibiotic. Antimicrobial resistance was defined as a four-fold increase in the MIC with stability after 10 serial passages on antibiotic-free media.
Results: A significant increase in resistance developed in 108 of 118 (91.5%) of the *P. aeruginosa* isolates. Resistance was stable in 81 (75%) of the isolates. A total of 87.5% of the isolates developed resistance to quinolones (i.e. gatifloxacin 88.2%, and moxifloxacin 83%), while 75% remained stable. All the strains tested acquired phenotypic resistance to aminoglycosides, which in 73.7% remained stable.
Conclusions: If exposed to subinhibitory concentrations *P. aeruginosa* quickly develops resistance to fluoroquinolones and aminoglycosides.

P1427 The antibiotic sensitivity of *Pseudomonas aeruginosa* isolated from various clinical specimens

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The number of *Pseudomonas aeruginosa* isolates in both from hospital and community acquired infections are increasing. The increasing resistance ratio of the antibiotics and the existance of the multiple resistance are serious problems. The goal of this study was to find out the multiple resistance *P. aeruginosa* strains isolated from various clinical materials and the antibiotic resistance pattern. A total of 182 strains isolated from various clinics was included in this study. In the period between January and December 2000, 35.5% of the strains were obtained from surgery clinics, 31.5% intensive care unit, 22.5% burn unit and 10% internal medicine clinics. The distribution of the strains according to the isolation sites were as follows: 46% of the strains were obtained from wound and abscess, 22% respiratory tract, 7.8% blood, 1.3% ear and 10.5% miscellaneous. The in vitro activity of cefoperazone, amikacin, ciprofloxacin, aztreonam and imipenem to *P. aeruginosa* were examined by microdilution broth test according to NCCLS-M7-A4. The resistance pattern was as follows: 48% to cefoperazone, 22% to amikacin, 33% to ciprofloxacin, 54% aztreonam, 18.5% to imipenem. Twenty-one percent of the total strains (182 strains) were found as multiple resistance strains which were isolated from burn unit (69%) and intensive care units (31%). Resistance to cefoperazone, ciprofloxacin and aztreonam of *P. aeruginosa* strains in our hospital were very high. This can be explained as the strains are mostly isolated from burn and intensive care units, also inappropriate antibiotic administration and not obeying to hospital infection control procedures.

P1428 Antibiotic-resistance pattern of clinical isolates of *Pseudomonas aeruginosa* in a university hospital, 1996–2001

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Objective: Description of resistance pattern of *Pseudomonas aeruginosa* (Ps.a), in clinically significant samples of patients remitted to the Laboratory of Microbiology during a 6-year period (1996–2001).
Material: The samples remitted have been evaluated for study during the period understood between 1996 and 2001. The samples have been processed for the isolation of Ps.a. The identification and susceptible to antibiotics has been performed in automated system MicroScan(c) Dade–Behring(c) through panels Combo Neg 6I (1997–1998) or panels Combo Neg 1S (1999–2001). The data were processed by the statistical system Statgraphics Plus 4.1.
Results: During the period of study has been isolated 1897 Ps.a. The percentages of isolates from samples was: respiratory samples 27.9%, urinary samples 26.4%, skin lesions 16.22%, wound 10.1%, ear discharge 8.2%, blood 3.3%, abscess 3% and other samples 4.88%. The resistance is shown in Table 1.

Table 1

	Ak	Aug	Azt	Cpe	Cfe	Cft	Caz	Cp	Fos	Gm	Imp	Mer	Ofl	P/T	Pi	Ti	To	T/S	Samples
1996	4	99	27	*	*	54	12	15	29	22	*	*	*	*	24	20	4	96	289
1997	5	100	22	*	*	88	13	13	67	23	*	*	*	*	24	11	4	93	315
1998	5	98	25	13	100	91	12	15	65	22	14	4	24	9	12	14	6	95	328
1999	4	99	27	15	97	92	14	15	74	24	17	10	24	8	11	13	3	95	287
2000	9	100	35	20	100	89	17	16	75	28	18	10	30	12	14	16	9	95	323
2001	15	99	27	26	100	91	19	22	79	30	19	10	34	12	14	16	10	95	355

Keys antibiotics: Ak = amikacin; Aug = amoxicillin/clavulanic ac.; Azt = aztreonam; Cfz = cefazolin; Cpe = cefepime; Cfe = cefixime; Cft = cefotaxime; Cfx = cefoxitin; Caz = ceftazidime; Cp = ciprofloxacin; Fos = fosfomycin; Gm = gentamicin; Imp = imipenem; Mer = meropenem; Ofl = ofloxacin; P/T = piperacilin/tazobactam; Pi = piperacillin; Ti = ticarcillim; To = tobramycin; T/S = trimethoprim/sul. (% of resistance for antibiotic and year).

The resistance of Ps.a have showed an important increased against Fos (50%), Cft (37%), Cpe (13%), Ak (11%) and Ofl (10%). A less increased have at Gm (8%), Caz (7%), Cp (7%), To (6%) Mer (6%) and Imp (5%). The rest of antibiotics have stayed in constant levels during these last years.
Conclusions: The isolates of Ps.a in the area of the university hospital have shown a significant increased in the resistance to cephalosporins 11th-generation, aminoglycosides and fluorquinolones. The increase in resistance pattern against this antimicrobial agents and cephalosporins third-generation is a very important event that requires of a strict surveillance in the microbiology laboratory.

P1429 Comparison of intravitreal ceftazidime and merpenem in treatment of experimental pseudomonal post-traumatic endophthalmitis in a rabbit model

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Objectives: We developed an experimental model of post-traumatic pseudomonal endophthalmitis in rabbits to compare the efficacy of intravitreal ceftazidime and three doses of meropenem.
Materials and methods: A penetrating eye injury was made in the right eyes of 35 rabbits. 0.1 mL from 104 cfu/mL of *Pseudomonas aeruginosa* ATCC 27853 were inoculated in the midvitreous body. Most animals showed the first signs of endophthalmitis in 14–16 h after inoculation. The animals were examined clinically at 2, 4, 12 and 22 h after surgery using slit-lamp biomicroscopy and indirect ophthalmoscopy. Rabbits were divided into *five treatment groups*. Seven rabbits received 0.1 mL intravitreal injection of ceftazidime (22.5 g/L); seven rabbits received 0.1 mL intravitreal injection of meropenem (0.5 g/L); seven rabbits received 0.1 mL intravitreal injection of meropenem (1 g/L), 7 rabbits received 0.1 mL intravitreal injection of meropenem (2 g/L) and seven rabbits received 0.1 mL of normal saline. At 2 and 24 h, two 100 µL vitreous samples were taken for high-pressure liquid chromatography analysis and colony counting in culture.
Results: At 2 h, vitreous levels of ceftazidime were above the two doses (0.5 and 1 g/L) of meropenem ($P < 0.05$). At 24 h, vitreous levels of ceftazidime were above the three doses of meropenem ($P < 0.05$). Culture results showed no difference between treatment with ceftazidime or three doses of meropenem. Clinical and bacteriological examinations revealed significantly less inflammation in rabbits treated with ceftazidime and meropenem than saline control groups.
Conclusion: Intravitreal antibiotic treatment both with ceftazidime and meropenem appears effective.

P1430 Old drugs for new use: in vitro activity of polimixins against Gram-negative bacilli

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Introduction: Polimixins are a group of cyclic basic polypeptides. Only polymixins B and E (colistin) are available for therapeutic use in humans. Emergence of resistance in many genera of Gram-negative bacteria emerged quickly and it is now found worldwide. In Brazil, this fact have not different aspects and in some cases we do not have any standard antimicrobial available to be used.
Objectives: Evaluation of antimicrobial activity of polimixin B and colistin against Gram-negative bacilli resistant to cefalosporin and sometimes even imipenem.
Methods: Susceptibility testing was performed by using the E-test (AB BIODISK, Solna, Sweden) and following NCCLS procedures. The MIC_{50} and MIC_{90} were determined with both antimicrobial. Thirty strains were analyzed and MIC_{50} and MIC_{90}.
Results: MIC_{50} and MIC_{90} against Gram-negative bacilli, in mg/L, were respectively: polimixin B, 1.5 and 3.0 (range from 0.75 to 4.0); colistin, 1.0 and 1.5 (range from 0.38 to 2.0).
Conclusions: Polimixin B and Colistin was found to be effective against most strains of Gram-negative bacilli. Although polymixins are very toxic, it could be reserved for serious infection due multiply resistant organisms.

P1431 In vitro activities of ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin and gemifloxacin against multiply resistant *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* strains obtained from cystic fibrosis patients

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Objectives: Aggressive antibiotic therapy is one of the main reasons for the improvement in life expectancy among CF patients. Due to repeated drug exposition *P. aeruginosa* becomes progressively resistant to several classes of antimicrobial agents. An increasing incidence of *S. maltophilia* isolates has been reported in some CF centers. This organism is highly resistant to many antibiotics. The aim of this study was to investigate the activity of the newer fluoroquinolones; levofloxacin, moxifloxacin and gemifloxacin in comparison with ciprofloxacin and ofloxacin against these two groups of multiply resistant bacteria obtained from CF patients.
Methods: Thirty-four multiply resistant *P. aeruginosa* isolates and 17 *S. maltophilia* strains obtained from respiratory specimens of 16 CF patients between 1997 and 2001 were included in this study. Susceptibilities of the strains to various fluoroquinolones were studied by the agar dilution procedure, according to the recommendations of the NCCLS.
Result: The table shows the results of the in vitro activity of the fluoroquinolones tested.

Microorganism (n^*)	MIC (mg/L) Quinolone	Range	MIC_{50}	MIC_{90}	S%	I%	R%
P. aeruginosa (34)	LEVO	0.25–128	4	16	21	32	47
	OFX	0.5–128	8	32	21	12	67
	CIP	0.06–64	2	4	15	38	47
	MXF	0.06–>128	8	32	15	15	70
	GEM	0.12–>128	2	8	12	–	38
S. maltophilia (17)	LEVO	0.25–2	0.5	2	100	–	–
	OFX	0.5–4	2	2	88	12	–
	CIP	0.25–4	2	4	18	35	47
	MXF	0.06–1	0.5	1	100	–	–
	GEM	0.03–2	1	2	35	–	65

S: sensitive, I: intermediate sensitive, R: resistant.

Conclusions: We conclude that the newer fluoroquinolones are not a good choice for the treatment of respiratory tract infections of CF patients with multiply resistant *P. aeruginosa* strains, but levofloxacin and moxifloxacin display potent and superior activity to those of other fluoroquinolones against *S. maltophilia* and could be considered a good option for the treatment of infections sustained by this bacteria in CF patients.

P1432 Antimicrobial activities of gatifloxacin, levofloxacin, ciprofloxacin and trimethoprim–sulfamethoxazole against nosocomial isolates of *Stenotrophomonas maltophilia*

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Introduction: *Stenotrophomonas maltophilia* (Sm) is a nonfermentative Gram-negative bacillus that is now emerging as one of the leading causes of nosocomial infection, especially in intensive care units. This organism usually affects immunocompromised, mechanical ventilated, use of intravascular devices, broad spectrum antibiotic prophylaxis and prolonged hospitalization patients. Management of Sm infections can be difficult due to its inherent multidrug resistance.
Objective: The purpose of this study was to evaluate in vitro activities of gatifloxacin (G), levofloxacin (L), ciprofloxacin (C) and trimethoprim–sulfamethoxazole (TMS).
Methods: Organisms were identified according to Manual of Clinical Microbiology Murray, 7th edition and confirmed by API 20 NE (bioMerieux). We determined MIC by agar dilution method with Mueller Hinton against 63 Sm isolated from 23 blood cultures (11 from venipuncture; 12 through catheter), 15 respiratory tracts, 4 urines, 21 other samples. Agar plates were incubated aerobically at 35 °C for 24 h. MICs were interpreted according to NCCLS 2001 guidelines criterium for *Pseudomonas aeruginosa* and other

non-Enterobacteriaceae. MICs break points used for G were: ≤2 μg/mL susceptible, 4 μg/mL intermediate and ≥8 μg/mL resistant (this breakpoint applies to isolates from the urinary tract only). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* 25922 were used for quality control.
Results: Out of 63 tested strains: 1 to G, 2 to L, 4 to TMS, and 36 to C were resistant (see table).

	MIC (μg/mL)				
Drug	Range	50	90	Break point	Susceptibility isolate (%)
Gatifloxacin	0.06–4	0.5	1	≤2	98.42
Levofloxacin	0.06–8	0.5	1	≤2	96.23
TMS	0.25–16	0.5	1	≤2/38	93.66
Ciprofloxacin	0.5–32	2.0	8	≤1	42.86

Conclusion: These results showed that G and L were more active in vitro than TMS (drug of choice) and C was the least effective against Sm.

P1433 In vitro synergy of colistin with rifampin and cotrimoxazole on multidrug-resistant *Stenotrophomonas maltophilia*

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Objective: Nosocomial infections by *S. maltophilia* are managed with difficulty due to the limited number of therapeutic options. Interactions of colistin with rifampin and cotrimoxazole were investigated as probable candidates active on that species.
Methods: Twenty different isolates were exposed over-time to the in vitro interaction of 1× and 4 × MIC of colistin with 2 μg/mL of rifampin and 2/38 μg/mL of trimethoprim/sulfamethoxazole, i.e. concentrations representing mean serum levels. All isolates were resistant to ampicillin/sulbactam, to rifampin (MIC > 64 μg/mL) and to cotrimoxazole (MIC > 4/76 μg/mL). Antibiotics were added with a 6 log-phase inoculum of each isolate in Mueller–Hinton broth and bacterial growth was determined at standard time intervals during incubation at 37 °C. Synergy was defined as any more or equal to a 2 log 10 decrease of viable cell counts compared to the most active single agent. A total of 180 time-kill curves were performed.
Results: Synergy between 1 × MIC and 4 × MIC of colistin and rifampin was found in eight (55%) and 10 (55%) isolates, respectively, after 4 h of growth, in 11 (55%) and 11 (55%) isolates, respectively, after 6 h of growth and in 11 (55%) and 13 (65%) isolates, respectively, after 24 h of growth. Synergy between 1 × MIC and 4 × MIC of colistin and cotrimoxazole was found in one (5%) and four (20%) isolates, respectively, after 6 h of growth and in 10 (50%) and 10 (50%) isolates, respectively, after 24 h of growth.
Conclusions: Colistin interacts synergistically with rifampin and to a lesser extent with cotrimoxazole on a considerable number of *S. maltophilia* isolates. That interaction occurs over the first hours of bacterial growth so as to become promising for the therapeutic management of nosocomial infections by these isolates.

P1434 In vitro activity of aminoglycosides on *Stenotrophomonas malthophilia* virulence factors

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Objectives: The aim of study was to investigate the effect of aminoglycosides (amikacin, gentamicin, netilmicin, tobramycin, streptomycin) at the subinhibitory concentrations (sub-MICs) on the cell-surface hydrophobicity, lipase activity and motility of *S. malthophilia* strain isolated from tonsil of patient with tumor disease.
Methods: The cell-surface hydrophobicity was evaluated by the method of bacterial adherence to hydrocarbon–xylene (BATH) and by the method of salt aggregation with ammonium sulfate (SAT). The lipase activity was measured spectrophotometrically in the culture filtrates by the use of polyoxyethylen-sorbitane (Tween 60). The motility assay was performed on the semisolid swarming agar medium (0.35%).
Results: The highest inhibition of adherence to xylene caused by both gentamicin and amikacin at 1/4 of the MIC was 68.6 and 69.9%, respectively. These data correlated with the results of salt aggregation test. All antibiotics tested at sub-MICs expressively inhibited the lipase production. Amikacin caused the total inhibition of this enzyme within the whole concentration range. Exposure of the bacterial cells to sub-MICs of aminoglycosides resulted only in moderate inhibition of motility of the strain tested compare to the unexposed cells.
Conclusions: The results obtained indicate the interference of aminoglycosides with pathogenic potential of *S. malthophilia* at sub-MICs of aminoglycosides, namely gentamicin and amikacin.

P1435 *Stenotrophomonas maltophilia* (SM) in an ambulatory patient population

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SM has become an important nosocomial pathogen in hospitalized, immunocompromised patients and has been noted to colonize the respiratory tract of patients with cystic fibrosis (CF) [J Hosp Infect 1995: 30: 453]. Little appears to have been written about SM in the non-hospitalized population. CLS is a large freestanding clinical laboratory in New York City serving an exclusively ambulatory population. Over a 7-month period (9/1/1999–4/1/2000), 30 patients had positive cultures for SM. Culture and identification of the organism used standard procedures [ASM: Manual of Clinical Microbiology (7th ed.), 1999] and Vitek instrumentation (BioMerieux; Hazelwood, MO). Antibiotic sensitivity studies used E-test strips (AB BioDisk; Piscataway, NJ). The positive cultures were approximately equally distributed by gender (17F) and appeared to come mainly from older adults with 22/30 (73%) from individuals ≥52 YO and 18/30 (60%) patients ≥62 YO. Two patients however, were 5 and 7 YO. Culture sources included sputum (14), mucocutaneous/soft tissue (9), genital (4), urine (2), ear (1). With respect to the sputum specimens 8/14 (58%) were from patients ≥62 YO. Associated conditions included cancer (3) [prostate, breast, non-Hodgkin's lymphoma], sarcoidosis (2) and COPD (1). The 9 cultures from mucocutaneous/soft tissues included 8 foot ulcers, 7 of which were from patients ≥72 YO, and 1 lip pustule (F, 7 YO). The four genital cultures included one case in which there had been a recent termination of pregnancy and one case of prostate cancer treated with radiation. One of the two urine cultures was associated with BPH and recent catherization. None of the patients had a history of CF. Sensitivity data indicated that 29/30 cultures were susceptible to trimethoprim/sulfamethoxazole (MIC ≤ 2/38 μg/mL), 27/30 to ticarcillin/clavulanic acid (MIC ≤ 16 μg/mL), 17/30 to Ciprofloxacin (MIC ≤ 1 μg/mL) but only 3/30 to Aztreonam (MIC ≤ 8 μg/mL). Of the 30 cultures, 27 were sensitive to at least two drugs but the remaining cultures were susceptible only to trimethoprim/sulfamethoxazole. Although the number of specimens was small, our study suggests that positive SM cultures occur mainly in older outpatients. Its role in soft tissue infections has previously been noted [Ann Int Med 1994; 121: 969]. There is a suggestion that the organism may colonize or infect the respiratory tract of older adults. Occasionally multidrug resistant organisms may be seen.

P1436 An epidemiological analysis of *Stenotrophomonas maltophilia* strains

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Objectives: *S. maltophilia* has recently emerged as an important cause of nosocomial infection. In this study, we aimed to determine whether there is a genotypic relationship between clinical isolates of *S. maltophilia* strains obtained from hospitalized patients.
Methods: *S. maltophilia* was isolated by standard microbiological methods using blood and EMB agar, and identified was with automatized Sceptor system. This identification was confirmed by PCR using SM1 and SM2 primers directed 23S rRNA of *S. maltophilia*. Forty-four clinical isolates from 41 patients were tested for antibiotic susceptibility and genotyped by enterobacterial repetitive intergenic consensus sequences PCR (ERIC-PCR). Susceptibility to antibiotics were evaluated by disk diffusion method and by E-test strips.

Results: Isolates from the first five patients were identical. Identical pattern was observed in two isolates from two different patients in the same clinic on the same day. Furthermore, the DNA binding patterns of five isolates were also identical.
Conclusion: Three nosocomial outbreaks were determined by *S. maltophilia* in our hospital using ERIC-PCR. These findings provoke new insights into occurrence of cross-contamination.

P1437 Is *Stenotrophomonas maltophilia* an emerging pathogen? Epidemiology and resistance to antimicrobials in a general hospital

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Objectives: To determine the prevalence and the susceptibility to antimicrobials of clinical isolates of *S. maltophilia* in a general hospital over a 5-year period.
Methods: From January 1996 to December 2000 all *S. maltophilia* recovered in our microbiology laboratory were studied. The isolates were identified using the MicroScan system and confirmed by the API 20 NE system. Over the entire study period, susceptibility testing was performed by the broth microdilution method using the MicroScan system. Antimicrobials evaluated were: piperacillin, ceftazidime, cefepime, gentamicin, amikacin, tobramycin, ciprofloxacin, imipenem, meropenem, and cotrimoxazole.
Results: A total of 690 *S. maltophilia* were isolated from clinical samples. *S. maltophilia* represented 1.8% of the total of Gram-negatives recovered over the study period and 10% of the total of nonfermenters. The origin of isolates was: lower respiratory tract (227), wounds (184), blood (101), urine (69), sterile fluids (47) and miscellaneous (62). The 690 isolates belonged to 42 different wards. A total of 515 corresponded to adult patients (75%), and 175 to children (42 isolates were from newborn patients). The number of isolates per year (n) and the percentages of resistance to antimicrobials are shown in the table.

	1996 ($n=120$)	1997 ($n=112$)	1998 ($n=160$)	1999 ($n=163$)	2000 ($n=135$)
Piperacillin	41	62	71	52	55
Ceftazidime	63	61	61	63	56
Cefepime	N/A	N/A	N/A	90	90
Gentamicin	89	79	85	79	76
Tobramycin	89	79	84	74	67
Amikacin	88	78	84	75	70
Ciprofloxacin	49	54	58	51	49
Cotrimoxazole	8	3	5	4	0
Imipenem	100	100	100	100	100
Meropenem	100	100	100	100	100

Conclusions: *S. maltophilia* is an important cause of nosocomial infection. In our hospital, resistance rates of this microorganism to different antimicrobials have remained stable over the years. Small percentages of resistance to cotrimoxazol have been detected, however, this antimicrobial remains the treatment of choice for *S. maltophilia* infections.

P1438 Bactericidal activity of human serum against *Stenotrophomonas maltophilia* isolated from blood

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Objectives: Purpose of this work was determination of all factors necessary to killing *Stenotrophomonas maltophilia* rods sensitive to the treatment of normal human serum (NHS).
Methods: The NHS susceptible rods were treated by different serum preparations: NHS with blocked one of the pathways of complement (C) activation, with removed lysozyme or with blocked one of the activation pathway without lysozyme.
Results: Fifty percent of studied *Stenotrophomonas maltophilia* strains were sensitive to the bactericidal action of NHS. For 15 studied *S. maltophilia* strains five different variants of the bactericidal action of complement and lysozyme were found. Eight strains was sensitive to the action of serum in which the C was activated via classical pathway without lysozyme or alternative pathway with lysozyme as well as simultaneously by both pathways without the participation of lysozyme. Three other strains were sensitive to the C activated via classical or alternative pathways with participation of lysozyme or to the C simultaneously activated by both pathways without the lysozyme. Two strains were killed by the C activated via the classical pathway with presence of lysozyme. One strain was killed by the C activated via alternative pathway with lysozyme and another one strain by the C activated via classical or alternative pathway always with participation of lysozyme.
Conclusions: Diversity of the mechanisms of bactericidal activity of serum is probably due to the different antigenic structure of studied bacteria.

P1439 In vitro activity of new sulbactam combinations against aerobic and anaerobic bacteria: results of a German multicenter study

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Objectives: Sulbactam is a β-lactamase inhibitor which is used in combination with β-lactams. In the present study the in vitro activities of sulbactam plus penicillin (PES), sulbactam plus cefuroxime (CFS) and sulbactam plus ceftazidime (CAS) against aerobic and anaerobic bacteria were compared to those of the three β-lactams alone (PEN, CFX, CAZ) as well as the commonly used antimicrobials piperacillin/tazobactam (PIT), imipenem (IMP) and metronidazole (MTR).
Methods: Between May 2000 and May 2001, a sample of 1934 clinical isolates (Gram-positive cocci, Enterobacteriaceae, *Acinetobacter* spp., anaerobes) recovered from hospitalized patients from 11 geographic areas in Germany was collected. Minimal inhibitory concentrations (MICs) using the broth microdilution method were determined according to DIN 58 940. Sulbactam was added to PEN, CFX, and CAZ at a constant concentration of 8 mg/L.
Results: The table shows MIC_{50} and MIC_{90} values for selected groups of bacteria (mg/L).

Isolates (n)	MIC	PEN	PES	CFX	CFS	CAZ	CAS	PIT	IMP	MTR
All aerobe isolates (1529)	50	32	2	4	1	0.5	0.25	1	≤0.12	n.t.
	90	≥256	≥256	≥256	≥256	≥256	≥256	16	1	n.t.
A. baumannii (35)	50	32	≤0.12	32	≤0.12	2	≤0.12	≤0.12	≤0.12	n.t.
	90	≥256	≥256	≥256	32	32	4	64	0.25	n.t.
S. aureus incl. MRSA (364)	50	8	≤0.12	1	0.5	8	4	1	≤0.12	n.t.
	90	128	8	128	64	64	64	8	1	n.t.
All anaerobe isolates (405)	50	8	≤0.12	16	≤0.12	8	≤0.12	≤0.12	n.t.	0.5
	90	64	≤0.12	128	1	128	2	2	n.t.	2
Bacteroides spp. (248)	50	8	≤0.12	64	≤0.12	64	≤0.12	0.25	n.t.	1
	90	64	0.25	128	4	≥256	2	2	n.t.	2
Prevotella spp. (71)	50	1	≤0.12	2	≤0.12	1	≤0.12	≤0.12	n.t.	0.5
	90	32	≤0.12	64	≤0.12	64	0.5	0.25	n.t.	2

n.t., not tested.

The addition of sulbactam enhanced the activity of β-lactams to varying degrees. Sulbactam/penicillin G showed high activities against *S. aureus* (MICs comparable to PIT and better than the cephalosporins), anaerobes (MICs better than metronidazole) and *A. baumannii* ($MIC_{80} \leq 0.125$ mg/L). Combined with cephalosporins, the effect of sulbactam was most pronounced in anaerobes (MICs comparable to metronidazole) and in *A. baumannii*, but there was only a moderate increase in the activity against other aerobic species.

Conclusion: The combination of sulbactam plus penicillin G represents a valuable addition to the armamentarium of approved sulbactam combinations. Pencillin G plus sulbactam is an interesting option primarily for the treatment of infections caused by *S. aureus* and anaerobes.

P1440 Problems in interpretation of carbapenem susceptibility results with *Acinetobacter baumannii* in automated systems

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Objectives: *A. baumannii* is a multidrug resistant pathogen often only susceptible in vitro to carbapenems and colistin. Inasmuchas they cause nosocomial infections associated with high mortality rates, accurate results by in vitro methods are essential to the administration of appropriate antimicrobial therapy. Although the MICs of *A. baumannii* to imipenem and meropenem have been shown to be similar using microbroth techniques, we observed differences using two semi-automated systems. This study (1) measures the extent of MIC discordance with the semi-automated systems when testing *A. baumannii* to these carbapenems, using the microbroth method as a gold standard (2) determines the impact on interpretation and (3) offers recommendations to improve accuracy of carbapenem reporting.
Methods: Carbapenem MIC results of 136 clinical isolates from patients at Columbia Presbyterian Medical Center were obtained by the MicroScan Walkaway SI (Dade Behring, IL) with neg combo 27 panels, the Vitek 2 (bioMerieux, MO) and microbroth (PML Microbiologicals, OR) systems.
Results: With the MicroScan Walkaway, the strains were 54% susceptible to imipenem and 27% to meropenem and the susceptibility with the Vitek 2 system was 52 and 31% for imipenem and meropenem, respectively.
Conclusions: Discordance of meropenem with imipenem was observed in 27% of isolates using the MicroScan Walkaway and 21% with Vitek 2. Broth microdilution confirmed that discrepancies occurred most often in the MIC range of 4–16 with both systems. It is recommended that breakpoint panels should not be solely used to determine susceptibility to carbapenems and reconsideration of breakpoints for these antibiotics with *A. baumannii* might be warranted.

P1441 Bactericidal activity and synergy against multiresistant *Acinetobacter baumannii*

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Objectives: The purpose of this study was to know the bactericidal activity and synergy of imipenem (IMP), meropenem (MRP), sulbactam (SB) and colistin (CL) against four strains of multiresistant *Acinetobacter baumannii* representing the four more frequent clones isolated in our center.
Methods: MIC and MBC were performed using microdilution method (NCCLS). Time-kill curves were used to evaluate the bactericidal activity and synergy of antimicrobial combinations (IMP + SB, IMP + CL, MRP + SB, MRP + CL, and SB + CL) against the multiresistant *A. baumannii* strains A, B, C and D. For the time-kill curves antibiotics concentrations were equivalent to MIC of each of antimicrobial agents and the C_{max} of IMP and SB obtained in mice (IMP 30 mg/kg and SB 60 mg/kg). Antibiotics were considered to be bactericidal against *A. baumannii* when there was a reduction of the original inoculum $\geq$3 log cfu/mL during the 24 h of incubation. Synergy was defined as $\geq$2 log decrease in cfu/mL when using the drug combination, relative to the most active component alone.
Results: MIC/MBC: IMP (32/32 mg/L), SB (32/32 mg/L), MRP (8/16 mg/L for strains A and C, and 16/16 mg/L for strains B and D), CL (0.5/2 mg/L for the strains A and C, 0.5/8 for the strain B, and 0.5/1 for the strain D). C_{max}: IMP (16.9 mg/L), SB (81.5 mg/L). Bactericidal activity and synergy: All antimicrobials were bactericidal against the strain B, reaching a bacterial concentration <2 cfu/mL; IMP (MIC) was also bactericidal against the strain C. The following combinations were synergistic: IMP(MIC) + SB (MIC) for the strain A, IMP(MIC) + CL for the strains A and C, IMP(C_{max}) + CL for the strains B, C and D, MRP + SB(MIC) for the strain A, MRP + CL for the strains A, C and D, SB(MIC) + CL for the strains B and C.
Conclusions: The carbapenems plus sulbactam are synergistic against selected strains of multiresistant *A. baumannii*. Also, the combination of colistin to carbapenems and sulbactam is synergistic against most of the strains of multiresistant *A. baumannii*. These results suggest that these combinations may be useful in the treatment of infections caused by this agent

P1442 Novel OXA-type carbapenemase in endemic carbapenem-resistant clones of *Acinetobacter baumannii* from Bilbao, Spain

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Objectives: To characterize a collection of carbapenem-resistant *Acinetobacter baumannii* isolates obtained from a single hospital in Bilbao over the course of 1 year, and to investigate their relationship with other strains of *A. baumannii* associated with worldwide outbreaks of nosocomial infection.
Methods: A total of 82 clinical isolates of *A. baumannii* obtained from patients with chronic bronchiectasis attending a single hospital in Bilbao over a 1-year period, were identified by tDNA fingerprinting. MICs were determined by the broth microdilution method and interpreted according to NCCLS criteria. Carbapenem-resistant isolates were examined for the presence of carbapenemases with phenotypic disk synergy tests, followed by PCR analysis and sequencing of PCR products. RAPD fingerprinting with DAF4, ERIC-2 and M13 core primers was used to study the population structure of the Bilbao isolates and their relationship with other worldwide nosocomial isolates of *A. baumannii*.
Results: Determination of MICs revealed that 49 (60%) and 39 (48%) of the *A. baumannii* isolates were resistant to imipenem and meropenem, respectively, at the NCCLS breakpoints and also showed multiple resistance to other main antibiotic classes. RAPD fingerprinting of carbapenem-resistant isolates revealed two major clusters (I and II) of isolates, of which cluster I could be further subdivided into a number of related subclusters depending on the precise RAPD primer used. One of these subclusters was related to two carbapenem-susceptible isolates found previously in Barcelona, Spain, and Trieste, Italy, but this was the only close relationship found amongst a collection of 48 *A. baumannii* isolates associated with worldwide outbreaks of nosocomial infection. Phenotypic disk synergy tests indicated the presence of carbapenemase activity in the isolates from Bilbao. PCR analysis followed by sequencing detected a novel integron-associated OXA-type carbapenemase in both major clusters.
Conclusions: The study revealed that two major clones of carbapenem-resistant *A. baumannii* isolates were endemic in a single Bilbao hospital. Resistance was associated with the presence of a novel integron-associated OXA-type carbapenemase that has spread between the different clones.

P1443 *Aeromonas* spp. an emerging enteric pathogen?

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Objectives: To investigate the role of *Aeromonas* spp. in adults gastrointestinal infections.
Methods: During a 3-year period stools from 587 (1999 : 190, 2000 : 192, 2001 : 205) patients were investigated for *Aeromonas* spp. The samples were plated in blood agar plus ampicillin 10 mg/L and incubated in ambient air for 48–72 h. The identification and susceptibility to antibiotics were performed by the commercial ID/MIC panel (Pasco, Difco).
Results: This retrospective study revealed an incidence of 1% (1999), 5.2% (2000), 6.3% (2001). *Aeromonas hydrophila* 17/25 (68%) was the most common isolated species followed by *Aeromonas caviae* 6/25 (24%). The clinical features were: diarrhea 20/25, abdominal discomfort 18/25, vomiting 17/25, fever 5/25 in patients who were suffering from diabetes mellitus, chronic renal

failure, alcoholism, intestinal malignancies. All the strains were susceptible to Ciprofloxacin and Trimethoprim/sulfamethoxazole.
Conclusions: (1) The isolation rate of *Aeromonas* species, as responsible pathogen of the intestine, showed an increasing trend. (2) *Aeromonas hydrophila* was the predominant species. (3) Diarrhea, abdominal discomfort and vomiting were the main symptoms, since fever was rather rare. (4) The most common used antimicrobial agents, Ciprofloxacin and Cotrimoxazole, showed a remarkable in vitro activity (100%) in these infections.

P1444 Pulsed-field gel electrophoresis analysis of *Aeromonas veronii* isolated from a patient with sepsis

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Objectives: Gram-negative bacilli of the genus *Aeromonas* are widespread in aquatic environments and are responsible for different human infections. In contrast to the recognized association of these bacteria with immunocompromised hosts, recent data indicate that *Aeromonas* spp. may also be primary causes of human infections in immunocompetent hosts. In recent years, *Aeromonas* extra-intestinal infections have been reported in growing frequencies. Nevertheless, *Aeromonas* bacteremia remains an uncommon finding, often associated with different underlying hepatic diseases and usually related to the species *A. hydrophila*. To date the etiologic role of *A. veronii* has been rarely described. Here we report a case of *A. veronii* sepsis associated with acute suppurative cholangitis in a patient with chronic hepatitis B.
Methods: The Sceptor (Becton Dickinson, Milan, Italy) and the API 20E (bioMérieux, Rome, Italy) systems were used to identify the bacteria recovered from peripheral blood and bile. The clinical isolates were analyzed by pulsed-field gel electrophoresis (PFGE) using the Chef Mapper System (Bio-Rad, Milan, Italy) after DNA digestion with *Spe* 1 and *Xba* 1 restriction enzymes (BioLabs, New England, USA). DNA fingerprinting gels were compared using the Molecular Analyst software for Macintosh (Bio-Rad).
Results: The two clinical isolates from blood and the isolate from bile were identified as *A. veronii*. PGFE analysis revealed their genetic homogeneity, as they were characterized by indistinguishable PFGE patterns, in spite of the different antibiotic susceptibility showed. The PFGE pattern was comparable to that of the 35624 ATCC *A. veronii* strain.
Conclusions: *A. veronii*, together with A. hydrophila, can be responsible for *Aeromonas* sepsis, associated with biliary tract infection and PGFE analysis can be an useful tool to demonstrate the genetic homogenicity of *Aeromonas* clinical isolates.

Fungal infections

P1445 Candidemia in a Greek hospital: 7-year analysis

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Objective: Candidemia is a serious cause of blood stream infection resulting in a mortality rate of nearly 40%. In this study, we determined the rate of blood isolation of different *Candida* species from hospitalized patients.
Methods: Between 1995 and 2001, 150 clinical strains of *Candida* spp. were isolated from blood cultures specimens (one isolate per patient). Identification of *Candida* was performed with Becton–Dickinson (BACTEC NR 9240) and BioMerieux (Yeast API 32ID) systems. The in vitro activity of amphotericin B, fluconazole, itraconazole, 5-fluorocytocine, ketoconazole and miconazole against *Candida* bloodstream isolates was tested by determination of minimum inhibitory concentration (MIC) using Sensititer panels.
Results: *Candida* spp. were identified as: *Candida albicans* 43.3%, *C. parapsilosis* 14.6%, *C. glabrata* 13.3%, *C. tropicalis* 12.6%, *C. krusei* 8%, *C. pseudotropicalis* 4.6% and *C. famata* 3.3%. The majority of *Candida* species was susceptible to amphotericin-B and fluconazole and moderate susceptible to other tested antifungal agents. The patients with candidemia were immunosuppressed patients of specific units 46.7%, medical wards 29.3% and surgical wards 24.0%.
Conclusion: In our hospital, *Candida albicans* was the yeast most frequently isolated from blood followed by *C. parapsilosis*, *C. glabrata* and *C. tropicalis*. The approved antifungal agents were generally active against *Candida* spp. The majority of candidemia cases were in high-risk patients with 32.6% mortality rate.

P1446 Fungal colonization of upper gastrointestinal tract in HIV-infected patients

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Objectives: The aim of the study was to analyze fungal strains cultured from oral and esophageal swabs as well as from gastric juice in HIV-infected patients (pts) in relation to CD4 lymphocyte count.
Methods: Mycological cultures from oral and esophageal swabs, and gastric juice were analyzed in 45 HIV-infected patients of whom 25 had CD4 < 200/mm^3 (group 1) and 20 had CD4 > 200/mm^3 (group 2). Samples were taken during gastroscopy performed in year 2001 because of dyspeptic symptoms. Fungal resistance to antimycotic drugs was estimated. Control group consisted of 21 non-HIV-infected patients (group 3).
Results: Significant fungal growth was found in oral swab cultures: in 14 pts (56%) in group 1, in 10 pts (50%) in group 2, in 3 pts (14%) in group 3; in esophageal swab cultures: in 11 pts (44%) in group 1, in 10 pts (50%) in group 2, in 6 pts (28%) in group 3; in gastric juice cultures: in 12 pts (48%) in group 1, in 7 pts (35%) in group 2, in 6 pts (28%) in group 3. Mycotic stomatitis was present in 14 pts (56%) in group 1 and in 6 pts (30%) in group 2. Fungal esophagitis was present in 10 pts (40%) in group 1 and in 2 pts (10%) in group 2. There was no symptomatic mycosis found in group 3. The following fungal strains were isolated in group 1: *Candida albicans* in 52% pts, *C. glabrata* in 8% pts, *C. kruzei* in 4% pts, *C. tropicalis* in 4% pts, *C. inconspicua* in 4% pts; in group 2: *C. albicans* in 55% pts, *C. glabrata* in 15% pts, *C. tropicalis* in 5% pts, *C. lusitaniae* in 5% pts, *Geotrichum* in 5% pts; in group 3: *C. albicans* in 47% pts, Geotrichum in 5% pts. The antimycotic drug-sensitivity of fungal strains in HIV-infected patients was as follows: clotrimazole (100%), nystatin (98%), ketoconazole (98%), miconazole (85%), fluconazole (72%). There were fewer drug-resistant fungal strains isolated from non-HIV-infected patients, among them all were sensitive to clotrimazole, nystatin and fluconazole.
Conclusion: Fungal colonization of upper GI tract mucosa is significantly more frequent in HIV-infected patients and is related to cellular immunity deterioration measured by CD4 lymphocyte blood count. There are often coexisting clinical symptoms of fungal infection. *Candida albicans* appeared to be predominant fungus cultured from all groups of patients. Drug resistance (especially to fluconazole) is more frequent in HIV-infected patients.

P1447 Investigation of *Candida dubliniensis* and identification of *Candida* spp. in oropharyngeal swabs of cancer patients

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Objectives: To investigate the presence of *C. dubliniensis*; a novel *Candida* species, and other fungal pathogens in cancer population.
Methods: The oropharyngeal swabs of 543 cancer patients were collected during their visit to oncology clinic in 9-month period. Samples were obtained with a sterile swab, which was immediately used to inoculate and streak on Sabouraud dextrose agar (SDA) plate for isolation. Cultures were incubated at 37 °C for at least 48 h in aerobic condition. Among *Candida* isolates, phenotypic characteristics of *Candida dubliniensis* was investigated by analysis of germ-tube formation and chlamydospore production, growth at 42 °C and 45 °C, colony morphology on Staib agar and intracellular beta-D-glucosidase activity. The carbohydrate assimilation patterns were studied using the API 20C AUX system.
Results: Totally 209 *Candida* species were isolated. Of the isolates 147 (70.3%) were *C. albicans*. The other species were *C. parapsilosis*, $n = 16$ (7.6%);

C. tropicalis, $n = 13$ (6.2%); *C. glabrata*, $n = 10$ (4.7%); *C. guillermondi*, $n = 5$ (2.3%); *C. krusei*, $n = 4$ (1.9%); *C. keyfr*, $n = 3$ (1.4%); *C. famata*, $n = 3$ (1.4%); *S. cerevisiae*, $n = 2$ (0.9%); *C. pelliculosa*, $n = 2$ (0.9%); *C. utiles*, $n = 1$ (0.4%); *C. Neoformans*, $n = 1$ (0.4%); *H. Polymorpha*, $n = 1$ (0.4%); and no *C. dubliniensis* was isolated.

Conclusions: Although colonization with *Candida* does not indicate clinical candidiasis, it may be the cause of candidemia with increased morbidity and mortality. Among *Candida* spp., *C. dubliniensis* is gaining significant importance because of its misidentification as *C. albicans* by routine protocols like germ-tube formation and chlamydospore production and its resistance to antimicotics like fluconazole. In our study, *C. dubliniensis* was not isolated in oropharyngeal specimens of the cancer patients. Further studies are needed to reveal the incidence of *C. dubliniensis* in various patient populations.

P1448 Trends in hospitalizations with candidiasis diagnoses in Australia, 1995–1999

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Objectives: Mycoses due to *Candida* are important causes of morbidity and mortality in hospitalized patients. This study examined the trends in hospitalizations with candidiasis diagnoses (CD) in Australia during the period 1995–99.

Methods: Data were extracted from the National Hospital Morbidity Database. A hospitalization with a CD was defined as any acute hospital-discharge diagnosis of: disseminated candidiasis (ICD-9-CM: 112.5); invasive candidiasis (ICD-9-CM: 112.4, 112.81, 112.83, 112.85, 112.89, 112.9, 771.7); or noninvasive candidiasis (ICD-9-CM: 112.0, 112.1, 112.2, 112.3, 112.82, 112.84). Costs of hospitalizations with a CD were estimated by multiplying length of hospital stay (LOS) by the average DRG cost per hospital day (1998 Australian dollars). Logistic, Cox, and linear regression models were used to examine the impact of different candidiasis diagnoses on mortality, LOS, and costs adjusting for age, gender, year, insurance, hospital type, and principal reason for admission.

Results: A total of 57 758 hospitalizations with a CD were identified (disseminated: 533; invasive: 11 885; noninvasive: 45 340). Hospitalizations with a CD increased annually by 3% from 1995 to 99. During this period hospitalizations with a CD accounted for 757 714 bed days and an estimated $520 million in hospital costs (disseminated: 16 361 days, $17 million; invasive: 198 421 days, $154 million; noninvasive: 542 932 days, $349 million). 9% of hospitalizations had candidiasis coded as the primary diagnosis (disseminated: 16%; invasive: 9%; noninvasive: 9%). In-hospital mortality was higher for hospitalizations with disseminated CD (OR 4.1, 95% CI 3.2–5.3) and invasive CD (OR 1.1, 95% CI 1.0–1.2) as compared to hospitalizations with noninvasive CD after adjusting for co-variates. The risk of being discharged was significantly lower for hospitalizations with disseminated CD (HR 0.39, 95% CI 0.35–0.44) and invasive CD (HR 0.78, 95% CI 0.76–0.80) as compared to hospitalizations with noninvasive CD. Hospitalizations with disseminated CD and invasive CD as compared to hospitalizations with noninvasive CD had increased hospital costs of 185% and 39%, respectively.

Conclusions: Hospitalizations with different CD were associated with differences in measured outcomes. Among hospitalizations with CD, disseminated CD occurred infrequently, however, had significantly greater burden with respect to in-hospital mortality, LOS, and costs.

P1449 *Candida* infections in high-risk patients in abdominal surgery

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Candida species are increasingly important nosocomial pathogens. The aim of the study was an analysis of the results of microbial cultures in patients who had been operated during the last 6 years. The usefulness of laboratory diagnostics (traditional laboratory methods and serological ones performed on some of the patients) has been valued. The risk factors for mycotis spread have been analyzed. *Candida albicans* was the third cause of infection (10.6%), 204 fungal species were detected in 123 patients, often *Candida albicans*. In 49 patients, *Candida* were cultured from several specimens. About 17.8% of the infection were found in patients with inflammatory bowel diseases, 15.4% in patients with peritonitis, 13.8% with acute pancreatitis, and 12.2% with neoplasms of upper gastrointestinal tracts, 13.8% neoplasms of colon, 12.2% neoplasms of biliary tract and in 8.1% with neoplasms of pancreas, the rest 6.5% included other clinical diagnosis. From peritoneal cavity *Candida* was raised in 48.2%, from-infected wounds in 17.9%, respiratory tract in 17.9%, urine in 5.4%, vascular catheters in 7.1% and blood in 3.6%. It had been called attention for the frequency of existence risk factors: broad spectrum antibiotics (100%), poor health state (66.7%), invasive monitoring (65.0%), neoplasm (46.3%), diabetes (20.4%), steroid medications (16.2%). More than three risk factors in all the analyzed group were in 76.4% (94/123) and in group with lethality in 91.0% (51/56) (n.s.). In all the groups the lethality has been stated as 45.5% and in group with positive blood cultures from 85.7%. In group with multiple positive cultures 35 patients (71.4%) died, and in group with single cultures – 21 patients (28.4%) ($P < 0{,}0001$). Clinical symptoms were generally unspecific. From all the serologic studies performed marking of anti-*Candida* antibodies by indirect immunofluorescency is worth attention (1 : 160), although serologic studies in systemic mycotic infections and their interpretation are difficult. On the basis of literature and our own studies we have accepted the rules of the antifungal systemic and early empirical systemic treatment offered by Anaissie and Solomkina.

Conclusions: Mycotic infection is related to considerable mortality, that rises in patients with co-morbidity and those subjected to intensive care. For taking rational measures, the awareness of the risk, adequate diagnostics and monitoring of the infections in the high-risk groups, are indispensable.

P1450 Molecular epidemiology of *Candida* blood isolates in a university hospital during a 6-year period

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Objective: *Candida* species currently are the second most common nosocomial pathogen in Marmara University Hospital, Istanbul with a significant increase in the rate of candidemia from 12 to 26% of all blood stream infections during the last 3 years. An epidemiological analysis of 148 *Candida* blood isolates between 1994 and 2000 was performed in order to check for clonality of the most profound *Candida* species.

Methods: *Candida* blood isolates were identified in the species level by standard methods. The DNA of *C. albicans* and *C. tropicalis* was digested with *Eco*R1, and that of *C. parapsilosis* with both *Eco*R1 and *Sal*I. The products were separated by electrophoresis in agarose gel and blotted onto a nylon membrane by standard methods. As the species specific probe, lambda-phage clones Ca3, Cp13–3, and Ct14 were used for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, respectively. The fingerprint patterns were analyzed using the Bionumerics software.

Results: *C. albicans*, *C. parapsilosis*, and *C. tropicalis* comprised 40, 31, and 12% of all isolates, respectively. Levels of clonality varied among three *Candida* spp. Of the 60 *C. albicans* isolates, there were six clear incidences of two or more different patients with isolates having identical fingerprints. Thirty of 42 *C. parapsilosis* isolates could be grouped into six clusters of strains that were indistinguishable, including 12 different isolates with the identical fingerprint in a cluster. Eleven of 17 *C. tropicalis* isolates clustered in groups of two or more identical strains.

Conclusion: In all, *Candida* species analyzed there was evidence of nasocomial transmission and this was at a higher level for *C. parapsilosis* and *C. tropicalis* than *C. albicans*.

P1451 Candidemia: an emerging nosocomial infection in the non-immunocompromised host

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Objectives: Nosocomial infections contribute significantly to increased morbidity, mortality, prolonged hospital stay and escalation of treatment

costs. The present study evaluated the prevalence, clinical profile and outcome of candidemia in immunocompetent hospitalized patients.
Methods: Non-neutropenic, immunocompetent hospitalized patients (with absolute neutrophil count >1500 cfu/mm) were screened for Candidemia in the presence of fever (>38.4 °C for >48 h) and/or hypotension and altered mental status. Blood cultures were drawn in brain–heart infusion and BACTEC media and were observed for the growth of *Candida* species. Additionally, sites suspected as serving as portal of entry like intravascular and urinary catheters, other indwelling lines and swabs were also cultured for *Candida*. All the patients found positive for candidemia received intravenous/oral fluconazole or itraconazole for a period till 14 days after resolution of signs and symptoms.
Results: Seven of the 91 patients screened over a 12-month period had candidemia incidence of 7.6%. Six (four female and two male) of these were evaluated in detail. All of them had prolonged fever (mean duration-32 days) which could directly be attributed to candidemia. Prolonged intravascular devices, urinary catheters, procedures like tracheotomy, parenteral antibiotics and bedsores were identified as the possible sources of entry for *Candida* in all the patients and *Candida* species were cultured from these in all the patients. Hospital stay was prolonged by a mean duration of 28.7 days in these patients. All the patients successfully cleared *Candida* from their blood on treatment with antifungal therapy. Although four of the six patients died, candidemia did not contribute directly to the death of any patient.
Conclusions: Candidemia is a remediable cause for prolonged hospital stay and morbidity in hospitalized immunocompetent patients and should be actively sought for and treated.

P1452 Randomly amplified polymorphic DNA in the genotyping of *Candida albicans*

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Objective: To genotype the *Candida albicans* isolates from blood and other body specimens by randomly amplified polymorphic DNA (RAPD).
Methodology: Three oligonucleotide primers were used in the RAPD analyzes. Initially, genomic DNA from six standard strains was used as template for the RAPD and the amplified products were resolved by agarose gel electrophoresis. After various parameters were optimized to obtain the maximum number of discreet bands with the standard strains, the method was applied to six *C. albicans* isolates obtained from blood cultures and other body sites of four patients in a hospital ICU and 11 *C. albicans* strains isolated from hands and mouths of nurses working in their vicinity. A total of 24 blood culture isolates obtained from various hospitals around Kuwait were also typed.
Results: The blood culture profiles of *C. albicans* isolated from two ICU patients differed from those of the nurses by at least one band. The genotypic patterns with primer AP3 obtained with isolates from one patient in the ICU matched exactly with a mouth isolate profile of a nurse. However, profiles of these isolates obtained with primer CARAPD1 were dissimilar. The 24 clinical *C. albicans* isolates gave a distinguishable pattern using the CARAPD1, AP3 and CT5 as primers, though CT5 was less discriminatory. Considering single-band differences to be significant, 22 patterns could be discerned from the 24 isolates tested. Interestingly, amplification patterns of the two isolates from the same individual were considerably different with both primers suggesting that at least two different strains were infecting at the same time.
Conclusions: This study demonstrates that RAPD is a simple and rapid technique for the typing of *C. albicans*, and thus has an application in the molecular epidemiology of nosocomial candidiasis.

P1453 Epidemiology of fungal organisms isolated and identified at a tertiary-care reference mycology center in northern Italy: a 12-month survey

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Objective: To perform a prospective surveillance study of fungal organisms cultured in a 1-year period from inpatients and outpatients referring to our university hospital.
Methods: Among 27 959 clinical specimens submitted for fungal search, 3781 (13.5%) proved positive. Samples bearing to a single patient (p) sent in the same day were regarded as 1 submission.
Results: *Candida* spp. largely prevailed over all other fungi: 3645 isolates of 3781 (96.4%). Although *C. albicans* still accounts for the great majority of cultured strains (2638 cases of 3645: 72.4%), the emerging of *C. glabrata* (286 episodes: 7.8%), *C. parapsilosis* (70), *C. kefir* (36), and *C. krusei* (21 cases) was notable, as well as the appreciable number of *C. lusitaniae*, *C. rugosa*, *C. guilliermondii*, *C. colliculosa* (5–13 isolates each), and *C. dubliniensis*, *C. incospicua*, *C. pelliculosa*, *C. famata*, *C. lipolytica*, *C. pseudotropicalis*, and *C. pulcherrima* (1–4 episodes each), while untypeable *Candida* spp. were cultured in the remaining 429 cases. Fungi other than *Candida* spp. included *Aspergillus* in 55 cases (1.5%) (*A. fumigatus* in 32 episodes, *A. flavus* in 15, and *A. niger* in 8), *Saccharomyces cerevisiae* in 53 cases, *Trichosporon* spp. in 10 p (*T. ashai* and *T. inkii* in 8 and 2 cases, respectively), and *Geotrichum* spp. in 10 p (*G. capitatum* in 6 cases, *G. candidum* in 1, and *Geotrichum* spp. in 3 cases). The isolation of *Cryptococcus neoformans* (4 cases), *Rhodotorula* spp. (3), and *Penicillium* spp. (1 episode), proved remarkably rare.
Conclusion: Although taking into account the difficulty to distinguish invasive infection from trivial colonization according to isolation only, and the significance attributable to the different isolation sites, underlying disease (if any), inpatient/outpatient origin, and concurrent antimicrobial administration, the large prevalence of *Candida* spp. over other fungal pathogens remains a major issue, with a rising frequency of some nonalbicans strains, which usually show unpredictable in vitro susceptibility to most imidazole antimycotic compounds, and a prominent association with concurrent illnesses, immunodeficiency, prolonged hospitalization, instrumentation, use of prosthetic devices, and parenteral nutrition. A quite stable number of *Aspergillus* spp. organisms is isolated predominantly from neutropenic hosts, while *Saccharomyces* spp. is an emerging colonizer and pathogen of the genital tract. The decline of *Cryptococcus* and *Penicillium* spp. is attributable to the sharp drop of frequency involving all AIDS-related opportunistic pathogens

P1454 Real-time detection of *Aspergillus* spp. using nucleic acid sequence-based amplification (NASBA) and molecular beacon detection

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Objectives: Invasive aspergillosis is a leading cause of morbidity and mortality in transplantation, cancer, surgical, and burn patients. The development of molecular based methods for both the diagnosis and preemptive monitoring of patients at risk for invasive aspergillosis will permit the early detection of fungal disease and the prompt initiation of antifungal therapy to improve survival.
Methods: Initial work by Loeffler et al. described a nucleic acid sequenced-based amplification (NASBA) method combined with electrochemiluminescence (ECL) end-point detection for the identification of Aspergillus sp. in whole blood samples. We have modified the assay for 'real-time' detection by replacing ECL detection with molecular beacon technology. Nucleic acids from fungal cultures were isolated using a freeze–thaw procedure followed by silica-based extraction. Nucleic acid extraction and NASBA amplification were performed by using NucliSens Basic Kit reagents (Organon Teknika/bioMérieux, Boxtel, The Netherlands). Isothermal (41 °C) amplification of a 18S ribosomal RNA region and molecular beacon fluoresence detection were performed simultaneously using a single-tube closed system. Continuous monitoring of emitted fluoresence was performed using a NucliSens EasyQ Analyzer.
Results: Specificity studies indicate the assay can efficiently detect all clinically relevant species of *Aspergillus*, with minimal cross reactivity with non-*Aspergillus* fungal isolates. *Aspergillus* sp. RNA can be detected as early as 30 min after the initiation of the amplification reaction. The sensitivity of the assay is in the range of 10–100 conidia per amplification reaction.
Conclusions: This assay has significant potential for the detection of invasive aspergillosis. Benefits of the assay over the original format include rapid and sensitive detection, significantly less technical time and a single-tube format that greatly reduces the risk of sample cross-contamination that can lead to false-positive results. Additional studies are being conducted to optimize assay conditions, restrict cross reactivity and enhance assay sensitivity.

P1455 Prospective comparison between the Nuclisens Basic Kit NASBA and a PCR–ELISA for the detection of *Aspergillus* spp

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Objectives: Invasive aspergillosis is a major cause of morbidity and mortality in immunosuppressed patients. Early diagnosis is mandatory for appropriate, successful therapy. In this study, a prospective comparison between a Nuclisens–nucleic acid sequence based amplification (NASBA)-based assay (detection of *Aspergillus*-RNA) and a previously published protocol for the amplification of fungal DNA by PCR–ELISA was performed. Blood samples from patients ($n = 21$) receiving an allogeneic stem cell transplantation (SCT) were analyzed twice weekly by both assays.
Methods: Serially diluted *Aspergillus fumigatus* conidia were used for sensitivity testing of the new molecular-based method. Primers and a specific probe were designed within the fungal 18S rRNA gene region. Blood specimens ($n = 245$) from SCT recipients were compared subsequently by PCR and NASBA assays. Isothermal amplification combined with electrochemiluminescence were performed by using NucliSens Basic Kit reagents (Organon Teknika, bioMérieux, Boxtel, the Netherlands) as described previously.
Results: The NASBA assay showed a detection limit of 1 cfu/mL blood (PCR: 10 cfu/mL). Thirteen patients were positive by both assays, 3/21 negative by both assays whereas 5/21 showed a positive result only by the NASBA test. Two patients suffered from documented invasive aspergillosis. They were both NASBA-positive over 3 weeks prior to radiological documentation of pulmonary infiltrates and remained positive for up to 31 days. In contrast, PCR remained positive only for 4 days. In patients with pulmonary infiltrates or amphotericin B therapy ($n = 10$), a mean number of 6.4 NASBA tests (range 1–13 tests) was positive, compared to 2.3 NASBA-positive tests (range 1–5 tests) in patients with febrile neutropenia only ($n = 6$).
Conclusion: The NASBA Basic Kit technology is a highly sensitive tool for the detection of *Aspergillus*-RNA in blood specimens of immunosuppressed patients.

P1456 Rapid identification of medically important yeasts by a multiplex PCR method

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Objectives: Yeasts are emerging as important etiological agents of nosocomial infections. The identification of yeasts by conventional morphological and metabolic characteristics may require from 2 to several days. The aim of this study was to evaluate a multiplex PCR method for the identification of *Cryptococcus neoformans* and *Candida* spp. that are frequently isolated from clinical specimens.
Methods: A total of 220 yeast strains were used in this study. These strains included the following species (strain number): *C. albicans* (94), *C. tropicalis* (38), *C. parapsilosis* (30), *C. glabrata* (28), *C. krusei* (9), and *Cryptococcus neoformans* (21). Nine primers were designed to amplify the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of the above yeast species that are commonly isolated in the hospital. The PCR products amplified from different species varied in size and could be effectively separated by disc-polyacrylamide gel electrophoresis. In addition, 49 strains of other yeast species, 22 strains of molds, and 13 strains of bacteria were also tested by the multiplex PCR method. The negative and positive controls were run in parallel with test samples.
Results: The precise lengths of the amplicons obtained from the six yeast species were as follows: *C. glabrata* (280 bp), *C. albicans* (254 bp), *C. parapsilosis* (189 bp), *Cryptococcus neoformans* (161 bp), *C. tropicalis* (127 bp), and *C. krusei* (117 bp). Of the 220 strains of yeasts tested by the multiplex PCR, all strains of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *Cryptococcus neoformans* were correctly identified. However seven out of the 30 strains of *C. parapsilosis* produced no PCR product and these strains were not identified. The overall sensitivity of the multiplex PCR method was 96.8% (213/220). All strains of other yeast species, molds, and bacteria produced negative results. The specificity of the method was 100% (84/84).
Conclusions: The multiplex PCR method had a high sensitivity (96.8%) and specificity (100%). The method could be completed within 8 h and is simpler than any previously reported molecular methods for the identification of yeasts.

P1457 Epidemiology of fungal urinary tract infections in a pediatric hospital in Greece during a five-year period

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Objectives: To evaluate the frequency, the causative agents and the main epidemiological characteristics of fungal urinary tract infections (UTIs) in children.
Methods: We retrospectively reviewed all cases of fungal UTIs during a 5-year period in a 500-bed pediatric hospital (1996–2000). The urine cultures were performed in the microbiology laboratory. Appropriate guidelines were followed for the specimen collection and transport. Isolation and identification of culture isolates were made by conventional methods and the minimum inhibitory concentrations of yeasts to antifungal agents (amphotericin B, fluconazole, itraconazole, ketoconazole and 5-fluorocytocine) was determined by the Alamar colorimetric method (Sensititre). The interpretation of results was based on (cfu/mL) counts of isolates, the presence of leukocytes in urine and additional clinical information.
Results: Thirty-two out of 3093 UTI episodes were caused by *Candida* species in 29 patients, aged 30 days to 14 years old (16 males, 13 females). All cases were considered as nosocomial infections. *Candida albicans* comprised 78% of fungal isolates, *C. tropicalis* 12.5%, *C. parapsilosis*, *C. glabrata* and *C. sake* 3.1% each. Most yeast isolates were found sensitive to all antifungal agents, while 100% were sensitive to amphotericin B (AB). All patients received antifungal therapy according to the results of the sensitivity testing (nine received AB; 12 fluconazole (FZ); five AB and FZ, two ketoconazole (KZ); one nystatin (NS); 1 NS and KZ; one FZ and 5-fluorocytocine; one FZ and KZ). The main predisposing factors for fungal UTIs were: antibiotic administration (100% of patients), invasive procedures (22/29, 76%), abnormalities of urinary tract (14/29, 48%), preceding surgery (8/29, 27.5%), malignancy (5/29, 17.2%) and renal failure (3/29, 10.3%). The highest incidence of candidal UTIs was noted in intensive care unit (ICU) (12/32, 37.5%) and in pediatric wards (11/32, 34.3%). The remaining cases occurred in surgical wards (4, 12.5%), in neonatal ward (2, 6.2%), in hemodialysis unit (2, 6.2%) and in oncology unit (1, 3.1%). Fungemia was diagnosed in six cases, all in patients of the ICU.
Conclusions: *Candida* species are important causative pathogens of nosocomial UTIs in children, frequently complicated by fungemia, especially when specific predisposing factors are present.

P1458 Fungi – isolated pathogens from hospitalized patients with diarrhea

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Objectives: Analysis of fungal isolations from the stool from the patients with diarrhea hospitalized on different hospital wards (1998–2000).
Methods: Fungal strains were cultured from the stool and swabs of the anus. The culture was done according to standard mycological procedures and commercially available tests (bioMerieux, Sanofi Diagnostics Pasteur). Susceptibility to antifungal agents was tested using Fungitest (Sanofi Diagnostics Pasteur).
Results: In a total of 299 specimens, among them 234 from the internal medicine wards and 65 from the surgical wards, were tested. From these specimens, 336 fungal strains were isolated, including 266 isolates from the internal medicine wards. The most common species were *C. albicans* – 164 strains (48.8%) and *C. glabrata* – 93 strains (27.7%). Fungi were found in specimens mainly from the hematological ward: *C. albicans* – 73 strains (21.7%) and *C. glabrata* – 59 strains (17.6%).
Conclusions:
1 Fungi were isolated from the patients with diarrhea more often from the internal medicine wards than from the surgical wards.
2 Diarrhea caused by fungi is observed mainly in hematological ward.

3 The most commonly isolated fungi from the patients with diarrhea are: *C. albicans* and *C. glabrata*.

P1459 Frequency of isolation of fungi from clinical specimens

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Objectives: To determine the spectrum of fungi in clinical specimens from patients in our hospital.
Methods: The study involved the clinical specimens recovered from patients hospitalized at the Public Hospital no. 1 in Gdansk from January 2001 to September 2001. A total of 1435 fungi isolates were recovered from respiratory secretions, blood, pus, wounds, urine, vaginal swabs, stool and other miscellaneous clinical specimens. The identification of fungi was done with the VITEK system (bioMerieux) YBC card and Chromagar ID (ABS Graso, Poland).
Results: We analyzed 32 014 clinical specimens recovered from Intensive Care Unit, Hematology Unit, Surgery, General Medicine and Pediatric Unit patients. We isolated 1435 fungal strains. 38.8% strains were isolated from respiratory secretions, 24.1% from urine, 13.2% from wound, 2.4% – blood, 16.6% – stool, 3.7% – peritoneal fluids, 0.6% – vaginal swabs, 0.6% – catheters. The frequency of isolation was as follow: *Candida albicans* 56.0%; *C. glabrata* 22.9%; *C. krusei* 4.2%, *C. tropicalis* 3.2%, *C. parapsilosis* 1.5%; Aspergillus spp. 1.8%; *C. lusitaniae* 0.6%; *Mucor* spp. 0.5%; and other *Candida* spp. 9.4%. The majority of isolates fungi were from: Intensive Care Unit (413) – 28.8%; Surgical (311) – 26.8%; Hematology Unit (241) – 16.8%, Pediatric (85) – 5.9%, and Internal Medicine (385) – 26.82%.
Conclusion: *Candida albicans* is the most common yeast in our patients. Molds were never so often isolated from clinical material.

P1460 *S. boulardii* fungemia in three intensive care unit patients not receiving *S. boulardii* therapy

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Objectives: *Saccharomyces boulardii* is a biotherapeutic agent widely used in the prevention of antibiotic-induced diarrhea in intensive care units (ICU). In humans, there are 17 cases of *S. boulardii* fungemia reported in the literature, almost invariably associated with oral administration of freeze-dried *S. boulardii*. The objective of the study is to describe the largest outbreak of *S. boulardii* fungemia in a single medical ward in patients that were not receiving *S. boulardii* (three immunocompetent ICU patients).
Methods: The three patients were on enteral nutrition, and had a central venous catheter (CVC). They had multiple blood cultures positive for *S. boulardii*; in one case, CVC tip was also cultivated and was positive. The two available strains and a strain from the commercial sample available in the ICU's pharmacy were identified as *S. boulardii* by biochemical tests and lack of spore formation. Mithocondrial DNA-restriction profiles analysis was carried out by pulsed field gel electrophoresis (PFGE).
Results: The fungal isolates were identified as *S. boulardii*. PFGE demonstrated that they were genotypically identical to *S. boulardii* obtained from the commercial sample stored in the ICU's pharmacy. The patient were cured with fluconazole therapy (200 mg bid) administered for 2–3 weeks, or by CVC removal.
Conclusions: CVC contamination was the probable source of fungemia for the three study patients. The inability of the routine identification methods to differentiate it from *Saccharomyces cerevisiae* has probably led to an underestimation of the real number of cases of fungemia in the literature. They can take place even in immunocompetent patients, and in patients not receiving *S. boulardii* therapy, by CVC contamination; it has been previously demonstrated that after opening a packet of freeze-dried *Saccharomyces boulardii*, aerosolized viable yeasts persist in the air and on room surfaces, and that they persist on undressed hands of operators even after vigorous hand washing. Colonization of CVC via aerosol dispersions must be prevented by preparing the biotherapeutic agent in a dedicated room separated from patients rooms. *S. boulardii* must be considered a potentially dangerous microorganism: two studies documented septic shock in the presence of positive blood cultures that yielded *S. boulardii* as the unique isolate. All cases described in the literature and our patients had a rapid response to therapy.

P1461 Infection due to *Cryptococcus neoformans* in Romanian adults

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Objectives: To analyze the clinical aspects, the treatment and the evolution of infections owing to *Cryptococcus neoformans* in Romanian HIV-infected adults.
Methods: The study involved the patients hospitalized in HIV Department of our hospital, between November 1999 and October 2001. Neurological changes were retrospectively analyzed. To establish the cause of the disease and the immunological status of the patients, clinical and laboratory investigations were performed. The classic India ink test from CSF smear, cultures from CSF and blood cultures were performed using BacT/Alert System, Auxacolor and ATB Expression for identification of yeast and fungi test or E-test to determinate the sensitivity of them.
Results: Among 318 hospitalized patients, 18 (5.66%) had cryptococcal meningoencephalitis. There were 7 males and 11 females, aged 16–46 years. Classic meningeal symptoms occurred in 8 patients. All patients were HIV +, class C (average value of CD4 = 68 lymphocytes/mm^3) at the moment of cryptococcosis. One patient had cryptococcal skin lesions and some of them had pulmonary symptoms before or after diagnosis of CNS disease. The India ink test from smears of CSF was positive in all patients, cultures from CSF were positive in 15 patients and five had fungemia. CT/MRI were performed in six patients (four patients with intracerebral cryptococcomas). Patients were treated with three different regimes: fluconazole, 10 patients; itraconazole, five patients (after the detection of fluconazole resistance); and three patients with combined treatment. Intermittent maintenance therapy was done in six patients. Six patients began an antiretroviral treatment before the onset of meningitis, three of them stopping it because of adverse events. Mortality was registered in 12 patients. The average time of survival was 5.6 months(range 4 days to 23 months).Two patients were lost from follow-up. Sixteen patients had simultaneous opportunistic infections (toxoplasmosis, septic shock, HIV encephalopathy and tuberculosis). Persistently elevated intracranial pressure (13 patients), relapses of the disease (nine patients with mean three episodes) and coma complicated the disease. Just one patient treated by fluconazole and receiving HAART for HIV infection is alive with negative CSF cultures at 13 months from the onset of the illness.
Conclusions: Because some of the prognostic factors in cryptococcal meningitis can be corrected, early diagnosis, early use of appropriate antifungal treatment, ARV treatment are important in management.

P1462 Disseminated cryptococcosis in an immunocompetent woman

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Introduction: Most patients with cryptococcosis have an underlying disease, though it does occur in patients who are not apparently compromised but much less frequently. We report a case of cryptococcosis in an immunocompetent patient which involved the lungs, CNS, eyes, skin and bones. A 53-year-old female patient was admitted to our hospital because of fever lasting for 45 days, and reaching 39 °C the last 10 days. The patient also reported nonproductive cough, a slight back pain and a mild headache. An early chest radiograph had revealed a well-defined, non-calcified, single lung nodule measuring 1 cm. Her medical history included scleroderma (CREST) for which she was taking ciapride and omeprazole. Upon admission, except for the skin signs of scleroderma, there were also diffuse rosy spots on the trunk. Nuchal rigidity and focal neurological symptoms were absent. Radiographic examination showed no change in the aforementioned lung nodule (chest radiograph and CT scan). A bone scan showed an osteolytic lesion of the right-arm. The eye examination revealed papilledema and a significant loss of visual acuity in the left-eye. An Indian ink preparation of spinal fluid showed yeast cells with capsules, characteristic of *Cryptococcus*. Antigen of *Cryptococcus neoformans* was detected both in serum and in spinal fluid. The patient with the diagnosis of cryptococcosis was administered liposomal amphotericin and flucytocine for 40 days. She was discharged in excellent condition, with restored visual acuity and with a significant reduction in the size of the lung nodule. She was subsequently given antifungal prophylaxis for 1 year. No cause of immunosuppression has been found, despite a thorough investigation.

Discussion: The described case is a disseminated cryptococcosis in an immunocompetent woman with clinical manifestations from central nervous system, lungs, skin, bones and eyes. Although our patient had a history of scleroderma neither the disease itself nor its treatment predispose to immunosuppression. The lung is the portal of entry and initial site of infection for *Cryptococcus neoformans*, which may subsequently disseminate to other sites, with predilection to the central nervous system. Although clinical manifestations tend to overlap between immunocompromised and immunocompetent hosts, a disseminated disease does not usually characterize cryptococcosis in immunocompetent hosts.

P1463 Rhinocerebral zygomycosis

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Introduction: Rhinocerebral zygomycosis is often found in patients with diabetes mellitus, particularly in the presence of acidosis. The most useful clue is the black necrotic eschar either in the nasopharynx or on the palate. This eschar or material sloughing from it may be mistaken for clotted blood. This is a case of rhinocerebral zygomycosis in a 39-year-old diabetic man, admitted to the emergency room after a car accident with hemopneumothorax and multiple rib fractures. Ten days later, he developed nonspecific neurological symptoms, mental status dysfunction, fever and epistaxis. Brain CT-scan revealed an infract on the right-bregmatic area and ultrasonography showed occlusion of the right-interior carotid. The para-nasal cavities appear normal. Over the following days, he developed an acute exophtalmus of the left-eye and full ophthalmoplegia in both eyes, which evolved into blindness. An angiography confirmed the carotid occlusion and a new CT-scan revealed opacities in all para-nasal cavities. At the time the patient was presenting bloody epistaxis and a black necrotic eschar had been formed on the palate. Nasal specimens and cerebrospinal fluid (CSF) were sent to the laboratory. Direct KOH wet preparation of the nasal material showed wide ribbon-like, hyaline, predominantly aseptate hyphae with wide-angle branching. Culture on common growth media allowed the growth of *Mucor* spp. The CSF count was 90 WBC/mm with lymphocyte predominance and the culture yielded *Mucor* spp. High-dose liposomal amphotericin-B was introduced. The patient died 20 days later.
Conclusions: Communication between physicians and the clinical microbiologists is essential. Specimens need to be accompanied by clinical details. Demonstration of large aseptate, twisted hyphae in the correct clinical setting constitutes strong evidence of zygomycosis. The rapid progression seen in the zygomycotic infection allows no delay in diagnostic efforts.

P1464 Dermatophytosis: a retrospective study

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Objectives: To determine the prevalence of dermatophytosis and the causative agents of this superficial infection in outpatients of our hospital, the last 10 years.
Patients and methods: During the period from 1991 to 2001 a total of 3269 clinically suspected cases of dermatomycoses were examined for causative fungi. Skin scrapings, hairs and nails were collected from relative anatomical sides with clinical signs of infection. After a direct microscopic examination the specimens, were cultured on a Sabouraud dextrose-agar plate with chloramphenicol for the isolation of yeasts and on a second plate with chloramphenicol and cycloheximide for the isolation of dermatophytes.
Results: According to this retrospective analysis, 821 patients had positive cultures for fungi. Among them 235 (29%) cultures were positive for yeasts and 586 (71%) cultures were positive for dermatophytes. *M. canis* was isolated in 340 (58%) cases, *T. rubrum* in 207 (35%) and *T. mentagrophytes* in 25 (4%). *E. floccosum*, *T. violaceum* and *T. tonsurans* were isolated from four cases each one. *M. gypseum* was isolated from two subjects.
Conclusions: Dermatophytosis (71%) was the most frequent superficial infection of dermatomycoses. *M. canis* (58%) and *T. rubrum* (35%) were the most prevalent species of dermatophytes which were isolated from cultures of outpatients with dermatophytosis.

P1465 Etiological agents of cutaneous mycoses in Bilbao (Spain) for 7 years

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Objectives: Knowledge of superficial mycoses epidemiology in our hospital. The hospital of Basurto is a primary and tertiary care, teaching hospital that provides care in the urban area of Bilbao (Spain).
Methods: We carried out a retrospective study of skin lesions suspicious of mycological etiology, received in our hospital between 1995 (September) and 2001 (November). Samples were observed for direct microscopy examination with 30% KOH and cultivated in Sabouraud + chloramphenicol/ciclohexamide agars for 15 days at room temperature. Moulds and dermatophytes identification was based on microscopic and macroscopic characteristics. For yeast identification, germ-tube test and Api C aux (bioMerieux) were performed.
Results: We received 2634 samples of 1827 patients, age range 20 months to 90 years old. A total of 1120 (42.52%) samples of 775 patients were scales of glabrous skin lesions, 1433 (54.40%) nails of 1010 patients and 81 (3.07%) hairs of 42 patients. We isolated 356 fungi:
1 58.14% dermatophytes,
2 36.51% yeast, and
3 5.33% *Scopularopsis* spp.
The most frequent isolated fungi was *T. rubrum* (31.61%), followed by *C. albicans* (16.01%), *M. canis* (14.60%), *C. parapsilopsis* (10.96%) and *T. mentagrophytes* (5.05%). *M. canis* was mainly isolated in patients less than 20 years old (65.38%) and *T. rubrum* between 21 and 60 years (73.39%). Yeasts were the most frequent isolation (70.54%) in patients older than 41 years. In-hand onychomycoses yeasts were the microorganisms more frequently isolated (98.04%), whereas in feet nails supposed 15.73% of isolations. In this sample, *T. rubrum* was predominant (44.94%) followed by *Scopulariopsis* spp. (21.34%) which was only isolated in this localization.
Conclusions: We observed:
1 a global predominance of dermatophytes;
2 anthropophilic dermatophytes incidence was higher than zoophilic;
3 usually in hand nails only yeasts were isolated;
4 in *Tinea capitis M. canis* was predominant;
5 increase in *T. rubrum* incidence;
6 not increase in African endemic dermatophytes despite immigration.

P1466 Clinical, mycological and epidemiological characteristics of toenail onychomycosis in Estonia

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Objectives: Studies during the recent years show an increase in the incidence of fungal nail disorders. The aim of our study was to evaluate the clinical features, predisposing factors and pathogens of toenail onychomycosis in Estonia.
Methods: Between January and May 2001, 436 patients (173 men, 263 women) with clinical signs of onychomycosis were interviewed and examined by dermatologist in all counties of Estonia. Specimens were collected by dermatologist for direct microscopy in 25% KOH with glycerol and cultures.
Results: Totally, 436 persons had 2344 infected toenails (mean number of affected toenails for one patient 5.4). At the time of investigation, 70% of patients had the signs of *Tinea pedis*. Higher age was risk factor for combined infection of skin and nails in both genders. Other foot diseases (psoriasis, eczema, viral warts, etc.) were diagnosed in 17% of patients. The coexistence of toe- and fingernail onychomycosis occurred in 14%, concomitant tinea manus was diagnosed in 6% and tinea cruris in 4% of patients. The factors associated with nail infections were prolonged antibiotic or immunosupressive therapy, diabetes, obesity, and impaired circulation. The most common subjective symptoms were embarrassment and discomfort of walking. The

diagnosis of fungal infection was confirmed by positive microscopy in 259 (59%) and by positive culture in 186 (43%). Of these positive samples, 23% cases were found to be positive by microscopy and 6% by culture only. Thirty-six percent specimens were positive by both methods. Eighty percent of isolates were dermatophytes. Yeasts were isolated in 11% (3% confirmed by distinctive microscopy) and non-dermatophytic moulds in 8% of the cases (2% confirmed by distinctive microscopy). *T. rubrum* was most common pathogen, followed by *T. mentagrophytes, T. interdigitale, Microsporum canis, T. violaceum* and *Epidermophyton floccosum. Candida albicans* and *C. parapsilosis* were agents of toenail candidosis.

Conclusions: Predisposing factors for onychomycosis are the diseases that impair the circulatory system, and concomitant treatment with antibiotics or immunosupressive drugs. The most frequently isolated pathogens were similar to the pattern in whole Europe. Our data show high prevalence of progressive onychomycosis and suggest the need for education of our patients.

Animal models and experimental treatment

P1467 The synergism between quinupristin–dalfopristin (Q–D) and β-lactams against methicillin-resistant *Staphylococcus aureus* (MRSA) requires blockage of bacterial penicillin-binding proteins (PBP)

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Background: Although not a cell wall inhibitor, subinhibitory concentrations of Q–D result in the production of a structurally and biochemically abnormal peptidoglycan in MRSA and enterococci. This might be the basis of the synergism between Q–D and several β-lactams that was previously demonstrated in vitro and in rats with experimental endocarditis (EE). However, the mechanism of this synergism is not known.

Methods: To evaluate the requirement for PBP blockage in this interaction we compared the effect of Q–D combined with either cefepime or aztreonam against a well characterized MLSB-resistant MRSA (strain P8). Cefepime blocks the normal staphylococcal PBPs, but not the low-affinity PBP2A of MRSA. Therefore, it is effective against methicillin-susceptible staphylococci but not MRSA. Aztreonam, on the other hand, blocks neither of the staphylococcal PBPs, and thus is ineffective against both methicillin-susceptible staphylococci and MRSA.

Results: In vitro, the minimal inhibitory concentrations in broth were 0.5 mg/L of Q–D, 32 mg/L of cefepime and >1000 mg/mL of aztreonam. On agar plates containing increasing drug concentrations, MRSA colonies grew on up to 500 mg/L of cefepime and >5000 mg/mL of aztreonam. In the presence of subinhibitory concentrations of Q–D (1/4 × MIC), these value decreased to 32 mg/L for cefepime, but were not affected for aztreonam. In vivo, rats with aortic EE were treated for 5 days (starting 12 h after inoculation) and received either a low dose of Q–D (7 mg/kg bid) alone or combined with human-like kinetics of 2 g of cefepime bid or 2 g of aztreonam qid. Controls were killed at the start of therapy. The results were as in the table (infected rats/total rats [median log cfu/g of vegetation]).

Control	Q–D bid	Cefepime bid	Q–D plus cefepime	Aztreonam qid	Q–D plus aztreonam
8/8 [7.9]	7/7 [9.1]	8/8 [9.0]	8/13 [3.6]*	10/10 [9.3]	10/10 [9.0]

*$P < 0.05$ compared to Q–D or cefepime used alone.

Conclusion: Aztreomam, which does not block *S. aureus* PBPs, did not synergize with Q–D. In contrast, cefepime that blocks the normal *S. aureus* PBPs interacted positively with Q–D. This suggests that some PBP blockage is required for synergy. Most β-lactams block the normal set of *S. aureus* PBPs. During such treatment MRSA are believed to use their low-affinity PBP2A to assemble the cell wall. Thus, the beneficial effect of Q–D on β-lactam-treated MRSA might result from some direct or indirect interference of Q–D with the function of PBP2A. The results provide a rationale for further studying the basis of such potentially useful interaction.

P1468 Failure of escalating doses of vancomycin (VAN) in the treatment of experimental endocarditis (EE) due to glycopeptide-intermediate *Staphylococcus aureus* (GISA)

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Background: It is generally agreed that the emergence of GISA is of clinical concern. Yet most isolates of GISA have minimal inhibitory concentrations (MIC) of VAN (8–16 mg/L) that can be achieved in the plasma during intravenous (i.v.) therapy. Therefore, it was proposed that VAN might still successfully treat GISA infections provided that appropriate amounts of the drug are delivered to the patient.

Methods and results: We tested this hypothesis in rats with aortic EE infected with either a Susceptible-susceptible methicillin-resistant *S. aureus* (MRSA 217; VAN MIC = 2 mg/L), or three consecutive clinical isolates of MRSA (MRSA P2, P3 and P3') with increasing levels of VAN resistance (MICs of 2, 8, and 16 mg/L, respectively). Although resistant to methicillin, all isolates had rather low MICs of amoxicillin/clavulanate (AC) (MIC < 16 mg/L), due to the intrinsic affinity of amoxicillin for penicillin-binding protein 2A (as previously demonstrated). Treatment started 12 h after inoculation and lasted for 5 days. Rats received human-like kinetics produced by either 1 g i.v. VAN/12 h (bid), or continuous VAN infusion producing constant plasma levels of 20 and 40 mg/L. Control treatment was A/C (2.2 g every 6 h). Results were as in the table (infected rats/total rats [median log cfu/g of vegetation]).

Isolates	Controls	VAN bid	VAN 20 (mg/L)	VAN 40 (mg/L)	AC
217 (VAN-S)	10/10 [7.5]	0/12 [2.0]	–	–	–
Pc-2 (pre-GISA)	6/6 [7.6]	8/8 [9.2]+	2/9 [2.0]+	–	4/13 [2.0]
PC-3 (GISA)	7/7 [6.82]	4/4 [9.0]+	–	7/9 [9.0]+	–
PC-3* (GISA)	10/10 [5.8]	7/8 [8.1]+	–	–	1/13 [2.0]

+Groups in which isolates with a further increase in Vanco MICs were selected.

Conclusion: VAN bid was only active against MRSA 217. Constant levels VAN were active against the pre-GISA PC-2, but failed against the most resistant GISA isolates. Moreover, VAN selected for variants with increased resistance (VAN MICs up to 16 mg/L) in all isolates of the PC series. In contrast, AC therapy was effective. These results contradict the optimistic view that high-dose VAN treatment may still be effective against severe GISA infection and highlights the need for developing alternative therapies.

P1469 Risk of pneumococcal resistance induction by short-term ciprofloxacin (CIP) or moxifloxacin (MXF) treatment in a bacteremic mouse model of pneumococcal pneumonia

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Objectives: MXF is a new quinolone with good activity in vitro and in vivo. Older quinolones such as CIP are well known to generate bacterial resistance, particularly when given in low doses. This capacity to induce bacterial resistance is a major impediment to use of quinolones as single-drug therapy. The aim of this study was to compare the in vivo ability of CIP and MXF to increase the minimal inhibitory concentration (MIC) against *Pneumococcus* in an experimental pneumonia model.
Methods: Female Swiss mice were infected with 1 000 000 cfu of a virulent *Streptococcus pneumoniae* (SP) strain (4241) by the intratracheal per oral route after general anesthesia. A 50-mg/kg dose of either antibiotic was given 18 h post-infection, followed 24 h later by a second dose. Controls were infected but not treated. After 48 h, the last antibiotic dose, lung cultures were done on blood agar containing the administered antibiotic in concentrations of 1/2 MIC, MIC, 2MIC, or MIC. The cultures were read after 24 and 48 h incubation in CO_2 at 37 °C.
Results: SP MIC before treatment was 1 mg/L for CIP and 0.25 mg/L for MXF. All the controls died, with no change in MICs of lung SPs (no growth at the MIC). In the CIP group, a mean of 25 cfu were found in the 1 mg/L agar, while cultures in the free CIP agar showed a mean of 100 000 cfu/lung, for the same mice. In contrast, with MXF, no growth occurred in the 0.25 mg/L agar. These results were confirmed by two successive experiments.
Conclusion: In this model of pneumococcal pneumonia, after only two doses, CIP selected resistant mutants, as shown by bacterial growth in agar containing CIP at the MIC. In contrast, MXF did not change the MIC of *Pneumococcus*. These results require confirmation in humans.

P1470 Efficacy of levofloxacin (LEVO) in a murine pneumonia model by *H. influenzae* in comparison with moxifloxacin (MOXI) and azithromycin (AZI)

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Background: *H. influenzae* is an important pathogen of upper and lower respiratory tract infections (RTI), including acute otitis media, sinusitis, bronchitis and pneumonia. Levofloxacin and moxifloxacin are two fluoroquinolones with good activity against RTI pathogens, including *Haemophilus* spp. Azithromycin is a macrolide antibiotic extensively used to treat community RTI.
Objectives: The aim of this study was to evaluate LEVO activity in an experimental pneumonia model of *H. influenzae* and to compare it with that of two other antibiotics used to treat RTI. Dosing regimens were chosen taking into account the PK/PD values linked to clinical efficacy in the literature (AUC/MIC > 100 for fluoroquinolones and AUC/MIC about 30 for AZT).
Methods: Bacterial inocula were prepared by harvesting *H. influenzae* ATCC10211 from chocolate agar plates and suspending it in saline solution. Ten-fold dilutions were prepared into cooled molten nutrient agar. Mice (C57BL/6 J, Harlan) were anaesthetized injecting i.p. 0.2 mL of a ketamine–xylazine solution and infected by intratracheal instillation of 50 µL of bacterial suspension (approximately 106 cfu/mouse). A single dose of antibiotics was administered orally 4 h post-infection. After 24 h, the start of therapy, lungs were removed and homogenized to determine viable bacterial numbers.
Results: MIC values were: 0.03 µg/mL for LEVO, 0.06 µg/mL for MOXI and 2 µg/mL for AZT. All animals developed a pulmonary infection 4 h after inoculation (about 5 × 106 cfu/lung). Following administration of LEVO (5, 0.5 mg/kg), MOXI (10, 1 mg/kg) and AZT (100, 200 mg/kg) bacterial counts were reduced to the limit of detection at the highest doses and remained close to start of therapy at the lowest ones. These results showed that LEVO and MOXI were effective in eradicating *H. influenzae* at doses corresponding to AUC/MIC values far lower than predicted while AZT behaved as expected.
Conclusion: A mouse model of pneumonia caused by *H. influenzae* was developed and validated by testing three antibiotics already on the market to treat RTI. In this experimental model LEVO showed to be more effective than predicted from PK/PD parameters in treating a pulmonary infection caused by *H. influenzae* pathogen.

P1471 Therapeutic effect of antibiotics within oxidative stress in acute septic peritonitis – a new rat model

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Objectives: Most animal models of septic peritonitis use cecal ligation and puncture or intraperitoneal injection of fecal inocolum. This may cause problems due to the inability to quantitate loading doses and a random combination of bacterial species involved. The purpose of our study was to establish a new animal model reflecting the situation in human septic peritonitis, which would combine systemic inflammatory response with chronic intra-abdominal infection and abscess-formation.
Methods: *E. coli*, *Bacteriodes fragilis* and *B. thetaiotaomicron* were cultivated in BHI. A dose-dependent mortality of 50% was found at injection of 5 × 108 cfu/kg BW. We investigated the models response to one β-lactam (piperacillin–tazobactam) and two quinolones (levofloxacin, moxifloxacin). The release of free oxygen radicals by macrophages is described as a pathogenic factor in lethal septic peritonitis. To simulate this oxidative stress (OS), ozonized oxygen was injected intraperitoneal. Ozone and antibiotic were given 1 h after bacterial inoculation alone and in combination. Furthermore, immuno-histochemical analyzes of pro-inflammatory cytokines (TNFα mRNA, IL-1β mRNA) were investigated by in situ hybridization of the rats spleen.
Results: We could show a significant higher mortality in animals exposed to OS. All antibiotics had a beneficial effect on mortality. The combination of antibiotics and OS led to a higher mortality in two cases. Only moxifloxacin did not reduce its beneficial effect on mortality in combination with OS. in situ hybridization showed a high expression of TNFα mRNA and IL-1β mRNA in the spleens of the control groups. The expression of the cytokines was even higher in the β-lactam group and significant lower in the quinolone group.
Discussion: Our standardized animal model is suitable to investigate the pharmacologic and pathophysiologic interrelations in human lethal septic peritonitis. The mortality rate was increased with piperacillin–tazobactam under OS, maybe due to LPS-liberation by destruction of bacterial cell walls. Probably because of their mode of action and a therefor lower and delayed LPS-release the quinolones were more effective in this animal model. As expected, OS raised mortality in the levofloxacin group. A higher release of proinflammatory cytokines could be the reason. The effect of moxifloxacin was not modified by the presence of ozonized oxygen in the peritoneum.

P1472 The effects of *Saccromyces boulardi* on bacterial translocation in rats with obstructive jaundice

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Background: The developing of bacterial translocation following obstructive jaundice (OJ) is an important cause of sepsis. Bacterial location has been consistently demonstrated in experimental models of OJ.
Objectives: The aim of this study was to investigate effect of *Saccromyces boulardi* treatment on preventing bacterial translocation in an OJ animal model.
Method: Sixty Sprague–Dawley adult rats were included in the study by 5 groups. Group 1 was the control group. In Group 2, 3, 4 and 5 common bile duct ligation was performed and either saline, ampicillin–sulbaktam (50 mg/kg/day), *S. boulardi* (100 mg/day) or ampicillin–sulbaktam plus *S. boulardi* via enteral catheter were given, respectively, for a 7 days of period. Biochemical and microbiological analyzes were performed from blood, mesenteric lymph nodes, liver and spleen samples. Histopathological examinations of terminal ileum specimen were also performed.
Results: The bacterial translocation rates were 0% in group I, 83% in-group 2, 42% in-group 3, 42% in group 4 and 33% in group 5. Bacterial translocation rate was significantly higher in-group 2 than groups 3, 4 and 5 ($P < 0.05$).

There was not a significant difference among groups 3, 4 and 5 for bacterial translocation rates. Serum bilirubin level, AP, ALT, and AST were significantly elevated in groups 2, 3, 4 and 5 compared to group 1 ($P < 0.001$), however, there was no significant difference among groups 2, 3, 4 and 5 ($P > 0.05$). Histopathological examination of ileum specimens revealed a significant decrease in the height of villi in group 3 when compared with the groups 4 and 5 ($P = 0.001$).
Conclusion: *S. boulardi* was effective in the successful control of translocation and improvement of intestinal barrier function.

P1473 Lipid peroxidation by multidrug-resistant *Pseudomonas aeruginosa* in the pathogenesis of nosocomial sepsis

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Objective: Concentrations of polyunsaturated fatty acids (PUFAs) are increased in serum during acute inflammation. Elevated levels of malonodialdehyde (MDA) as a result of the peroxidation of PUFAs have been found in the blood of patients with sepsis (Goode et al. Crit Care Med 1995; 23:646). In order to investigate whether peroxidation may be triggered by multidrug-resistant *P. aeruginosa* (MDPA), one isolate was ex vivo exposed to sera derived from animals intravenously administered γ-linolenic acid (GLA).
Methods: An emulsion of GLA was administered iv at a dose of 25 mg/kg over 10 min by the left jugular vein of seven rabbits and blood was sampled at 15-min intervals by catheters inserted in the left carotid and in the hepatic veins. Blood was centrifuged and 1 mL of serum was added in 9 mL of Mueller broth containing a six log-phase inoculum of one MDPA blood isolate. The applied isolate was resistant to ceftazidime, imipenem, amikacin and ciprofloxacin. During 3, 5 and 24 h of incubation at 37 °C aliquots were sampled for estimation of viable cell counts and for treatment with trichloroacetic acid. Sera were then centrifuged and MDA was determined by the thiobarbiturate assay. Levels of acids in blood samples were measured by GC–MS.
Results: Added sera did not have any effect on MDPA growth and mean viable counts were 6.4, 8.0 and 9.0 log of cfu/mL after 3, 5 and 24 h of incubation, respectively. Mean MDA after 3 h of growth in the presence of sera sampled from the hepatic veins before infusion of GLA and 15, 30, 45 and 60 min after infusion of GLA were 604, 564, 1450, 465 and 870 mM, respectively, and in the presence of sera sampled from the carotid artery 590, 887.5, 935, 1256.7 and 2305.7 mM, respectively. These concentrations decreased considerably at 5 and 24 h of growth. In blood samples, GLA was found metabolized to arachidonic acid at concentrations ranging between 18.6 and 30.6 mM which are within those detected in sepsis (Falconer et al. Br J Cancer 1994; 69: 826).
Conclusions: Lipid peroxidation of sera supplemented with PUFAs occurs ex vivo over the first hours of rapid growth of MDPA, so as to implicate lipid peroxidation triggered by MDPA as a probable pathogenetic mechanism of nosocomial sepsis.

P1474 Incidence of renal infarcts in *Pseudomonas* experimental endocarditis in rabbits

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Introduction: Kidneys are usually affected during the course of infective endocarditis. Glomerulonephritis, infarcts and abscesses are the most usual types of pathologic processes, but clinical or biochemical evidence of renal involvement may be lacking in spite of the presence of such a pathology. Autopsy studies demonstrated infarctions in about half of the cases.
Objectives: The present study was conducted in order to evaluate the frequency of renal infarcts during the course of experimental *Pseudomonas aeruginosa* (PA) endocarditis in a rabbit model.
Materials and methods: Forty four surviving rabbits with left sided PA endocarditis were sacrificed on day 8 (10 controls, 14 receiving ceftazidime plus tobramycin (TOB), 10 ciprofloxacin, 11 imipenem plus TOB). The kidneys were excised and sent to the pathology laboratory. The specimens were fixed using buffered formalin. Hematoxylin and eosin sections were examined under light microscopy. Special histochemical stains such as d-PAS and Gram were performed in selected cases.
Results: 39/45 (86.6%) of cases showed single or multiple unilateral or bilateral wedge-shaped white (anemic) septic infarcts with the apex of the wedge pointing towards the focus of vascular occlusion. The infarcts were visible macroscopically as sharply demarcated pale, yellow-white areas with the base against the cortical surface and the apex pointing towards the medulla. Microscopically they were accompanied by calcification of the surrounding cells.
Conclusions: Renal infarcts are almost a constant feature of PA experimental endocarditis in rabbits. This surprisingly high tendency for embolization of a distant organ emphasizes the need for early initiation of antibiotic therapy and early valve replacement when it is indicated.

P1475 Protective effect of lactobacilli in experimental model of salmonellosis

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Introduction: Several studies have shown immunomodulative effect of lactobacilli in infections and their antagonistic activity against pathogens. Still there are few data about protective effect of lactobacilli against oxidative stress in case of salmonellosis.
Objectives: The aim was to determine microecologically, morphologically and by means of oxidative stress parameters the influence of several strains of lactobacilli on experimental salmonellosis.
Methods: NIH mice ($n = 47$) used in this study were divided into four groups: (1) *S. typhimurium*-challenged mice ($n = 16$), (2) lactobacilli (*L. acidophilus* and *L. fermentum*) administered mice ($n = 10$), (3) salmonella-challenged mice pretreated with lactobacilli ($n = 14$) and (4) control group ($n = 7$). After sacrification the counts of salmonella were estimated in intestine and blood, biochemical and pathohistological changes (scores: 0 = no changes to 5 = severe changes) were evaluated in distal part of ileum.
Results: Mortality rate and the intestinal and blood salmonella counts didn't differ in groups 1 and 3. Pathohistologically in group 3 intestinal lymph node hyperplasia was less expressed than in group 1 (medians 1 vs. 1.5, $P < 0.01$). *Suppurative lymphadenitis* was found in group 1 animals but not in group 3 ones (medians 1.1 vs. 0, $P < 0.01$). Oxidative stress parameters were more expressed in group 1 than in 3: concentration of intestinal lipid peroxides (LPO) was higher in group 1 (mean 297 pmol/mg prot) comparing with group 3 (224, $P < 0.01$), also the free iron and saturation of iron-binded proteins were higher in salmonella group (medians 22.5 μM and 31.5%) vs. group 3 (13 and 22%).
Conclusion: Although administering of lactobacilli does not reduce salmonella concentration in intestine nor decrease mortality rate, it still express protective effect against mucosal inflammatory injury and oxidative stress.

P1476 Kinetics of ceftazidime and pro-inflammatory cytokines in neutropenic mice during *E. coli* sepsis

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Objectives: During sepsis cytokines are produced by the host, in response to endotoxin (LPS). LPS is liberated from bacterial cell-walls during lysis. Ceftazidime (Cz) is a β-lactam-antibiotic with a dose-dependent pattern of binding to penicillin-binding-proteins (PBPs). At low dose-levels PBP-3 binding occurs, leading to filament-formation. During filament-formation large amounts of LPS are incorporated in the cell-wall. At higher concentrations Cz binds to PBP-1, leading to a quick bacterial lysis. We compared the effects of dosing-schedules of Cz on morphology, growth and the pro-inflammatory response.

Methods: We studied kinetics of Interleukin-6, TNF-α and Cz in neutropenic Swiss mice during sepsis, treated with Cz ($N=18$) or saline ($N=10$), during the first 4 h of treatment. On $t=0$ mice were challenged with 107 cfu *E. coli* ATCC 25922 in thigh muscles. After 2 h, they were treated with 2 mg Cz (80 mg/kg), intravenously, either as bolus-injection or continuous infusion. On $t=3$, 4, 5 and 6 h mice were sacrificed for collection of plasma-samples, bacterial counts and morphological studies. Kinetics of IL-6, TNF and Cz were also measured in mice without infection ($N=10$).
Results: During continuous infusion only rods were seen, whereas after bolus-injections massive filament-formation occurred. In both groups, a significant reduction of cfus in thigh muscle was measured compared to controls ($P=0.006$). In continuously treated mice a peak-level of TNF was reached 1 h after start of the infusion, significantly higher than bolus-treated animals ($P=0.018$) and controls ($P=0.004$). The bolus-group also produced significant higher TNF, compared to controls ($P=0019$). IL-6 showed a fast decrease in untreated mice from 2916 to 336 pg/mL, whereas in continuous-treated mice continuous high levels were measured (2026–1717 pg/mL). After bolus-injections a decrease occurs, comparable to controls. In septic mice plasma-Cz-levels were lower and clearance was slower, compared to controls. In mice without infection IL-6 and TNF were below detection-limits at all time-points.
Conclusions: During continuous infusion of 80 mg/kg Cz filament-formation is prevented. This leads to bacterial lysis and LPS-production, which is reflected as high cytokine-responses during the first hours of treatment. After bolus-injections filament-formation occurs. This results in the risk of a postponed, but much higher LPS-release and therefore should be prevented.

P1477 Penetration of moxifloxacin (MXF) into the vitreous humor in an endophthalmitis rabbit model

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Objective: Bacterial endophthalmitis is a serious complication of ocular surgery and of penetrating eye trauma. Leading causative organisms are Gram-positive pathogens, i.e. *Staphylococcus aureus* and *Staphylococcus epidermidis*. Treatment remains difficult because most antibiotics show poor ocular penetration when administered by systemic route. MXF is a new fluoroquinolone, with enhanced activity against Gram-positive organisms including *Staphylococcus* spp.
Methods: The penetration of MXF into the vitreous humor (vit) of normal and 24-h *S. aureus* infected eyes of New Zealand albino rabbits was evaluated, following an IV administration of two doses (5, 20 mg/kg). MXF MIC and MBC for the *S. aureus* V8 strain used were 0.125 and 0.250 μg/mL, respectively.
Results: A good intravitreal penetration of MXF was observed whatever the doses in infected and uninfected eyes. For the 5-mg/kg dose, the mean vit AUC 0.5–5 h (area under curve) was 1.73 ± 0.37 μg h/mL and the mean ser AUC 0.5–5 h was 5.40 ± 3.26 μg h/mL in uninfected rabbits ($n=5$). At the same dose in infected rabbits ($n=6$), the vit AUC 0.5–5 h was 2.38 ± 0.74 μg h/mL and the ser AUC 0.5–5 h was 8.55 ± 3.72 μg h/mL. The penetration ratios determined by vit AUC 0.5–5 h/ser AUC 0.5–5 h were 0.32 and 0.28 for uninfected and infected rabbits, respectively (no significant difference). For the 20-mg/kg dose, in uninfected rabbits ($n=5$), the mean vit concentrations (0.5 h = 1.56 ± 0.39, 1 h = 1.67 ± 0.17, 2 h = 1.66 ± 0.33, 3 h = 1.48 ± 0.22, 5 h = 1.11 ± 0.23) resulted in a mean vit AUC 0.5–5 h of 6.64 ± 0.76 μg h/mL, the mean ser AUC 0.5–5 h was 19.43 ± 11.16 μg h/mL. The mean vit AUC 0.5–5 h increased to 9.12 ± 1.81 μg h/mL (concentrations: 1.75 ± 0.52, 2.03 ± 0.64, 2.50 ± 0.67, 2.02 ± 0.44, 1.54 ± 0.35) in infected rabbits ($n=8$) while the mean ser AUC 0.5–5 h was unchanged. The penetration ratio was then 0.34 for uninfected rabbits and was significantly improved for infected rabbits ($P<0.02$), reaching 0.47. The ser AUIC was 152.48 μg/mL/h for the 20-mg/kg dose and 63.92 μg/mL/h for the 5-mg/kg dose. The vit AUIC of 68.48 μg/mL/h for the 20-mg/kg dose was associated with a 3.5-log decrease of viable bacteria in the vitreous. The vit AUIC of 14.56 μg/mL/h for 5-mg/kg dose was associated with a 1.6-log decrease. The vit IQ_{max} (C_{max}/MIC) were 20 and 5.44, respectively.
Conclusions: The penetration of MXF in the vitreous of rabbits is high, and MXF concentrations are higher than the MICs against major pathogens, including meti-S *S. aureus* strains.

P1478 Experimental model of *Staphylococcus aureus* conjunctivitis in the rabbit: efficacy of netilmicin solution and gel

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Objective: To set up a reproducible rabbit model of *Staphylococcus aureus* conjunctivitis and evaluate the efficacy of Netilmicin in two ophthalmic formulations.
Methods: Animal model: An abrasion along the inner surface of the lower lid and a radial 4 mm incision near the medial canthus was effected on both conjunctivae of 15 white New Zealand rabbits. Two 50 μL drops of a 1×10^8/mL suspension of an ocular isolate of *S. aureus* were administered in the cul-de-sac of both eyes at 2 h intervals for a total of three applications. Clinical evaluation of the infected eye was performed with a slit lamp, and clinical signs were monitored daily for 5 days. Groups of three rabbits were sacrificed with Tanax, at 24–48–72–96–120 h after injury and infection, and their conjunctivae removed to determine the bacterial load. Efficacy test: The experimental model described above was used to test the efficacy of a new gel formulation containing xanthan gum, sodium hyaluronate and Netilmicin sulphate versus Netilmicin sulphate eye drops. Both formulations contained the equivalent of 0.3% Netilmicin base. At 24 h after the infection 30 rabbits with evident clinical signs were randomly distributed in two groups and treated. The right eye of each animal was treated three times a day for 2 days with 50 μL of Netilmicin eye drops (group 1) or Netilmicin gel (group 2). The left eyes were treated with vehicles.
Results: At 24 h from the injury *S. aureus* caused in the rabbit a conjunctivitis that persisted for the 5-day period of ocular observation. Hyperemia and purulent discharge were the most pronounced scored signs. Analysis of the bacterial burden at 24, 48, 72 and 120 h yielded a mean ± standard deviation of 4.9 ± 0.089; 3.6 ± 0.504; 3.2 ± 0.103; 4.2 ± 0.971 log colony forming units/g of tissue, respectively. Statistical analysis (*t*-test) of the treatment groups confirmed that the two pharmaceutical formulations, administered three times a day for 2 days, were equivalent, both producing a 98% reduction of the bacterial load.
Conclusion: We have established a rabbit model of *S. aureus* conjunctivitis with persistence of the infection and relative clinical signs over a 5-day period. This animal model was useful to test topical ophthalmic formulations against the most common pathogen of the eye surface. The highly significant *t*-values found for both treated groups confirm that the antibiotic is equally active in both formulations.

P1479 Experimental group B streptococcal arthritis: role of interleukin-10

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Objective: Septic arthritis is one of the clinical manifestations of group B *Streptococcus* (GBS) infection in neonates and is often associated with age and serious underlying diseases in adults. Intravenous inoculation of CD1 mice with type IV GBS results in high incidence of diffuse septic arthritis, associated with high systemic and local production of IL-1β and IL-6. Aim of this study was to assess the role of the anti-inflammatory cytokine IL-10 in the evolution of GBS systemic infection and arthritis.
Methods: CD1 mice were inoculated intravenously with arthritogenic strain 1/82 of type IV GBS. Mice were administered intraperitoneally with anti-IL-10 monoclonal antibodies (1 mg/mouse) at the time of infection, or with exogenous murine IL-10 (400, 200 or 100 ng/mouse) for 5 days, starting 1 h after bacterial challenge. Mice were monitored daily for survival and arthritis. In a subsequent set of experiments, mice were killed at selected times for examination of cytokine production, bacterial clearance and joint histopathology.

Results: Detectable levels of IL-10 were evident in sera and joints of GBS-infected mice as early as 4 h after challenge, and a peak value was reached on day 4, followed by a progressive decrease in the subsequent days. Neutralization of endogenous IL-10 by administration of anti-IL-10 antibodies (1 mg/mouse) at the time of infection resulted in worsening of articular lesions and 60% mortality, associated with early sustained production of IL-6, IL-1β and TNF-α. Treatment with exogenous IL-10 had a beneficial effect on GBS arthritis, with a clear-cut dose dependence. The decrease in pathology was associated with a significant reduction in IL-6, IL-1β and TNF-α production. Histological findings showed a limited periarticular inflammation and a few cell influx in the articular cavity of IL-10-treated mice, confirming clinical observations.
Conclusion: This study provide the first evidence of a regulatory role for endogenous IL-10 in GBS disease and calls attention to the potential therapeutic use of this cytokine in GBS arthritis The beneficial effect of exogenous IL-10 on GBS arthritis appear to be associated not only with its ability to suppress cytokine production, but also with its effect on leukocyte recruitment at joint level.

P1480 Efficacy of moxifloxacin and cotrimoxazole in the experimental pneumonia caused by *Stenotrophomonas maltophilia*

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Objectives: To compare the activity of moxifloxacin (MX) and cotrimoxazole (TS) in monotherapy and in combination in an experimental pneumonia murine model caused by *S. maltophilia*.

Materials and methods: In vitro studies: nine different isolates from bacteremias were used. The following parameters were determined: MICs, MBCs, C_{max} in serum, bactericidal activities and synergy (checkerboard, time-kill curves). In vivo studies: two strains (A and B) were used; strain A was susceptible to both antimicrobials, and strain B was susceptible to TS and intermediate to MX. An experimental pneumonia model in mice with an intratracheal inoculum of 50 µL was developed. Two types of experiments were carried out: mortality study (treatment during 72 h and evaluation of the survival) and study of bacterial clearance from lungs (treatment each 2 h during 12 h and sequential sacrifice of three mice each 2 h). In the mortality study, the animals were grouped in CON (controls, no treatment), MX (40 mg/kg/day), TS (40 mg/kg/day), and MX + TS. In the clearance study, the groups were: CON, MX (20 mg/kg/2 h), TS (10 mg/kg/2 h), and MX + TS. Variables: mortality and bacterial clearance from lungs. Statistical analysis: Fisher, χ^2, ANOVA, and post hoc Tukey and Dunnett tests.
Results: In vitro: eight out of nine strains were susceptible to TS (NCCLS 2000), with a MBC range of 3.2/64–6.4/128; for MX MIC_{90} was 16 mg/L (range 1–16), with a MBC range of 16–64. In serum mice C_{max} of MX and TS were 6.84 and 3.27(62.1) mg/L, respectively. For both strains, only MX showed bactericidal activity and, besides, synergy was found using MX + TS combination to concentrations equal to C_{max}. In vivo: mortality studies: only the combined therapy decreased the mortality respect to CON (strain A: 13.3% vs. 86.6% and strain B: 0% vs. 93.3%, $P = 0.000$), being better than monotherapies ($P < 0.01$); bacterial clearance: as in mortality study, the combination was the only treatment that cleared the lungs respect to CON (strain A: 4.98 vs. 8.44 log cfu/mL, $P = 0.037$, and strain B: 6.16 vs. 8.37 log cfu/mL, $P = 0.008$). Comparing treatment groups, MX was better than TS in monotherapy for strain A ($P = 0.000$), and combination was better than monotherapies for both strains ($P < 0.01$).
Conclusions: MX shows bactericidal in vitro activity against *S. maltophilia*. The association of MX to TS improved the in vivo survival and the bacterial clearance from tissues in a model of severe experimental murine pneumonia caused by *S. maltophilia*.

Mycobacteria: diagnostic methods

P1481 Detection of *Mycobacterium* using a laser scanning cytometer

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Introduction: In the last decade, the reemergence of tuberculosis has emphasized the importance and the need of implementing and maintaining effective public health approaches to prevent transmission of the disease. A quick diagnosis is essential for an effective treatment and reduction of transmission. Direct detection methods, although very quick, suffer from the drawback of low sensitivity and of being too time consuming. Cultivation still remains a delayed method for diagnosis. The laser scanning cytometer (LSC) (Compucyte Corporation) is a new technology, which to our knowledge was hereby used for the first time in microbiology. LSC is a new apparatus similar to flow cytometer but with considerable advantages regarding clinical and research applications.
Objective: To develop a method of detection of *Mycobacterium* using acridine-orange and an LSC.
Material and methods: Five hundred smears from clinical samples (198 positive and 302 negative), were stained in parallel by Kinyoun acid-fast stain (the method routinely used in our laboratory) and by acridine-orange fluorescent stain. Smears stained by Kinyoun were analyzed by light microscopy, as usual, and smears stained by acridine-orange were maintained in the dark until cytometric analysis, according an optimization protocol. Discrepant results were re-tested and if disagreement still persisted, data were compared with the results of the culture.
Results: Smears Kinyoun positive were all positive on LSC, even those showing scarse bacilli. Fifty smears Kinyoun negative (4.96%) were positive by LSC but only 11 became positive on culture. Only four (1.32%) were considered false positive.
Conclusion: Observation of Kinyoun stained smears is time consuming, needs considerable observer experience and shows low sensitivity, although with a high specificity. LSC analysis is automated, allowing the observation of all the cells present in the smear, in real time or later on, due to the available software. Fluorescent staining proved to be more sensitive than Kinyoun staining. We believe that the detection of *Mycobacterium* by LSC is an important new methodology that needs further improvement.

P1482 A new serological test for tuberculosis diagnosis

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Objective: We have evaluated a new serological test for tuberculosis (TB) diagnosis. It is an enzyme linked immunosorbent assay (ELISA) which detects the presence of TB-specific immunoglobulin G (IgG) antibodies. It uses a mixture of protein- and glycoprotein antigens obtained from culture supernatant of *Mycobacterium tuberculosis*. We have evaluated if serological detection of TB is beneficial in case of sputum-negative TB patients, e.g. extrapulmonary TB and children TB.
Methods and results: The study was performed using 1332 sera from which 489 were found positive for TB by culture. The TB-positive sera were obtained from the World Health Organization of which 39% ($n = 190$) were tested positive for HIV. All negative sera were from Dutch origin and included 554 healthy blood donor sera and 279 sera from patients found positive for other infectious diseases than TB (sick, non-TB). Using a cut-off set at specificity of 95%, the sensitivity for the HIV-negative, TB-positive panel was 80% and positive predicted value (PPV) is 83% and negative predicted value (NPV) is 93% (prevalence TB + is 26%). No difference was found with respect to specificity when healthy and sick, non-TB sera were compared. The sensitivity of the HIV- positive TB sera was 52% at a lower cut-off which allows a specificity of 90%. We compared ELISA data with results obtained from Ziehl-Neelsen (ZN) stained sputum smears (according to DOTS). A sensitivity of 72% at a specificity of 95% was found for ZN-negative patients. This means that at an average sensitivity of 70% for ZN on sputum smear, a combined analysis of patients using ZN and ELISA results in a sensitivity of 92% and a specificity of 95% (see table).

	Positive in culture	
	ZN positive	ZN negative
Positive in ELISA	58.4%	21.6%
Negative in ELISA	12.3%	7.3%

Conclusion: The described ELISA test for the serological detection of TB has a significant added value in the diagnosis of TB especially for ZN-negative patients.

P1483 Value of repeated sputum specimens sent to the laboratory in the diagnosis of pulmonary tuberculosis

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Objectives: The presumptive diagnosis of active disease depends on the demonstration of acid-fast bacilli by microscopy, with definitive diagnosis by subsequent culture. How many sequential sputum specimens are really necessary for the diagnosis of tuberculosis? To answer this question, we retrospectively evaluated the results of 6844 respiratory specimens sent to our laboratory between 1998 and 2001.
Methods: All of the specimens were tested by acid fast bacilli (AFB) smear and BACTEC 460 culture system and then we examined the laboratory records of the patients.
Results: A total of 785 (11%) specimens of 353 patients were positive for *Mycobacterium tuberculosis* complex. For 76% (270/353) of these patients the organism was detected from one or more sputum specimens. Of the patients with sputum specimens submitted to the laboratory, 16% (42/270) had only a single specimen sent for examination, 11% (29/270) had two specimens collected, and 74% (199/270) had at least three specimens sent to the laboratory. The AFB smear was found as positive for 55% (23/42) of patients of whom only a single sputum specimen was sent whereas the AFB was detected at least once for 55% (16/29) of persons who had two specimens sent to the laboratory. AFB smear was positive for 69% (137/199) of the patients who had three or more sputum specimens collected. *M. tuberculosis* was isolated in the first, second and third samples of the majority (98%, 195/199) of the patients of whom three or more sputum samples were sent to the laboratory. Our results indicate that the diagnostic value of four or more sputum specimens submitted to the laboratory was very low (2%) and we could carry out *M.tuberculosis* isolation in the first, second and third sputum samples of the overwhelming majority (98%) of the patients.
Conclusions: We recommend that at least three sequential sputum specimens be collected for all patients suspected TB, which is necessary for definitive and cost-effective diagnosis of pulmonary tuberculosis. The examination of four or more specimens is an insufficent use of laboratory resources and will increase the hospital expenses.

P1484 Evaluation of FAST PlaqueTB test for the rapid diagnosis of *M. tuberculosis*

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Objectives: Acid fast bacilli (AFB) smear and culturing the organism are conventional methods for diagnosis of mycobacterial infections. FAST PlaqueTB is a rapid test utilizing bacteriophage amplification technology for the detection of viable *Mycobacterium tuberculosis* in clinical specimens. This test can detect 100–300 bacilli/mL and the results are available in 24 h. We evaluated the performance of the FAST PlaqueTB test by comparing with BACTEC 460 TB culture system, polymerase chain reaction (PCR) and AFB smear methods.
Methods: All of the specimens were evaluated by AFB smear, BACTEC 460 culture system, polymerase chain reaction (PCR) and FAST PlaqueTB test.
Results: We investigated 74 sputum specimens sent to our laboratory. Twenty-two AFB smear positive specimens were also positive by FAST PlaqueTB test, BACTEC 460 TB culture system and PCR method. Twenty-three of 52 specimens that were negative by microscopy were positive by BACTEC 460 TB culture system and 20 of 52 specimens were found as positive by FAST PlaqueTB test and 18 of 52 specimens were positive by PCR. One specimen was evaluated as positive only by PCR whereas one specimen was positive only by FAST PlaqueTB test. One of the specimens was found as positive by BACTEC 460 TB culture system and FAST PlaqueTB test. The sensitivity of AFB smear, PCR and FAST PlaqueTB test were 48.9%, 86.7% and 88.9%, respectively, when we accepted BACTEC 460 TB culture system as gold standard.
Conclusions: We concluded that FAST PlaqueTB test has a good potential for rapid diagnosis of *M.tuberculosis* as a result of the evaluation of these three tests according to BACTEC 460 TB culture system.

P1485 Isolation of nanobacteria from kidney stones

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Introduction: Nanobacteria were observed by Robert Folk in 1990 in Italy while studying mineral samples. The size of these microorganisms is 0.1–0.5 μm and they are not able to grow in common bacteriological media. They are included in the alpha-2 subgroup of Proteobacteria as well as *Brucella* and *Bartonella*. Their principle characteristic is the capability of forming apatite crystals at neutral pH and in physiological calcium and phosphate concentrations. Their implication in several pathologies has been suggested, all of them being peculiar due to the calcification of different structures, such as in the case of kidney stones, etc.
Materials and methods: From February to August 2001, 21 stones from patients of the Urology Service were collected. The extracted stones were triturated in a sterile glass mortar, and whenever possible, part of the sample was preserved for chemical analysis, and the rest was demineralized by means of HCl 1 M. The resultant material was filtered through 0.22-μ pore filters and cultivated at 37 °C in DMEM supplemented with FBS gamma-irradiated. After 6–8 weeks of incubation, the tube bottom was scraped with glass beds and the sediment were inoculated in tubes and cultured as described. The result of this culture was dried and shaded with gold for analysis by transmission (TEM) and scanning electron microscopy (SEM).
Results: Twenty-one stones obtained from patients, 52.4% belonging to men and 47.6% to women. According to the age distribution, there was no age group especially significant in number. The mean age was 57.09 years. The chemical composition of the stones was, in most cases, mixtures of calcium phosphate and calcium oxalate. After 4–6 weeks of culture, in seven of the initially inoculated tubes appeared a whitish precipitate which was delicately granulated and firmly adhered to the tube walls. The use of Gram, Ziehl-Neelsen, and acridine orange stains to visualize this precipitate gave negative results. In the subcultures the precipitate appeared in a shorter period of time and higher quantity, and where analyzed by TEM and SEM, showed particles with a thick cellular wall and a capsule with variable thickness, with pleomorphic sizes and shapes. The cellular wall and the capsule are clearly separated structures. The total thickness varies from 20 to 200 nm. The X-ray analysis gave a composition of the structure formed by Si, Au, P, Ca, and K. Si corresponds to the glass support, and Au to the shadys.

P1486 A rapid test for detection of *Mycobacterium tuberculosis* in body fluids and aspirates

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Introduction: The Aga Khan Hospital, Nairobi has a large number of immunosuppressed patients admitted with extrapulmonary tuberculosis posing diagnostic challenges.
Objective: To evaluate the sensitivity and specificity of performance of Fast Plaque TB assay (Biotec) with body fluids and aspirates (excluding sputa).
Methods: Since June 2001, 112 body fluids consisting of CSF (21), pleural fluids (32), ascitic fluids (5), lymphnode aspirates (6), tracheal aspirates (7), bronchoalveolar lavage (16), and various other aspirates including bone marrow were subjected to Fast Plaque TB assay following the manufacturer's instructions. All the specimens were cultured by Bactec 460 and on conventional Lowenstein Jensen's slopes. Cultures were followed upto 16 weeks on solid media and 12 weeks in liquid media.
Results: Thirty-one specimens were tested positive by Fast Plaque TB assay. Only 6 of these were ZN smear positive. Twenty-nine of these specimens grew MTB in culture. Eight specimens grew nontubercular mycobacteria in

culture. None of this tested positive with Fast Plaque TB assay. Three of them were ZN smear positive. A false positive assay rate of 6.4% is observed in the results so far.
Conclusion: Almost the same false positive assay rate (6.25%) was observed in our earlier study with sputa samples. Obviously with body fluids a large sample size is necessary for any realistic impressions. Our study is going on.

P1487 Evaluation of mycobacteria growth indicator tube (MGIT) for susceptibility testing of *Mycobacterium tuberculosis*

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Introduction: Mycobacteria growth indicator tube (MGIT) is a nonradiometric broth-based culture system suitable for susceptibility testing of *M. tuberculosis*. However, the absence of antimicrobial agents in the MGIT medium can cause, in some cases, the repetition of the test due to bacterial or fungal contamination of the medium.
Objectives: To evaluate MGIT for susceptibility testing of *M. tuberculosis* to streptomycin, isoniazid, rifampicin and ethambutol using PANTA (polymyxin B, amphotericin B, nalidixic acid and azlocillin) as inhibitor of bacterial and fungal growth.
Methods: We selected for the evaluation a total of 102 clinical isolates of *M. tuberculosis* and the reference strain H37Rv. The proportions method (PM) was used as the reference method (NCCLS, M24-T2) with the following drug concentrations (μg/mL): S (2 and 10), I (0.2 and 1), R (1) and E (5 and 10). MGIT susceptibility testing was performed according to the manufacturer's recommendations except for the use of PANTA as inhibitor of bacterial and fungal growth. For analysis purposes, strains were considered as intermediate to one drug when resistant to the low concentration and susceptible to the high concentration, and susceptible or resistant when susceptible or resistant to both concentrations, respectively.
Results: There were 43 intermediate or resistant strains to at least one drug by PM. MGIT system had a mean time-to-result of 6.8 days (5.1–11.3 days). Agreement between both methods was of 98.0% (S), 99.0% (I), 100% (R) and 96.1% (E). Discordant strains for S were two PM-susceptible and MGIT-intermediate, for I one PM-intermediate and MGIT-resistant, and, for E four PM-intermediate and MGIT-resistant. During the test, no MGIT-tubes were contaminated.
Conclusions: MGIT is an excellent and rapid method for the susceptibility testing of *M. tuberculosis*. The use of PANTA can avoid bacterial and fungal contamination without affecting the validity of the method.

P1488 Evaluation of MB/Bact medium, Middlebrook 7H11 medium and Löwenstein-Jensen medium for isolation of mycobacteria from drinking water

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Objective: To evaluate three different culture methods for isolating non-tuberculous mycobacteria (NTM) from water in a geographical area with a high incidence of clinical NTM isolates.
Methods: Drinking water samples (5 L) from 8 public distributions systems were weekly collected between April and October 2001. The specimens were filtered (0.2 mm) and the membranes were mixed with 10 mL of physiological serum for 24 h. The suspensions were decontaminated and concentrated with the *N*-acetyl-L-cysteine-NaOH procedure, and the sediment were used for acid-fast staining and for inoculation into MB/BacT (MB) bottle, Middlebrook 7H11 (7H11) medium, and Löwenstein-Jensen (LJ) medium. All isolates were identified by biochemical tests, specific DNA probes, and PCR-RFLP of the hsp65 gene.
Results: A total of 26 NTM (12%) were isolated from 216 water specimens studied: 20 *M. gordonae* (MGOR), 1 *M. chelonae* (MCHE), and 5 *M. fortuitum* (MFOR). The table summarizes the mycobacteria recovery rates from each medium alone or in combination.

NTM	Total	MB	7H11	LJ	MB + 7H11	MB + LJ	7H11 + LJ
MGOR	20	14	3	5	17	17	8
MCHE	1	1	0	0	1	1	0
MFOR	5	0	3	2	3	2	5
Total (%)	26 (100)	15 (57.7)	6 (23.01)	7 (26.9)	21 (80.1)	20 (76.9)	13 (50)

All specimens were smear negative for acid-fast bacteria. Rates of contamination for MB, 7H11 and LJ were 0.46%, 4.63%, and 2.31%, respectively.
Conclusion: The MB medium shows the highest sensitivity for recovery of NTM from water samples, except for MFOR. Therefore, an additional solid medium should be used in combination to improve NTM recovery.

P1489 SDS-PAGE for identification of species belonging to the *Mycobacterium fortuitum* complex

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Objectives: We performed a study to determine the usefulness of SDS-PAGE of whole cell proteins for characterization of species of rapidly growing mycobacteria that belongs to the *Mycobacterium fortuitum* complex.
Material and methods: Tested strains were 37 *M. fortuitum*, 32 *M. chelonae*, 10 *M. peregrinum*, 5 *M. abscessus* and 3 *M. mucogenicum*. Eight collection strains (including type strains of the five species) were also included in the study. Identification of the strains was achieved by common biochemical tests. SDS-PAGE was performed according to the common protocols and the gels were stained with Coomasie blue, photographed and scanned for comparison with the Scion Image software. The average similarity between patterns were calculated by using the Dice coefficient.
Results: All strains yielded between 44 and 58 bands in the electrophoretograms. Intraspecies similarity showed Dice coefficients higher than 95%, with only one strain of *M. fortuitum* with 6-band difference (Dice coefficient 87.75%). However, interspecies similarity was always below 75%, the similarity being higher between *M. fortuitum* and *M. peregrinum* (75.51%) and between *M. chelonae* and *M. abscessus* (54.9%). Visual examination of the electrophoretograms was enough for species characterization when type strains are included in the test.
Conclusions: SDS-PAGE of whole cell proteins is a useful technique for identification of isolates of the *M. fortuitum* complex, and is easy to perform without the need of complex or expensive equipment.

P1490 Rapid identification of mycobacteria species by automatic sequence analysis of *hsp65* gene

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Objective: The purpose of this study was to evaluate the partial hsp65 sequencing for the rapid identification of mycobacteria species.
Methods: Thirty-four clinical isolates used in this study were grown in a liquid medium (MB/Bact system; Organon Teknika Corp., Durham, NC) or on Lowenstein-Jensen medium (Biotest, Heidelberg, Germany). Nine strains were from the American Type Collection (Rockville, MD). Bacterial DNA was prepared as described by Van Soolingen and a segment of the hsp 65 kDa heat shock protein gene (*hsp65*) was amplified as described by Telenti. The PCR product was purified with silica gel columns (Qiagen; M-Medical-Genenco, Florence, Italy). Sequencing reactions were done by a standard sequencing method with a DNA sequencing kit (Deaza Dye Primer Kit – Visible Genetics Inc.) with the fluorescent primers Cy5.0-Tb11 e Cy5.5-Tb12 on a Opengene Long Read Tower sequencing system (Visible Genetics Inc., Toronto, Ontario, Canada). The sequences were analyzed by using our Gen-library. This database was obtained by alignment with Gene Base software Version 1.0 (Applied Maths-Belgium). A phylogenetic tree of the mycobacteria was constructed by using the same program.
Results: The strains analyzed were identified correctly and each species had an unique sequence. Nucleotide differences were found along the length of the amplified *hsp65* fragment but were particularly frequent in 4 regions: positions 122–125, 218–224, 287–292 and 355–359 which represent the hypervariable

regions of the *hsp65* gene and possible species-specific regions. Among isolates classified as the same *Mycobacterium* species the analysis identified *hsp65* allelic diversity. For example, 28 isolates of *Mycobacterium tuberculosis* had 6 distinct *hsp65* alleles. The phylogenetic analysis based of *hsp65* gene confirmed the phylogenetic analysis of the genus *Mycobacterium* obtained with 16S rRNA sequencing.
Conclusions: Automated *hsp65* sequencing is a rapid and reliable method for the identification of mycobacteria; allelic diversity within *hsp65* does not preclude the use of this target as the basis for recognition of medically important subspecies strain group.

P1491 Molecular typing of *Mycobacterium avium* strains isolated from HIV-positive patients

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Objective: *M. avium* is an important cause of opportunistic infection in HIV-positive patients with low CD4 cells count ($< 100 \times 10^6$ cells/L). The aim of the study was the molecular typing of *M. avium* strains isolated from these subjects.
Methods: Forty-two strains of *M. avium* (identified by biochemical tests and Accu Probe) isolated from different samples (blood, sputum, brochoalveolar lavage, feces) of 16 HIV-positive patients were examined. DNA plasmids were extracted by alkaline lysis (after growth with D-cycloserine and ampicillin), analyzed by electrophoresis in 0.7% agarose gel and visualized by ethidium bromide. The strains were also typed by RAPD-PCR (random primer amplified polymorphic DNA) using three primers (Leg 1, A 1245, B 1245); the amplified products were analyzed by electrophoresis on polyacrylamide gel and silver staining and by electrophoresis in 2% agarose gel.
Results: Two strains isolated from different samples of each two patients showed one small (< 30 kb) plasmid, while four strains isolated from different samples (blood, sputum, bronchoalveolar lavage, feces) of one patient showed small and large (> 150 kb) plasmids. Interpretation of RAPD-PCR patterns of different patients strains was sometimes difficult. Isolates of the same patient showed the same profile by RAPD-PCR. Strains analyzed with B 1245 primer showed the same profile in nine subjects and three different patterns in plasmid carrying strains of three patients.
Conclusions: *M. avium* isolates belonging to a patient had same molecular profile (RAPD-PCR and plamids), suggesting that disseminated infection was caused by the same strain. As some isolates of different patients shared the same profile, suggesting a common source, relatedness between strains and patients need to be further investigated.

P1492 Integrated procedure for mycobacteria and corynebacteria identification in clinical specimens

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Introduction: Nowadays one of the urgent problems of clinical microbiology is a problem of identification of atypical microorganisms, causing infections in human beings. Recently a lot of cases of tuberculosis, mycobacterioses, diphtheria and diphtheria-like diseases have been reported to be caused by atypical bacteria like nontoxigenic corynebacteria and nontuberculosis mycobacteria, etc., mostly in immunocompromised persons. Most part of these cases were not diagnosed at early stages because of absence of adequate clinical laboratory methods.
Objective: To develop a simple, universal and reproducible method of identification and differentiation of *Mycobacterium* and *Corynebacterium* species in clinical samples like sputum, pharyngeal washes, mucous scrapes, etc.
Material and methods: Specimens of sputum from 50 smear-positive and 38 smear-negative patients, including those in which antituberculosis therapy was not effective; specimens from mucous membranes of genitalia of 23 patients with genital tuberculosis; pharyngeal washes from persons with diphtheria, tonsillitis, angina, reference strains of mycobacteria and corynebacteria. Mycobacteria isolation from sputum probes was carried out by its absorption on magnetic corpuscles with polyclonal antibodies and concentration in small volume. Then we extracted DNA by detergent processing. Pairs of specific primers to these mycobacteria and corynebacteria species and universal primers (UP-PCR, RAPD) were used.
Results: We established a high correlation between bacteriological methods and PCR in identification of *Mycobacterium tuberculosis* in smear-positive specimens of sputum. Specific DNA fragments of *Mycobacterium tuberculosis* were found in 83.5% of smear-negative specimens, that approximately twice exceeds the effectiveness of bacteriological methods. Additionally in 12.8% of smear-positive and in 18.7% of smear-negative patients nontuberculosis mycobacteria were found. In specimens with nontoxic corynebacteria 15.6% of other corynebacteria species were found out. Alternative method includes mycobacteria cultivating on Lowenstein-Jensen agar or corynebacteria cultivation followed by UP-PCR for species differentiation. Mathematical data treatment was made by special programs, which allow comparing and clusterizing PCR patterns. This PCR procedure is an effective method for revealing agents in clinical samples decreasing time expenditure with higher effectiveness and sensitivity.

P1493 Identification of mycobacterial species isolated from patients suspected of pulmonary tuberculosis based on the 16S rDNA sequence analyses

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Objectives: To identify the mycobacterial species isolated from the patients suspected of having pulmonary tuberculosis.
Methods: A polymerase chain reaction (PCR) was established for amplification of 5′-end fragments (~680 bp) of 16S rRNA genes of nontuberculous mycobacterial strains isolated from the sputum specimens of patients clinically suspected of having pulmonary tuberculosis in the pulmonary hospital in Chongqing of China. The 16S rDNA fragments were directly sequenced by dideoxynucleotide methods with an automatic DNA sequencer. Phylogenetic analyses were made based on the 16S rDNA sequences.
Results: In 16S rDNA sequence comparisons of 23 strains of the present study with the known mycobacterial species, the sequences of five stains (22%) were identical to that of *M. gordonae* and the sequences of four strains (17%) identical to that of *M. fluoroanthenivorans*. However, the sequences of other 10 strains were mostly similar to that of *M. kansasii*, *M. flavesscens*, *M. fuerth*, and *M. monancens*, respectively. The sequences of other 4 strains were unique and higher divergent (> 4%) from that of the known mycobacterial species, indicating that they may be new species of genus *Mycobacterium*.
Conclusion: The phylogenetic analysis on the basis of 16S rDNA sequences reveals that at least 4 new species of *Mycobacterium* are found in human pulmonary infection. The species of nontuberculous mycobacteria causing pulmonary infection of humans in Chongqing area are different from that reported in other areas. The analysis of 16S rDNA sequences is a quick and accurate way to identify mycobacterial species isolated from the patients.

P1494 Role of gene amplification methods in the diagnosis of patients infected with *Mycobacterium tuberculosis*

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Objectives: The purpose of the study was to evaluate two gene amplification methods for *Mycobacterium tuberculosis* (MTB) detection in both pulmonary and extrapulmonary clinical samples.
Methods: Eighty-one clinical specimens (71 respiratory and 10 extrapulmonary) from 80 patients suspicious of being infected with MTB were tested by microscopy/smear (Ziehl-Neelsen, Z-N), culture (Bactec, 9120 System, Becton Dickinson, USA), polymerase chain reaction (PCR, Roche Diagnostic System, USA), and Gen-Probe Amplified Mt Direct Test (AMTD, Gen-Probe, USA). All respiratory and urine samples were decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH prior to testing. The results obtained by gene amplification were compared with those by traditional methods.
Results: About 11/81 clinical specimens were positive. In particular, 8/11 specimens were positive for all the tests performed but one negative for Bactec culture and two negative for Z-N/Bactec culture, respectively. Of the remainder three samples, two were Bactec positive and Z-N/PCR/AMTD negative, and one was PCR positive and Z-N/Bactec/AMTD negative.

Conclusions: Results show that both nucleic acid amplification methods appear highly sensitive and specific for the detection of MTB. Moreover, in the presence of smear-and culture-negative samples, AMTD which is based on rRNA testing might represent in the presence of positive PCR a useful means in clarifying patient response to antimicrobial therapy.

P1495 Analysis of a 7-year experience with '*Mycobacterium tuberculosis* Direct Test' (MTD) on respiratory and nonrespiratory specimens

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Objectives: In our hospital, since January 1994 we have been using the '*Mycobacterium tuberculosis* Direct Test' (MTD test), manufactured by Gen-Probe Inc. (San Diego-CA), a rapid diagnostic test for cases of doubtful positive specimens for *M. tuberculosis*. This test was introduced to make up for the extremely long growth times of mycobacteria. Before the introduction of this test, the only rapid test available was a microscopic exam, which, unfortunately, offers a low sensitivity, allowing the detection of about 5×10^4 bacilli/mL in the case of staining with Ziehl-Neelsen or 2000–10 000 bacilli/mL with a fluorescent stain. This brings the percentage of positivity to 60–75% in the best case scenario, a value too low considering the elevated infectivity of *M. tuberculosis* for which 50% of the infective dose is <10 bacilli. Thus, the introduction of a rapid diagnosis using molecular biology based on amplification of RNA ribosomal fractions. We analyzed the results obtained over a period of 7 years on about 2500 specimens to verify the validity and economic impact on our hospital.
Methods: Using the MTD test, we tested various specimens: sputum, bronchial aspirates, pleural fluid, urine, seminal liquid, sterile body fluids, gastric aspirates, tissue specimens such as endopleuric fiber, etc. Particular attention was given to pretreatment of the various materials in order to have ideal specimens for amplification. Testing was performed using the manufacturer's directions. In addition to the amplification procedure, all specimens were cultured on solid media and in broth media. A microscopic exam was also performed.
Results: From the results obtained from about 2500 MTD tests performed, we found a high correlation with culture methods. In another 100 cases of bacterioscopic examination negative or doubtful, the MTD test provided an immediate diagnosis or exclusion of TBC without waiting for the culture results.We were also able to follow some cases of recidive indicated by the MTD test before the microscopic exam became positive.
Conclusion: The analysis of the results obtained over a 7-year period confirm the validity of the MTD test from specimens of different origin. It seems that the Amplified MTB Direct Test could be useful both for the positive effect on the patient, thanks to the rapid diagnosis and initiation of therapy, as well as for the cost savings afforded the hospital through reduced length-of-stay.

P1496 Randomly amplified polymorphic DNA PCR for comparison of strains of type I *Mycobacterium kansasii*

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Objectives: We studied the feasibility of using RAPD-PCR for comparison *M. kansasii* type I strains. This included development of optimal PCR conditions and primers. RAPD-PCR was then applied to 122 clinical isolates of *M. kansasii* type I, previously typed by PCR-RFLP.
Methods: Isolates: 122 clinical isolates of *M. kansasii*. Identification was performed by DNA probe (AccuProbe). Molecular typing was performed by PCR-FLP of the *Hsp65* gene. The type strain *M. kansasii* CECT3030T and one strain belonging to genotype I (provided by Dr V. Vincent, Institute of Pasteur, Paris) were used as control strains. DNA was obtained as follows: One loopful of bacteria was suspended in a mixture of TEN buffer and phenol:-chloroform:isomyl, subjected to sonication for 15 min and precipitated with isopropanol and sodium acetate. The concentration and the purity of the samples were determined by spectophotometry. 9 random primers were chosen for evaluation (MPTR-1, MPTR-F, INS-2, IS986-fp, R-4, P-2, Pntb-1, Pntb-2 y RISK-1). The amplification included 40 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C; this was followed by 10 min of extension at 72 °C. The amplification product was loaded onto 2% MS-8 agarose gel and the fragments were visualized by ethidium bromide staining and UV light.
Results: Twenty isolates and the control strains were used in the initial studies to develop optimal conditions for performance of the RAPD-PCR. With the 9 primers tested in this study, 7 produced satisfactory amplicons with all tested isolates. Two primers (MPTR-1 and INS-2) generated the best amplification patterns and produced more different patterns with the best reproducible results. These primers were used thereafter. Both primers produced three different patterns: the MPTR-1 produced three patterns which were named as A, B and C. The INS-2 primer produced three patterns which were marked as 1, 2 and 3. Five clusters were identified: A1, B1, A2, A3 and C1. The cluster A1 was formed by the majority of the isolates (87 isolates) and by the type strain CECT3030T. The second cluster (B1) was formed by 30 isolates. The cluster A2 was formed by 4 isolates. The pattern A3 was produced by only one isolate. The pattern C1 was produced by the control strain provided from the institute of Pasteur, and none of our collection produced the same pattern.
Conclusion: The RAPD-PCR technique appears to be simple and rapid compared with many complicated molecular typing methods

P1497 Restriction fragment length polymorphism of *gyrB* for the diagnosis of dysgonic *Mycobacterium tuberculosis* complex strains

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Objective: The aim of the study was to apply the PCR Restriction Fragment Length Polymorphism of *gyrB* recently described by S. Niemann et al. (J Clin Microbiol 2000; 38 : 3231–3234) for the discrimination of dysgonic *Mycobacterium tuberculosis* complex (MTBC) strains.
Methods: Eighteen dysgonic MTBC strains and four 'classical' *M tuberculosis* strains previously isolated from human clinical specimens and identified by biochemical and genetic tests were studied.The gene *gyrB* (1020 bp) was amplified by PCR using primers: MTUBf (5'-TCG GAC GCG TAT GCG ATA TC-3') and MTBUr (5'-ACA TAC AGT TCG GAC TTG CG-3'). Polymorphism of *gyrB* was analyzed using RsaI, TaqI and SacII according to the previously described algorithm (Niemann et al.).
Results: Sixteen of the 18 dysgonic MTBC strains were correctly discriminated using the analysis of *gyrB*: (i) *M. bovis* (pyrazinamide resistant) $N = 7$; (ii) *M. africanum* $N = 8$; (iii) *M. microti* $N = 1$. For to2 *M. africanum* strains (resistant to thiophen-2-carboxylic acid [TCH]), PCR-RFLP pattern was similar to the unique pattern of the 'classical' *M. tuberculosis* strains ($N = 4$).
Conclusion: The analysis of the *gyrB* polymorphism by PCR – RFLP is a rapid and simple technique to identify the majority of dysgonic *M. tuberculosis* complex strains. However, *M. africanum* subtype II (resistant to TCH, more frequently seen in West Africa) could not be distinguished from *M. tuberculosis*.

P1498 Assessment of a rapid molecular method for the identification of *Mycobacterium tuberculosis*

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Eight million people contract tuberculosis every year, and one-third of the world's population is infected with *M. tuberculosis*. Annually tuberculosis causes 3 million deaths. The main cause of dissemination is the absence of early diagnosis and prompt medical attention. Probes and DNA-amplification procedures, represent new technologic developments in clinical microbiology.
Objectives: We evaluated the specificity and sensitivity of a rapid method for the direct detection of microrganisms in clinical samples proposed for laboratory diagnosis of *M. tuberculosis* vs. standard procedures.
Methods: In the period between February and November 2001 we investigated for the presence of *M.tuberculosis*, 154 biological specimens, collected from patients recovered in medical and surgical wards at the University Hospital of Pavia (northern Italy), using both molecular and traditional methods. Each sample was submitted to a decontamination/dygestion by using the BBL MycoPrep kit. Fast extraction of mycobacterial DNA was

successively performed by using the '*Mycobacterium* gene releaser' (Euroclone Ltd., UK) kit. The method is based on a nested PCR. Each sample was also examined after Ziehl-Neelsen stain to detect and estimate the number of acid fast bacilli and cultured in double on Lowenstein-Jensen Medium (Difco Laboratories, USA); incubation was performed at 37 °C. The cultures were examined firstly after 10–14 days and then every week until a total of 8 weeks.
Results: The samples were from: respiratory tract 75%, urine or other urogenital tract specimens 19.7%, pus or from other sources 5.3%. Two of 154 specimens processed for the presence of *M.tuberculosis* provided positive result with both molecular and cultural methods, while they were not with microscopic examination. The remaining 152 samples always resulted negative with all procedures. The assessed molecular method showed high specificity (100%) and sensitivity (100%).
Conclusions: Because of their high specificity, sensitivity, simple applicability and high velocity, the molecular methods are a wise investment and should be routinely considered for the detection of *M.tuberculosis* in clinical laboratory, especially in the management of patients with active disease to evaluate the effectiveness of antituberculosis treatment.

P1499 Rapid detection of *Mycobacterium tuberculosis* resistance mutations in sputum samples by LyghtCycler PCR

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Objectives: To evaluate a new rapid method based on real-time PCR for the detection of mutations in the *rpoB* and *katG* genes in sputum samples.
Methods: One hundred sixty sputum samples with documented auramine counts, obtained from 69 patients diagnosed as having tuberculosis attended at the H.U.V. del Rocío in Seville were studied. DNA was extracted from the samples using DNA IV method (Unipath SA). The extracts were amplified with the LightCycler system, using two primers and two short oligonucleotide probes (fluoresceinated anchor and Red640 sensor labeled probes). For the *rpoB* gene we used two pairs of probes, one covering the region spanning codons 526 and 531, and a second one covering codons 513 and 518. The *katG* gene probe covered the region containing codon 315. All the samples were decontaminated by NALC-NaOH method, and inoculated onto Löwestein-Jensen media and a MGIT tube. Susceptibility testing was performed in clinical isolates from 33 patients by the Bactec MGIT 960 method.
Results: One hundred fifty-five (96.8%) samples were positive by PCR with both pairs of primers. Six samples had a mutation in the *rpoB* gene, two at position 531 and four at position 516. There was 100% agreement between phenotypic and genotypic susceptibility tests for RMP. In the case of INH, all the 10 genotypic resistant strains showed phenotypical resistance, while one phenotypic resistant strain could not be detected by PCR (false negative).
Conclusion: This is a rapid, sensitive and highly specific method to detect mutations at the *rpoB* and *katG* genes of *M.tuberculosis* directly in acid fast positive clinical samples, allowing discrimination between susceptible and resistant strains in less than 3 h.

P1500 Comparison of the Roche Amplicor *Mycobacterium* assay and BD Probe Tec system for the detection of *Mycobacterium tuberculosis* in respiratory specimens

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Objectives: Retrospective evaluation of two amplification techniques in their capacity to detect organisms of the *M. tuberculosis* (Mt) complex in respiratory specimens.
Methods: Three hundered ninety-eight sputum, bronchoalveolar lavage and bronchial and tracheal aspirate specimens from 297 patients were tested for the presence of Mt complex by auramine fluorochrome staining, strand displacement amplification technique (Probe Tec, Becton Dickinson) and PCR (Roche Amplicor Mt test). All specimens were decontaminated and digested with 2% NaOH in 0.5% *N*-acetyl-l-cysteïne and concentrated by centrifugation. Pellets were resuspended in 2 mL PBS and divided for culture and amplification techniques. The results of the amplification methods were compared with the reference, i.e. liquid culture by BACTEC or MGIT technique.
Results: Of the 398 clinical specimens, 47 specimens from 13 patients were culture positive for *M. tuberculosis*. Of these 47 specimens, 31 were smear positive, 33 were positive by the Roche Amplicor Mt test and 39 by the BD Probe Tec. Of the 351 culture negative specimens, five were positive by the auramine staining, six were positive by the Roche Amplicor Mt test and 11 were positive by the BD Probe Tec. After discrepancy analysis and review of the patients' clinical data, 58 specimens from 13 patients were considered as 'true-positive' specimens, meaning that two of five culture-negative smear positive specimens, six of six culture-negative Roche Amplicor Mt test positive specimens and nine of 11 culture negative BD Probe Tec positive specimens were diagnosed as 'true-positive' specimens. These data compared with the number of 'true-positive' specimens results in the following sensitivities:microscopy, 56.9%; for Roche Amplicor Mt test, 67.2% and BD Probe Tec, 82.8%. The specificities were 99.1%, 100% and 99.4%, respectively.
Conclusions: Both amplification techniques give rapid results and are very specific for the detection of Mt complex in respiratory specimens. The BD Probe Tec technique detects more 'true-positive' specimens than the Roche Amplicor Mt test, but the differences are not significant.

P1501 Isolation of *Mycobacterium* nucleic acids (RNA & DNA) from sputum and cultured strains using magnetic silica particles

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Objectives: bioMerieux is developing a novel isolation procedure that uses Boom chemistry in combination with magnetic silica particles. In principle, the isolation procedure is generic and can be applied for a broad range of different sample matrices. The objective of this study was to validate the method for the recovery of *Mycobacterium* nucleic acids from different samples.
Methods: In total, 19 AFB-smear positive sputum samples were used for RNA study. Sputum samples were decontaminated with NALC-NaOH and incubated for 20 min at 95 °C to inactivate the bacteria. Next, 1 mL of lysis buffer was added to the samples. Control RNA was spiked to the lysed samples to monitor isolation efficiency. *Mycobacterium tuberculosis* ribosomal RNA was detected with the NucliSens Basic Kit. Control RNA was detected in a separate amplification reaction using real time NASBA and a molecular beacon probe. Additionally 10 AFB-smear positive sputum samples and 10 *Mycobacterium* strains (Tb complex and MOTT) isolated on LJ slants were also tested for DNA extraction. Same protocol was applied on all samples. Sensitivity study was also performed on genomic DNA. All DNA samples were PCR amplified and detected on DNA chip.
Results: *Mycobacterium tuberculosis* RNA was detected in 18 samples (95%). One sputum sample scored negative for *Mycobacterium tuberculosis*. However, in this particular sample also the control RNA was not detected. This sample contained a large amount of cell debris, which was not removed adequately during sample pretreatment. Incorporation of a simple extra step, clarification by centrifugation, resulted in a more robust extraction of nucleic acids from sputum samples. All DNA samples (either from sputum or strains), and whatever the strain involved, were well identified on chip. Furthermore sensitivity was shown to be at least 50 copies of gDNA per assay.
Conclusions: The new nucleic acid extraction method was applied successful for the detection of *Mycobacterium* species in AFB-smear positive sputum samples or cultured strains. However, an extra step in the pretreatment procedure seems necessary for efficient nucleic acid recovery from sputum samples.

P1502 Four years' experience using MTD2 test as an amplification method for direct detection of mycobacteria

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Objectives: The purpose of this study was to evaluate results using MTD2 test (Gen-Probe Inc.) obtained from 1997 until now for the diagnosis of tuberculosis.

Methods: Since 1997, diagnostic protocols for detection of mycobacteria provided direct smear examination by staining with Auramine-Rhodamine, and culture in liquid (MGIT-BD) and on solid (Lowenstein-Jensen) media; the MTD2 test was used since there was a specific requirement for this type of test by clinicians in suspicious cases of tuberculosis or by the laboratory on all positive smear samples (in order to confirm the presence of *M.tuberculosis* complex).
Results: From April 1997 until November 2000 *M. tuberculosis* was isolated by culture techniques from 63 patients (49 cases of pulmonary tuberculosis and 14 cases of extrapulmonary tuberculosis). Microscopic examination was positive for 34/63 patients (53.9%): for respiratory samples the sensitivity was 59.2% and for other sample types it was 35.7%. MTD2 test was performed on 46/63 cases having a positive culture and it was positive in 41 cases, demonstrating a global sensitivity of 89% (91.9% in respiratory specimens and 77.8% in other specimens). In our analysis the average time to have a positive result using culture techniques was 12.3 days (4–34 days) in smear-positive samples (Auramine-Rodhamine staining) and 27.3 days (10–62 days) in smear-negative samples. Considering the lengthy times to obtain results using culture methods, it is important to verify the MTD2 sensitivity in patients with smear-negative results: during these 4 years, out of 10 patients with a negative smear and positive culture, for which an amplification test was done, seven resulted positive with the MTD2; this allowed a faster diagnosis. Finally we analyzed the use of MTD2 test during the year 2000: out of a total of 1123 detection tests for mycobacteria, MTD2 was required 275 times, about 24.5% of the total cases. In addition this analysis demonstrated that MTD2 test was required on the 50% of patients who had culture positive results, showing the appropriateness of the use of the test.
Conclusions: Our analysis confirms the effectiveness of amplification test employment for direct detection of mycobacteria in selected patients, as suggested by others.

P1503 Diagnosis of extrapulmonary tuberculosis: traditional methods and molecular biology

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Objectives: To evaluate the reliability of PCR related techniques in diagnosis of tuberculosis in extrapulmonary samples.
Methods: Fifty-seven cases of extrapulmonary tuberculosis were collected and analyzed in our institute from 1991 to 2201. Twenty-one patients were females and 36 were males. Age ranged from 22 to 89 years (mean value 62.3 years, median 70 years). The samples examined were 10 pus, 10 biopsies, 8 urines, 5 swabs and 24 biological liquids (18 pleural effusions, 1 blood, 2 liquors and 3 synovial or peritoneal fluids). All samples were cultured in Lowenstein Jensen medium; 36 were assayed by acid-fast bacilli stain and 23 by molecular test (Transcription Mediated Amplification TMA, Gen Probe and Ligase Chain Reaction LCx, Abbott).
Results: All samples had positive cultures (Gold Standard), 16 (44%) of the microscopical examinations and 16 (70%) of the PCR assays were positive. PCR and microscopy showed the same sensitivity (100%)for detecting *M.tuberculosis* in pus samples, but PCR was more sensitive than microscopy in biopsies (75% vs. 50%, respectively) and urines (100% vs. 28%, respectively). Sensitivity of PCR and microscopy is lower in swabs and liquids (0% vs. 20% and 25% vs. 0%, respectively) than in pus, biopsies, urines but in pleural effusions the sensitivity of PCR reached 66.7% vs. 0% of microscopy.
Conclusions: *M.tuberculosis* is a very fastidious microrganism because microscopical observation is not a sensitive and specific assay and culture takes several weeks. PCR assay is a more sensitive and rapid test. Actually it is evaluated both in clinical trials of pulmonary and extrapulmonary tuberculosis. Nevertheless, the commercial kits are generally not standardized for biological samples other than respiratory fluid or urine. Our study indicates that PCR is a rapid and a very good tool for diagnosis of extrapulmonary tuberculosis in pus, biopsy, urine and pleural effusion. The lack of sensitivity in swabs and liquids other than pleural effusion is probably due to a casual repartition of the very small number of tubercular bacilli (below 10 colonies) in the tested samples.

P1504 Combined use of BACTEC MGIT 960 System and a PCR assay, for the detection and identification of *Mycobacterium tuberculosis* from clinical specimens

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Objectives: Evaluation of the combined use of the BACTEC MGIT 960 System and a polymerase chain reaction assay for the detection and identification of *Mycobacterium tuberculosis* in a routine clinical laboratory.
Methods: Seven hundred and sixty specimens (539 sputum samples, 29 bronchial washings, 76 body fluids, 8 gastric fluids, 96 urine, 6 tissue biopsies, 1 lymph node biopsy, and 5 other specimens) submitted to the mycobacterial laboratory, were processed with the NALC-NaOH method recommended by CDC, stained and cultured in parallel on the Bactec MGIT 960 system and on Loewenstein–Jensen medium. The performance of two culture systems has been compared. In positive MGITs, the presence of mycobacteria was confirmed by Ziehl-Neelsen stain, and a PCR assay was applied for the identification of *Mycobacterium tuberculosis*. DNA extraction and PCR conditions have been performed as described previously. The results of PCR assay have been compared with those obtained by classical biochemical identification for mycobacteria tests.
Results: Eighty-two mycobacterial isolates – 51 *Mycobacterium tuberculosis* and 32 MOTT (mycobacteria other than tuberculosis) – have been detected. All of them were detected in MGIT (100% sensitivity) while 5 six (68% sensitivity) on Loewenstein–Jensen medium. The mean time of detection of *Mycobacterium tuberculosis* by BACTEC MGIT 960 System was 9.4 days while 20.5 days for Loewenstein–Jensen medium. The PCR assay identified all *Mycobacterium tuberculosis* isolates (100% sensitivity) while gave rise negative results for the remaining MOTT (specificity 100%).
Conclusions: The study indicates that the sensitivity and the detection time of mycobacteria in BACTEC MGIT 960 system were higher than that of Loewenstein–Jensen medium. The identification of *Mycobacterium tuberculosis* by PCR assay was in excellent concordance with that by the classical biochemical tests. We propose that PCR assay is faster, easier and cheaper for identification of *Mycobacterium tuberculosis* in a routine clinical laboratory.

P1505 *M. genavense* directly identified in stool specimen with HPLC

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M. genavense is a slow-growing fastidious mycobacteria wich has been reported to cause disseminated infections in patients with advanced HIV infection. The identification of *M. genavense* is a challenge for the mycobacterial laboratory due to its failure to grow in conventional solid media and its long incubation time to grow in liquid media. HPLC Mycolic acid analysis is a rapid, standardized, and economic system, which identify a wide mycobacteria species by their characteristic mycolic acid pattern. Although identification of *M. genavense* has been documented in culture media, to our knowledge this is the first report in which identification of *M. genavense* is performed in a stool specimen by HPLC. We present a case of 26-year-old-patient with AIDS with abdominal pain, diarrhea and wasting syndrome. A year ago he was previously diagnosed of disseminated mycobacteriosis without microbiological confirmation. Initial antimicobacterial therapy was effective and continued with secondary prophylaxis (ethambutol and clarithromicin) during one year. In a new admittance abundant small clumped acid-fast coccobacilli were seen in the stool samples from this patient. Different decontaminations methods were used for repeated culture in solid and liquid media, however, mycobacteria failed to grown and contamination by other bacteria was encountered. HPLC with fluorescence detector was used in order to identify directly the mycobacteria in stool specimen. Stool specimen (10 mL) was cleaned by means of two washes with saline solution. Sediment obtained by centrifugation was subjected to saponification and derivatised to 4-bromomethyl-6,7-dimethoxycoumarin derivatives. A linear gradient of chloroform/methanol was used in the HPLC analysis. To confirm the HPLC identification, PCR-restriction analysis was performed in 0.25 mL

of diluted stool specimen. With HPLC and fluorescence detector we obtained a triple cluster of micolic acid characteristic of *M. genavense* and the patient was diagnosed of disseminated mycobacterial infection with enteritis by *M. genavense*. On the other hand PCR-restriction enzyme analysis (PRA) revealed the presence of the characteristic pattern assigned to *M. genavense* (two DNA restriction fragment of 325 and 15 bp for BstEII and two of 140 and 105 bp for HaeIII). This result indicate that HPLC with fluoroscence detector could be used in stool in direct identification of fastidious mycobacteria as *M. genavense* or *M. avium* that produce disseminated infections whose reservoir for this bacterium seems to be the gut.

Infections and medical devices

P1506 Clinical interest of the type of bacteremia inpatients with permanent endocardial pacemakers

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Background: Bacteremia inpatients with permanent endocardial pacemaker (PEP) is a serious problem. Literature is scarce and agreement about management *does not exist.*
Methods: The files of the 1182 PEP implanted between May 1987 and January 1999, were revised. The criteria for bacteremia was two positive blood cultures for the same microorganism. Patients with one positive blood culture were included if the same microorganism was recovered from the system. Bacteremias were classified as early onset (EOB) occurring within the first 6 months after implantation and late onset (LOB) occurring thereafter. The source of the bacteremia was recorded.
Results: Twenty-six cases of bacteremia inpatients with PEP were studied. Eighteen bacteremias were PEP-related, the other eight, the source was not the pacemaker. The prevalence of PEP-related bacteremia in our hospital was 1.52%. EOB ($n=6$) and LOB ($n=12$) disclosed differences in diagnostic difficulty: All EOB had local signs of infection versus 27% in LOB group ($P<0.001$). *S. aureus* caused 83.3% of EOB and 16% of LOB ($P<0.005$). Mortality was higher in EOB patients (50% EOB vs. 0% LOB). Among patients with LOB 45% had been previously diagnosed of pneumonia. Endocarditis was diagnosed in 75% (9/12) with LOB. In the two groups, total extraction of the system was necessary. In any case of bacteremia arising outside the PEP, the system became involved.
Conclusions: EOB and LOB are different in the type of microorganism, diagnostic difficulty, and prognosis. Extraction of the pace-system is necessary, except in nonstaphylococcal bacteremias arising out of the system.

P1507 Conservative management of early vascular prosthesis infections

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Background: The use of synthetic vascular prosthesis has supposed an important advance in vascular surgery. However, infection of these prosthesis is a serious complication with high morbidity and mortality and, for most authors, complete extraction of the vascular graft plus parenteral antibiotic treatment are needed for cure.
Methods: We prospectively studied all patients with infected vascular prosthesis attended at our hospital since May 1998 until November 1999. Prosthesis was considered infected when spontaneous drainage through the surgical wound was observed, plus a CT-scan showed a periprosthetic collection. Only patients with patent prosthesis and without septic shock were included. We tried a conservative approach keeping the prosthesis in place, with surgical or radiological guided drainage plus antibiotic treatment.
Results: Fourteen patients with early prosthesis infection have been included (one of them with periaortic infection, and the others with extracavitary infection). All patients, except one, presented fever and spontaneous purulent drainage. Microorganisms were recovered from the pus in 10 patients. (definite infection) Only one microorganism was isolated in seven patients (*S. epidermidis* ($n=4$), *S. aureus*, *E. coli* and *B. cepacia*) and mixed flora in the other three patients (*Streptococcus simulans* + *E. faecalis*; *S. epidermidis* + *E. faecalis* and *S. epidermidis* + *Proteus mirabilis*). After two years of follow-up without antibiotic treatment, complete clinical, radiological and analytical cure was achieved in 11 patients (78.6%) and in other three (11.6%) the conservative management failed (under antibiotic treatment in two of them and one year after antibiotics were withdrawn in the other).
Conclusion: *Most patients with early vascular prosthesis infections, can be* cured without surgical extraction of the prosthesis.

P1508 Successful treatment of a VA shunt infection complicated by shunt nephritis with linezolid: a case report

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Although the incidence of shunt infection has decreased this complication is a constant source of concern. Rarely, shunt nephritis can result as a sequela of a chronic infection. In August 2001 a 40-year-old-woman was admitted because of seizures. Few days later she developed fever and mental deterioration. Blood cultures yielded methicillin-resistant coagulase-negative staphylococci without any evident source. The patient also suffered from deterioration of her renal function, edema and severe hypertension. The patient's medical history was uneventful until October 1998 when she had a subarachnoidal hemorrhage due to an aneurysm. Three months after clipping a VP shunt was implanted. The peritoneal had to replaced by an atrial part 2 weeks later. Now the patient did not improve in spite of adequate therapy. The hypothesis of shunt nephritis was generated though there was no evidence of shunt malfunction on the CT scans at admission. Pleocytosis, elevated protein and decreased glucose were found in the lumbar tap, but no bacteria. Complement factors C3 and C4 were decreased, circulating immune complexes and proteinase-3 antibody were elevated. Consequently the complete shunt system was removed. *S. epidermidis* was cultivated from all intraoperative materials. With respect to the superb penetration of linezolid into the CSF fluid and the impaired renal function the antimicrobial treatment consisted of linezolid 600 mg bid and 10 mg vancomycin administered intraventricularly. Immediately after surgery the patient's neurological condition improved. Two weeks later hydrocephalus developed. CSF fluid to monitor therapy prior to the insertion of a new shunt could only be gained by a suboccipital tap: Pleocytosis and hypoglycorrhachia had resolved, protein was normal, there was no bacterial growth. Thus a ventriculoperitoneal shunt was inserted. Six weeks after removal of the shunt creatinin has decreased from 4.5 to 2 mg/dL, the complement factors C3 and C4 are within the normal range, circulating immune complexes and proteinase-3 antibody are decreasing. In conclusion, improvement in renal function after removal of the contaminated shunt is the rule and occurred also in this patient, therefore no renal biopsy was performed. Therapy of bacterial meningitis due to resistant enterococci with linezolid was successful recently. Linezolid therapy for 4 weeks was also effective and well tolerated in this case of shunt infection and shunt nephritis.

P1509 Colonization/infection of ventriculoperitoneal shunt (VPS) by *Propionibacterium acnes*: a frequent cause of VPS failures

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Background: Study of cases of repeated ventriculoperitoneal shunt (VPS) failures and shunt colonization by Propionibacterium acnes.
Methods: Inclusion criteria: adult patients with VPS and multiple cerebrospinal fluid shunt failures and *P. acnes* CSF isolations. We have studied number of shunt revisions, intervals between revisions, symptoms, characteristics of CSF,

microorganisms isolated of CSF, and treatment of *P. acnes* VPS colonizations. All CSF were immediately processed and cultivated in aerobic and anaerobic media.

Results: *P. acnes* was isolated from 11 patients with multiple VPS failures.

- Numbers of revision: 4.4 (r. 2–8).
- Time of VPS failure: 4–46 months.
- Preoperative diagnosis: sterile VPS malfunction (100%).

There are a progressive shortening of the intervals between revisions.

Clinical presentation: Eight patients showed clinic of valve malfunction (headache, somnolence), and five abdominal pain. No fever. CSF showed no abnormalities. All patients were treated with intravenous penicillin (50.000 UI/kg q4h) during 15–21 days, removal of VPS, placed an external ventricular drainage device, and a new VPS implantation. No relapse or VPS malfunction were observed during a monitoring time of 30 months (*r. 12–46 m*).

Conclusions:

1 This results suggest that the colonization of VPS by *P. acnes* is associated with multiple VPS failures.
2 In this cases CSF cultures in aerobic and anaerobic media are warranted.
3 Replacement of the all shunt components and appropriate intravenous antimicrobial treatment are necessary.

P1510 Safety of heart-lung machines during prolonged stand-by

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Objectives: Heart–lung machines (HLM) are increasingly used 'off-pump' in open heart surgery. The tubes are discarded after every single operation even when the HLM has not been used. This represents a loss of $1500 for the disposable tubes and a loss of time for HLM installation. A prolonged stand-by period for HLM would therefore save time and money. We investigated whether the fluid circulating in the tubes remains sterile up to 72 h.

Methods: Samples of intratube fluid were taken under aseptic conditions after 0, 12, 24, 36, 48 and 72 h. Samples from the fluid bags were also taken. Microbiological testing was performed using a combined method of direct inoculation and membrane filtration (Steritest System, Millipore®). Endotoxin levels were measured in order to rule out any contamination.

Results: Four machines of the type SARNS 9000 were examined. Within an incubation time of 14 days, no (0%) bacterial growth was observed in the 44 samples taken from the tubes. The endotoxin results are pending.

Conclusion: HLMs can be kept in stand-by for up to 72 h, given aseptic assembly and handling of the tube system. The prolonged use of HLMs will save time and considerable amounts of money.

P1511 Micro-organisms from peritoneal dialysates inpatients with continuous ambulatory peritoneal dialysis

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Objectives: To evaluate the frequency of microorganisms isolated from peritoneal fluids patients with continuos ambulatory peritoneal dialysis (CAPD)

Methods: From CAPD patients with peritonitis symptoms 137 peritoneal fluids samples were collected. The specimens were inoculated on solid media after 10-min centrifugation. Aerobic, anaerobic and broth cultures in Bact/Alert (Organon Teknika) were started. The pellet was Gram stained.

Results: In the period from January to November 2001, 49 from 137 dialysates were positive (37.5%), six of the positives were polymicrobial. Following microorganisms were obtained: *Staphylococcus epidermidis* (20), *Enterobacter cloacae* (3), *S. aureus* (3), *S. viridans* (3), *Klebsiella oxytoca* (2), *Acinetobacter* sp. (2), *Pseudomonas* sp. (2), *Enterococcus faecalis* (2), *E. faecium* (1), *Pseudomonas aeruginosa* (1), *Serratia marcescens* (1), *Citrobacter braakii* (1), *Morganella morgannii* (1), *Proteus mirabilis* (1), *S. agalactiae* (1), *Aeromonas* sp. (1), *Bacteroides ovatus* (1), *Fusobacterium nucleatum* (1), *Candida albicans* (4) *C. tropicalis* (3). In polymicrobial infection: two *S. epidermidis* (doxycycline resistant and sensitive), *S. epidermidis* + *S. aureus*, *K. oxytoca* + *S. agalactiae*, *S. marcescens* + *C. tropicalis*, *E. faecium* + *C. albicans*.

Conclusion: The most prevalent etiologic agents of peritonitis inpatients with CAPD were *S. epidermidis* (20), *C. albicans* (4), *S. aureus* (3), *S. viridans* (3) and *C. tropicalis* (3). Utilization of different solid and broth media enabled good recovery of various microorganisms and appropriate antibiotic therapy based on microbiologic results.

P1512 Analysis of microbial causes of peritoneal dialysis-related peritonitis

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Background: Peritonitis remains a common and serious complication of PD. Because of the increasing antimicrobial resistance of isolated microorganisms some authors emphasize that current antimicrobial therapy procedures needs to be revised. In this study, we evaluated the microbial causes of peritonitis in our PD program and analyzed their antibiotic susceptibilities.

Methods: During a 22-month period 186 positive cultures, isolated from 152 patients' periton dialysate samples, were evaluated by means of microbial agents and their susceptibility patterns.

Results: The culture positive peritonitis rate was 0.66%/patient/year. There were 198 microorganisms isolated from 186 positive samples. The rate of infections caused by multiple pathogens was 6.4%. The ratio of Gram-positive, Gram-negative and fungal pathogens were 71.7, 26.2, and 2.1, respectively. *S. epidermidis* and *S. aureus* were the most common causes of peritonitis isolated in 47.7 and 12.6% of culture positive cases. *P. aeruginosa*, *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were the most common causes of Gram-negative peritonitis identified in 6.5, 5.0, 4.5, and 4.0% of culture positive cases, respectively. Methicillin resistance to *S. aureus* and *S. epidermidis* were 8 and 35%, respectively. All methicillin resistant strains were sensitive to vancomycin and 70% of them were sensitive to gentamicin. All Enterobacteriaceae strains isolated were sensitive to imipenem. Cefepime, ceftriaxone, cefoperazone, cefazoline susceptibilities of these isolates were: 85, 78, 74, and 52%, respectively. Also 85% of the isolates were sensitive to ciprofloxacin and 92% to gentamicin and amikacin. None of the *P. aeruginosa* isolates were resistant to ceftazidime, cefepime, imipenem and piperasilin tazobactam. One strain was resistant to aminoglycosides (amicasin, tobramycin, netilmicin), and another to ciprofloxacin.

Conclusions: Our data showed a significantly low rate of antimicrobial resistance in the strains isolated from peritonitis patients compared to the nosocomial isolates. According to what we have found we insist on the ampiric intraperitoneal administration of a cephalosporin and aminoglycoside, inspite of combinations including vancomycin in the management of peritonitis unless the previous profile of the microbial distribution and antimicrobial susceptibility patterns of peritonitis are known.

P1513 Biofilm-producing pathogens isolated from patients affected by metabolic disorders

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Objectives: Patients affected by metabolic disorders are easily infected by a wide range of opportunistic pathogens causing severe clinical symptoms. In many cases these bacteria are resistant to antibiotics and produce biofilm, factors that might increase the risk of a therapeutic failure. In this study, we have analyzed the pathogens isolated from patients affected by different metabolic disorders (hyperaminoaciduria, hypercholesterolemia, sugar intolerance, cystinuria, hyperlactacidemia, gammopathy, homocystinuria, calculosis).

Methods: the presence of microorganisms in the urine of patients affected by metabolic disorders was explored and the biofilm production was verified in Congo Red agar plates, according to the method described by Freeman.

Results: among 18 patients studied, 10 demonstrated bacterial growth of one or more species from urine samples. The microorganisms isolated showed in five cases the presence of biofilm-producing organisms, they were: *Klebsiella ornithinolytica*, *Proteus mirabilis* (two strains), *Alcaligenes faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, *S. simulans*, *S. haemolyticus*, *S. epidermidis*. Considering their metabolic disorders, four patients resulted affected by impaired sugar metabolism and one suffered hypercholesterolemia. Biofilm production was observed in one or more species isolated from the same patient affected by

these latter metabolic disorders. In no case, we detected biofilm producing and non producing strains from the same patient.
Conclusions: The present findings indicate that patients affected by disorders in sugar metabolism are more easily infected by biofilm-producing pathogens, while inpatients with other metabolic diseases, microorganisms involved did not produce slime. Further studies to detect factors that facilitate the colonization of these patients by biofilm producing strains are under way.

P1514 Ventilator associated pneumonia in intensive care units

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Introduction: The aim of this study was to evaluate the incidence, risk factors, etiologic agents, antimicrobial susceptibility, source of agents and mortality rate of ventilator associated pneumonia (VAP) in ICU.
Methods: The adult patients who received more than 48 h ventilatory support in four intensive care units of government hospital were evaluated prospectively. Among the consecutive 100 patients who had received ventilatory support, 25 were excluded from study as they died or separated from ventilator in 48 h. VAP was defined according to presence of CDC criteria. Etiologic agents were detected by quantitative culture of endotracheal aspirate and antimicrobial susceptibility tests were performed by disk diffusion test. Age, sex, accompanying diseases (such as COPD, DM), surgical procedures, nutritional status, antibiotic usage, APACHEE II, approach to gastrointestinal protection, period of ventilation were evaluated as the risk factors for VAP. Cultures were taken from each ventilator to detect the source of infection. Student's *t*, Mann–Whitney *U* and Kaplan–Meier log range tests were used for statistical evaluation.
Results: VAP was diagnosed in 26 of 75 patients (32%). There were no statistical difference between age, sex and APACHEE II of VAP(+) and VAP(–) patients. Relevant risk factors were surgical procedures, usage of H2 receptor blocker, duration of mechanical ventilation ($P < 0.001$). Etiologic agents of VAP were polymicrobial in 46% of patients and *Acinetobacter* spp. isolated in 27.5%, *Pseudomonas* spp. in 20%, MRSA in 22.5% and *Klebsiella pneumonia* in 10% of aspirate cultures. Gram-negative bacteria were multiresistant at the rate of 85%. Any contaminated ventilator was detected for being the source of these agents. Mortality rate of patients with VAP (100%) was significantly higher than that of VAP(–) patients (85%) ($P < 0.05$).
Conclusion: VAP is the most important complication of mechanical ventilation in ICU and the role of VAP on mortality rate is important. Surgical procedures, usage of H2 receptor blocker, duration of mechanical ventilation are the risk factors of VAP. Multiresistant Gram-negative bacteria and MRSA are the main problem microorganisms in VAP in ICU. It was concluded that the education of healthcare workers on hand washing and contact isolation precautions will help to decrease the incidence of VAP in ICU.

P1515 Ventilator associated pneumonia (VAP) in a university hospital

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Objective: To evaluate the rate of ventilator associated pneumonia (VAP) in the four intensive care unit (ICU) and determine causative pathogens and their susceptibility patterns.
Methods: Baskent University Hospital is a tertiary care hospital having 23 beds in ICU. All patients intubated in four ICU (neurology–neurosurgery, internal medicine, surgery–reanimation, and cardiovascular surgery) during one month surveillance period were enrolled. We used diagnostic criteria of CDC for diagnosis of VAP. Samples of deep endotracheal aspiration taken from patients cultured quantitatively in serial dilutions of 10–5 mL. Antibiotic susceptibility testing was performed by disk diffusion method according to the NCCLS standards.
Results: During the study period 43 patients were intubated in four ICU. Ten of them developed VAP. VAP rate was 43.2 per 1000 ventilation days. Four of the patients with VAP have other accompanying infections (two urinary tract, 1 surgical site, 1 necrotizing fascitis). VAP developed at mean 6.9 ± 5.3 days after intubation (median 7). In infected group 80% of patients had one or more underlying chronic disease such as diabetes mellitus, cerebrovascular disease and transplantation while this percentage was 39.3% in noninfected patients. Mean APACHE score was 9.60 ± 5.02 in infected group and 6.88 ± 4.14 in noninfected group. There was no APACHE score and mean age difference between infected and noninfected groups ($P > 0.05$, Mann–Whitney *U*-test). Isolated pathogens were *Acinetobacter* spp.(4), *E. aerogenes* (2), *Pseudomonas* (2), *Morexella* spp. (1), MRSA (1). We observed that three of four *Acinetobacter* spp. were resistant to meropenem, amikacin, ciprofloxacin, ceftazidime, piperacillin–tazobactam and susceptible to cefepime and sulbactam–cefoperazone and one of the two *Pseudomonas* was resistant to all tested antibiotics. Both of *E. aerogenes* were resistant to ciprofloxacin, amikacin and ceftazidime. Mortality rate attributable to VAP was 30%.
Conclusion: Reported rate of VAP commonly 10–15 cases per 1000 ventilator days based on a study population. We detected higher rate of VAP. Although there was no difference in APACHE score between the groups, high rate of underlying disease in infected group might have contributed to this high rate of VAP. We also observed a very high antibiotic resistance.

P1516 Quantitative cultures of bronchoalveolar lavage fluids (BAL) in mechanically ventilated patients

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Objective: The purpose of this study was to perform quantitative bacterial cultures on bronchoalveolar lavage fluids obtained from mechanically ventilated patients from Intensive Care Unit.
Methods: Two hundred bronchoalveolar lavage specimen from patients (20–60 years) requiring mechanical ventilation for 3–32 days, were quantitatively cultured for aerobic, anaerobic microorganisms and fungi in order to confirm any specific infection of lower respiratory tract. Concentration 10^4 cfu/mL was considered as a positive culture. Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method according to NCCLS guidelines.
Results: From 200 BAL cultures a percentage of 39% were sterile. From positive cultures a percentage of 20% grew *Acinetobacter anitratus*, 12% *Pseudomonas* spp., 18% *Enterobacteriaceae* (17 specimens *Klebsiella pneumoniae*, five *Escherichia coli*, eight *Enterobacter* spp., six *Serratia* spp.), 2% *Enterococcus* spp., 5% *Staphylococcus aureus* and 4% *Candida* spp. (eight specimens *Candida albicans* and two *C. glabrata*). The 78% of the strains of *Acinetobacter anitratus* and *Pseudomonas* spp., were susceptible to aminoglycosides and 50% to aminoglycosides and penems. Twenty-eight percent of Gram(–) bacteria were susceptible to aminoglycosides and penems and 55% of the *Staphylococcus aureus* strains were susceptible to teicoplanin-vancomycin. Seventy percent of the *S. aureus* strains were resistant to methicillin.
Conclusion: The infections in ICU patients are caused by multiresistant strains and the mortality is high. Therefore, the identification of lower respiratory tract infections by bronchoalveolar lavage fluids has a great diagnostic value.

P1517 Inhaled ampicillin/sulbactam against *A. baumannii* in bronchial secretions of ICU patients: a preliminary report

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Introduction: *Acinetobacter baumannii* is an important cause of nosocomial pneumonia, particularly in the intensive care unit (ICU) setting. Ventilator-associated pneumonia (VAP) caused by *A. baumannii* carries a high (43%) attributable mortality.
Objectives: The aim of this study was to assess the effectiveness of aerosolized ampicillin/sulbactam in ICU patients with multiresistant *A. baumannii* colonization of the respiratory tract.
Methods: Eleven intubated patients on mechanical ventilation participated in the study. Multiresistant *A. baumannii*, sensitive only to ampicillin/sulbactam, was isolated from the bronchial secretions ($10^7 - 10^8$ cfu/mL). All 11 patients were subsequently treated with intravenous ampicillin/sulbactam, whereas eight of them were also given the same antibiotic in aerosolized form.

Quantitative cultures of bronchial secretions, routine blood chemistry, as well as chest radiographs were obtained on a daily basis.
Results: A decrease (from 10^8 to 10^2 cfu/mL) in the number of *A. baumannii* colonies was observed, following 2 to 4 days of combined treatment with both intravenous and inhaled ampicillin/sulbactam. None of the eight patients developed VAP. In the three patients who only received the antibiotic intravenously, a decrease from 10^8 to 10^5 or 10^4 cfu/mL was observed after 7 days of treatment. One of the three patients developed VAP.
Conclusions: Our results suggest that the administration of aerosolized ampicillin/sulbactam represents an effective means of preventing ventilator-associated pneumonia caused by *A. baumannii*.

Catheter-related infections

P1518 Impact of antiseptic impregnation on central venous catheters: the rate of microbial colonization of antiseptic impregnated catheters

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Objectives: Prospective trials have shown that antiseptic impregnation of central venous catheters reduce the incidence in catheter associated infections. The aim of this study was to investigate the microbial colonization-rate between a nonimpregnated catheter (ARROW-HOWS™, Crosstec; type A), a silver-submicron impregnated central venous catheter (SICURIS™, Siemens; type B) and a new catheter with small amounts of silver-sulfadicin and chlorhexidine on its surface (ARROW gard Blue™, Crosstec; type C).
Methods: After removing the catheter, the distal segment was cut to a length of about 3–4 cm under aseptic conditions. The microbiological treatment consisted of quantitative analysis of colonization from the catheter-outside and qualitative analysis including the whole tip.
Results: A number of 400 catheters were investigated during a period of 18 months. By comparing the rate of colonization of the three catheters (A, B, C) significant differences were found ($P < 0.05$). Types A and B catheters represented the maximum-rate of microbial colonization (type A 32/125; type B 41/146). Type C appeared to be the catheter with the lowest number of colonization (14/129). Microbiological investigations showed coagulase-negative staphylococci with a rate of 71% of isolates followed by Gram-negative rods with 15%, *Staphylococcus aureus* with 7% and yeasts representing 7%.
Conclusion: The results of this study demonstrate that impregnation with silver-sulfadicin and chlorhexidine appear to protect the catheter from bacterial contamination.

P1519 Retrospective study on microbial colonization of central vessel catheters in pediatric ICU patients

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Objectives: Our targets were to determine the isolation rate of microorganisms which were recovered from central vessel catheters from ICU patients aged between 15 to 18 years, as well as their resistance to antibiotics.
Materials and methods: A total of 276 samples of central vessel catheters from ICU patients were sent for culture to our laboratory during the last decade (1991–2000). Catheters were removed from patients 3–15 days after their introduction. Culture was performed by the Maki semiquantitative method and all samples, after enrichment in cooked meat broth, were subcultured on selective media. All microorganisms were identified by classical microbiological methods and susceptibility to antibiotics was tested according to NCCLS instructions.
Results: A total of 114 cultures were positive (41.3%) and 162 were negative (58.7%). One microorganism was isolated in 87 samples (76%), two microorganisms in 19 samples (17%) and three or more microorganisms in eight samples (7%). The microbes most commonly isolated were the following: *Staphylococcus* co (48%), *Candida* spp. (17%), *Acinetobacter* spp. (6%), *Klebsiella* spp. (5.5%), *Enterobacter* spp. (5%) and *Enterococcus* spp. (5%). Resistance to antibiotics of *Staphylococcus* was as follows: erythromycin (55.5%), oxacillin (52.8%), clindamycin (45.8%), gentamicin (43%), netilmicin (30%), ciprofloxacin (27.8%), amikacin (18%), rifampicin (11%), and tetracycline (4%). Increased resistance to several antibiotics was also observed for *Acinetobacter* spp. isolates.
Conclusions: CNS and *Candida* are still the commonest microbes colonizing central vessel catheters from ICU pediatric patients. The high colonization rate found in these patients might be due to the difficulty to replace catheters. Regular replacement of central catheters may contribute to the decrease of blood infections induced by catheters.

P1520 Bacterial species isolated from central venous catheter-tips and the susceptibility to antibiotics

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Objectives: To determine the bacterial species isolated from central-venous catheter tips in a 3-month period from ICU patients of the hospital. The resistance pattern also was studied.
Materials and methods: A total of 415 catheter tips from ICU patients were investigated by the semiquantitative (Maki) method. Identification and susceptibility testing (MIC) were performed by semi automated system (Pasco, Difco Laboratories). For resistance determination of staphylococci and enterococci to glycopeptides E-test was used.
Results: A total of 200 (48.2%) clinical strains were isolated (see table).

Gram-positive cocci	85	Gram-negative bacilli	115
Coag. neg. staphylococci	56	*A. baumani*	29
S. epidermitis	13	*K. pneumoniae*	17
Enterococcus spp.	10	*S. maltophilia*	15
S. aureus	9	*P. mirabilis*	13
		C. violaceum	12
		P. shigelloides	8
		E. cloacae	7
		P. aeruginosa	7
		P. fluorescens	3

C. violaceum and *S. maltophilia* were the most resistant. GISA/GISE strains have not been isolated. Imipenem and vancomycin remain the more effective antibacterials against Gram-negative and Gram-positive pathogens, respectively.
Conclusion: The percentage of bacterial colonization of venous catheters in ICU is high. The increasing resistance of the bacteria to antibiotics is the major problem of ICU in spite of restricted policy use of antibiotics in our institution.

P1521 Reduction of central venous catheter-related infection (CRI) in cancer patients

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Background: Central venous catheters (CVCs) impregnated with chlorhexidine and silver-sulfadiazine (CH-SS) CH-SS have recently been introduced for the prevention of CRI. The purpose of this study was to assess the efficacy of CH-SS impregnated CVCs (ARROW gard Blue Plus™) to prevent CRI in patients with hematological malignancies undergoing chemotherapy followed by severe neutropenia (<100/μL). Proven CVC-related blood stream infection (BSI) was defined as the isolation of the same species from peripheral blood culture and CVC-tip (>15 cfu Maki technique).
Methods: A randomized, prospective clinical trial was carried out in consecutive cancer patients to compare CRI using an CH-SS impregnated CVC ($n = 51$) with that using a standard uncoated triple-lumen CVC ($n = 55$)

between March and October 2000. Patients were treated for leukemia ($n = 92$), lymphoma ($n = 10$), myeloma ($n = 3$), and myelodysplastic syndrome ($n = 1$).
Results: Study groups were balanced regarding to sex, age, underlying disease, insertion site, and duration of severe neutropenia. The CVCs were in-situ a mean of 14.3 (SD 8.2) d in the study group versus 16.6 (SD 9.7) d in the control arm. CVC tip colonization was observed less frequent in the study group (7 patients vs. 17 patients; $P = 0035$). CVC-related BSI were significantly less frequent in the study group (1 patients vs. 8 patients; $P = 0.02$). All CVC-related BSI infections were related to *Staphylococcus* spp.
Conclusion: In patients with severe neutropenia, CH-SS impregnated CVC yield a significant antibacterial effect against *Staphylococcus* spp. Resulting in a significantly lower rate of CVC tip colonization as well as CVC-related BSI.

P1522 Taqman quantitative PCR as a promising tool for the diagnosis of staphylococcal catheter infections

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Objectives: Coagulase negative staphylococci (CNS) are the main cause of catheter related bloodstream infections. Quantitative catheter culture (QCC) is the gold standard for diagnosing catheter infections. The current study compares the reliability of QCC and a newly developed method based on quantitative PCR (QPCR) in the diagnosis of catheter infection.
Methods: One hundred and two catheter segments were inoculated in vitro with *S. epidermidis* and implanted subcutaneously in rats. After 2 weeks, all catheters were removed. QCC of 56 catheters was performed after sonication of the samples as previously described. Cultures were counted after 1, 2 and 5 days. Instant RNA and DNA isolation was performed for the other 56 catheters using a FastPrep™ based protocol. Both the gmk (guanylate kinase) and 16S gene were quantified on the RNA (translated in cDNA; to check for viable bacteria) and genomic DNA (gDNA) sample with Taqman quantitive PCR. The theoretical detection limit was 20 cfu for QCC and 750 copies of gDNA for QPCR.
Results: QCC yielded an average of 1.4×10^5, 2.2×10^5 and 3×10^5 cfu after, respectively, 1, 2 and 5 days of incubation. QPCR yielded an average of 1.8×10^7 copies of gmk gDNA at the day of catheter explantation. These differences were significant ($P < 0.0001$; Kruskall–Wallis test). When comparing QPCR and QCC, QPCR had higher yield (Dunns Multiple Comparison; $P < 0.001$) and a lower variability (Bartlett test for equal variances; $P < 0.001$) than QCC. When using QCC, 22, 19, and 5 catheters were negative after, respectively, 1, 2 and 5 days. When using QPCR, just 1 catheter was negative for both gmk RNA and DNA. There was no RNA/gDNA mismatch in the QPCR group. Significantly more catheters were negative with QCC counted after 1 and 2 days than with QPCR (chi-square; $P < 0.001$).
Conclusion: QCC has an only moderate reliability in the diagnosis of catheter infections, especially when plates were just cultured for 1 or 2 days, or when high dilutions were used. Instant nucleic acid extraction with QPCR is a promising method for the diagnosis of catheter infections, with a higher sensitivity, a higher yield, and a smaller intersample variability. The clinical usefulness has however, yet to be established.

P1523 Successfully treated *Candida* CVC-related pneumonia in an immunocompromised patient

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Objectives: *C. albicans* and *C. tropicalis* are common agents of sepsis and secondary hematogenous pulmonary involvement even in immunocompromised patients, but *Candida pneumonia* is unusual. Imidazole antifungal agents, such as ketoconazole, fluconazole, itraconazole and voriconazole, have been successfully used in small series against some *Candida* species. Here, we describe a peculiar case of primary mycotic pneumonia in an immunocompromised patient. Clinical case: L.V., Caucasian male aged 14, affected by a metastasised EBV-related rhynopharyngeal carcinoma. After application of a Central Catheter he was treated with chemo and radiotherapy. He developed bone marrow aplasia; under prophylactic treatment with fluconazol and nevertheless he had a mucosal colonization due to a *Candida albicans* and a *C. cruzei*. An episode of FUO was successfully treated with ceftazidime, amikacin, teicoplanin. Three days later, fever with chills reappeared. Central and peripheral blood cultures were positive for *Candida albicans* and crusei, resistant to Fluconazole. An X-ray film showed a bilateral pneumonia. The CVC was removed and Ampho B plus Flucitosine were started. After 15 days clinical and X-ray pictures were unchanged. Antibiotics were stopped and treatment was replaced with Voriconazole, 4 mg/kg/day. Fever disappeared after 5 days and gradually the radiological picture improved. Two weeks later a new CVC was applied and CT started again. Voriconazole was continued orally and the patients definitely improved.
Conclusions: CVC-related mycotic infections in aplastic oncologic patients may show a good response to the treatment if it is appropriately and quickly started, and if the source is removed.

Clostridium difficile

P1524 PCR-ribotyping of clinically important *Clostridium difficile* strains from Hungary

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Objectives: Detection of the source of toxin-producing *Clostridium difficile* strains is of great importance for the control of the nosocomial spread of this microorganism. Many different techniques have been employed to help to determine genetic relationship among hospitally acquired *C. difficile* isolates.
Methods: Isolates of *C. difficile* originated from different hospital wards of University Hospital of Szeged were typed by PCR amplification of rRNA intergenic spacer regions (PCR ribotyping). The aim of our study was to determine: (1) whether isolates are genetically similar to *C. difficile* isolates collected in United Kingdom, Anaerobe Reference Unit, Cardiff during an international surveillance; (2) which are the most common ribotypes of *C. difficile* in our hospital.
Results: A total of 15 different ribotypes were detected among the 65 isolates tested. The most predominant PCR ribotype in the Hungarian survey of these isolates was 087, which accounted 39% of all isolates in contrast with international typing study where the the most common type was ribotype 001. We found two nontoxigenic *C. difficile* strains showing the same pattern, which were distinct from all the ribotypes previously described, suggesting a new type.

P1525 Clindamycin resistance (*ermB*), toxigenicity and genotype of *Clostridium difficile* strains isolated from patients with antibiotic associated diarrhea (AAD)

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Background: High level resistance to clindamycin is related to the presence of the *ermB* genes. We observed that isolated *C. difficile* strains highly resistant to clindamycin are belonging to different toxinotypes. The aim of the study was to define the relationship between clindamycin resistance, toxigenicity and genotype od *C. difficile* strains isolated from AAD.
Material and methods: Thirty-five *C. difficile* strains isolated from patients with AAD hospitalised in five units of the academic hospital have been studied. For comparative reasons we used *C. difficile* reference strains. Toxigenicity of the isolates was determined by means of EIA, cytotoxicity assays and PCRs for the detection the nonrepeating sequences and repeating sequences of toxin A and B genes. Susceptibility to clindamycin was investigated using the E-test. Detection of the *ermB* gene was performed by PCR using the 2980/2981 primer pair. Ribotyping was performed by PCR amplification of rRNA intergenic region spacer.
Results: Among 35 strains of *C. difficile* isolated from patients with AAD 26 strains belonged to toxinotype A+B+ and nine strains belonged to

toxinotype A − B+ 0. PCR ribotyping generated 13 different ribotypes. Within isolates A − B+, two differrent types of *C. difficile* strains were defined: eight strains of ribotype A and one strain of ribotype E. Within toxinotype A + B+, we observed high differentiation among ribotypes A-M. Among 35 strains of *C. difficile* 22 were high level resistant to clindamycin (63%). Interestingly most strains representing highly resistance to clindamycin belonged to ribotype A. All strains A − B+ were higly resistant against clindamycin. All strains resistant to clindamycin harbored *ermB* gene.
Conclusion: Different toxinotypes od *C. difficile* strains were observed among clindamycin resistant strains. Among clindamycin resistant strains a single ribotype dominates.

P1526 Evaluation of immunoassay methods for detection of *Clostridium difficile* toxins

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Objectives: To evaluate four commercial enzyme immunoassay methods (EIA) and the culture for the diagnosis of *C. difficile* associated diarrhea (CDAD).
Methods: The detection of toxins in the stools was performed in 114 samples from 103 patients by 4 EIAs: CdTOXA OIA ($n = 114$); Vidas *C. difficile* Toxin AII ($n = 114$); Premier Toxins A and B ($n = 89$); Immunocard Toxin A ($n = 23$). The reference assay was the demonstration of stools toxicity (ST) in cell lines Hep2. The stool culture ($n = 114$) was carried out on cycloserine-cefoxitin-fructose agar and the toxigenicity of the isolated strains was determined by the reference assay (strain citotoxicity-SCT) and EIA Vidas. Toxin A and B genes were examined by PCR method ($n = 28$). PCR ribotyping was also performed in 28 strains.
Results: Reference assay (ST) was positive in 28 (24%) of the samples and 36 (31.6%) were positive in some of the 4 EIAs. Sensitivity (S) and specificity (SP) of EIAs were, respectively: CdTOX A, 82 and 95%; Vidas: 89 and 96%; Premier, 84 and 95%; Immunocard, 76 and 100%. The percentage of false-positive and false-negative were, respectively: CdTOXA, 14.8%and 5.7%; Vidas, 11.1 and 3.5%; Premier, 12.5 and 6.1%; Immunocard, 0 and 23.1%. *C. difficile* was isolated from 31 (27%) specimens (S: 89%; SP: 94%). The mean correlation between the culture and EIAs was 83.5% (range: 78–88%). In the four EIAs, the mean percentage of samples with negative result and positive culture was 16.6% (range 9.3–30.7%). The mean percentage of toxigenicity of these strains was 61.4% (range 44.4–75%). In 26 (92%), *C. difficile* strains toxin A and B genes were detected and none were detected in the remaining two. Thirteen different ribotypes were detected, 43 percent corresponding to types 1, 20 and 78. Two ribotypes had never been described before and they were assigned as new types 126 and 127.
Conclusions: Except for Premier, EIAs were sensitive, rapid and easy to use for the diagnosis of CDAD. The culture and demonstration of strain toxigenicity in cell lines have improved diagnosis of the samples with false negative results of EIAs.

P1527 MLSB-resistance in *Clostridium difficile* is associated with resistance to moxifloxacin

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Objectives: *C. difficile* remains the leading cause of nosocomial-acquired diarrhoea. *C. difficile* usually exhibits resistance against β-lactam-antibiotics, whereas susceptibility to others may vary. This study investigated whether antimicrobial susceptibility of *C. difficile* has changed in a defined time frame.
Methods: *C. difficile* strains ($n = 178$), recovered from patients in two University hospitals in Germany between 1986 and 2001, were investigated for their susceptibility to erythromycin (ERY), clindamycin (CLIN), moxifloxacin (MXF), vancomycin (VAN), and metronidazole (MET) by E-test. The genetic background for resistance was investigated using PCR and DNA-sequencing. One hundred fifty-six isolates were toxigenic strains, determined by PCR amplifying fragments of *tcdA* and *tcdB*.
Results: All strains were susceptible to VAN and MET. Resistance rates to ERY, CLIN, and MXF at different times are shown in the table. All but three strains exhibiting resistance to antimicrobials were toxigenic strains

	1986–1995 (n=46)	1996–2000 (n = 83)	2001 (n = 41)	Σ
Only MLS_B-R	14 (30.4%)	39 (47%)	21 (051%)	74
Only MXF-R	2 (4.3%)	11 (13.2%)	9 (22%)	21
MLS_B + MXF-R	0	10 (12%)	9 (22%)	19

According to DIN recommendations resistance was defined as follows: CLIN, >8 μg/mL; ERY, >8 μg/mL M; recommendations do not exist for MXF, the MIC value published for trovafloxacin is >4 μg/mL.
Conclusion: (A) The results indicate that prevalence of resistance against MLSB- and fluoroquinolone-antibiotics in *C. difficile* is increasing. (B) Fluoroquinolone resistance is associated to resistance against MLSB-antimicrobials. (C) Antimicrobial resistance might be related to toxigenicity. Due to the low number of nontoxigenic strains included in this study further analysis of nontoxigenic strains are required to prove this hypothesis.

P1528 Toxin A-negative toxin B-positive *Clostridium difficile* strains as aetiologic agents in antibiotic associated diarrhoea (AAD) among children hospitalised in a haematology unit

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Purpose: We report cases of antibiotic associated diarrhoea (AAD) due to A − B+ *Clostridium difficile* strains in a hematologic unit and discuss the implications of diagnostic testing in suspected cases of *C. difficile*-associated AAD.
Material and methods: Stool samples of 19 children (hospitalised between 1999 and 2000), suspected for *C. difficile* associated diarrhoea were investigated. For direct detection of toxin A in faecal samples and in isolated strains, the EIA 'Culturette Brand Toxin CD' TCD (Becton–Dickinson) was used. The cytopathic effect examined by bacterial culture supernatants on McCoy cell line was used for detection of toxin B. PCR for detection nonrepeating and repeating sequences toxin A gene and nonrepeating sequences toxin B gene was used.
Results: Directly in feces toxin A was detected in three cases by using EIA and 16 sapmles were toxin A negative. Any other enteric pathogens as *Salomonella, Shigella,* enteropathogenic *E. coli* and rotaviruses were not detected in these stools. Nine strains of *C. difficile* were isolated. Of these strains, three were toxin A positive as demonstrated by the TCD test. The remaining six toxin A negative strains demonstrated cytopathic effect on McCoy cells. The non-repeating regions of both toxin genes were detectable in all strains. The PCR product obtained upon amplification of the repeating unit of DNA isolated from A − B+ strains was the same the product derived from the reference strain A − B+ GAI 95601 (700 bp). By PCR ribotyping we distinguished four different types among nine *C. difficile* strains. Within A − B+ strains we distinguished two ribotypes (A and E).
Conclusion: A − B+ *C. difficile* strains are capable to cause AAD in children of hematology unit. Clinical laboratories which use test for detection only toxin A need to be aware that results may be falsely negative.

P1529 Detection of *Clostridium difficile* strains with a variant pathogenicity locus

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Objectives: The genes for toxins A and B (*tcdA* and *tcdB*) of *C. difficile* are part of a 19.6-kb genetic locus that includes 3 additional open-reading frames (ORFs), *tcdD, tcdE* and *tcdC*. The acquisition of this pathogenicity locus (PaLoc) is a prerequisite for virulence. *C. difficile* strains, variant for the pathogenicity locus, have been already described. Despite the modified PaLoc, these strains can still be associated with clinical diseases, but few epidemiological data have been reported. The difficulty in the identification of variant *C. difficile* strains is due to the inability to recognise these strains by the commonly used clinical tests. Infact,with the exception of few strains, both toxin A and B produced by variant strains react with commercial kits for

toxin detection. Therefore, PCR-based methods seem the most appropriate approach to identify *C. difficile* strains with a variant PaLoc.
Methods: In this study we analyzed 49 toxinogenic *C. difficile* strains to identify variant strains for the pathogenicity locus and to investigate this genetic unit. *C. difficile* strains were examined by three PCR-based methods: a PCR multiplex assay detecting the three accessory genes *tcdD, tcdE* and *tcdC* together with *cdu2* and *cdd3* that are located upstream and downstream the PaLoc; a RFLP-PCR differentiating *C. difficile* strains according to changes in their toxin genes when compared to the reference strain VP I10463; a specific PCR assay for the detection of the genes encoding the enzymatic (*cdtA*) and the binding (*cdtB*) components of the binary toxin, an actin-specific ADP-rybosyltransferase recently found in the majority of variant strains.
Results and conclusion: We identified nine (18%) *C. difficile* strains with a variant PaLoc and seven of these strains, variant for the toxin A and B genes, could be classified in already known toxinotypes: four belonged to toxinotype V and three to toxinotype VI. One of the other two strains was variant only for the *tcdC* gene and the other for the *tcdC* gene plus some minor modifications in the toxin A and B detected by the RFLP-PCR. This last strain may represent a new toxinotype. All the strains with variations in toxin A and B genes produced also the binary toxin suggesting a vicarious role for the latter.

P1530 Multicenter typing analyzes of *Clostridium difficile* isolates from geographically diverse hospitals in Japan

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Objectives: *Clostridium difficile* is well known as the principle pathogen causing nosocomial diarrhea. Recent reports have documented particular types of *C. difficile* are associated with active diseases. We investigated isolates from *C. difficile*-associated diarrhea (CDAD) in geographically diverse hospitals in Japan.
Methods: A total of 183 *C. difficile* from six hospitals epidemiologically unrelated were examined. Toxigenicity of isolates was screened by PCR detecting the toxin A and B genes and determined by enzyme-linked immunosorbent assay for toxin A production and cell culture assay for toxin B production. Two typing systems, PCR amplification of rRNA intergenic spacer regions (PCR ribotyping) and pulsed-field gel electrophoresis (PFGE), were used to type *C. difficile* isolates.
Results: A single PCR ribotype, type smz, accounted for 47% of the total isolates and was predominant at all six hospitals examined in the range of 31 to 65%. All isolates typed as PCR ribotype smz were not typeable by PFGE because of DNA degradation. Although 14 isolates which were identified as PCR ribotype gr corresponding to the type endemic in US and UK, were recovered from five of the six hospitals, this type did not predominate at any of the hospitals examined in Japan. Of the 183 isolates tested, 30 isolates were identified as toxin A-negative, toxin B-positive (A − B+). A − B+ isolates were recovered at all six hospitals, and accounted for 3, 4, 8, 11, 18, and 39%, respectively. At the hospital with the highest prevalence of A − B+ isolates, the 19 A − B+ isolates represented two types by PCR ribotyping, types fr and trf. All 12 isolates which were typed as trf were classified into the same major PFGE type (defined by three or fewer than three fragment differences), but the remaining seven fr isolates displayed different seven PFGE patterns.
Conclusions: These typing results indicate the endemic strain (PCR ribotype smz) may have specific potential for nosocomial infection in Japan. A − B+ *C. difficile* was recovered from the all six hospitals, indicating isolation of A − B+ from CDAD patients is not rare. In addition, typing analysis by PFGE suggests that there was a nosocomial spread of a clone of A − B+ at a hospital.

Bacteremia and endocarditis

P1531 Blood cultures at a teaching hospital from 1997 till 2001

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Objectives: The aim of the study was to review and compare the results of blood cultures performed manually from 1997 until mid 1998 and automatically with Bactec 9120 (BD, Cockeysville, MD, USA) from mid 1998 onwards, and to determine any advantages of continuous blood culture monitoring in a laboratory manned only 8 h/day.
Methods: In 1997, all blood culture bottles were routinely subcultered after 24 h and 5 days on enriched blood agar, chocolate agar and *Brucella* agar plates and incubated at 37 °C in ambient air, with added 5% CO_2 and anaerobically, respectively. Blood culture bottles that were flaged positive by Bactec 9120 were subcultured using the same protocol as above. Also Gram stain was performed.
Results: In 1997, we determined 54 positive from 1126 blood cultures drawn. from 1998 till the end of October 2001 there were 118 117 105 and 100 blood cultures positive from 1318, 1385, 1315 and 1266 drawn, respectively. The positive rate has been constant (7–9%). The most common isolates have been *Streptococcus pneumoniae* ($n = 112$) and *Escherichia coli* ($n = 139$) followed by coagulase-negative staphylococci (CoNS) ($n = 69$) *S. aureus* ($n = 50$), enterobacteria ($n = 44$). Ninty-eight percent of first positive blood culture bottles were detected by 17 h after insertion into Bactec 9120. Most (78%) of the presumed contaminants were detected after more than 17 h of incubation.
Conclusions: With automated, continuous blood culture monitoring system, we determined more positive blood cultures than manually and time interval to detection has shortened. Number of presumed contaminating bacteria (CoNS, *Corynebacterium* sp., *Propionibacterium acnes*) increased in first year of employing automated system, but have come down again after auditing the blood drawing procedures.

P1532 *Moraxella lacunata* bacteremia in elderly patient with chronic obstructive pulmonary disease

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Objectives: The presentation of an unusual case of *M. lacunata* bacteremia in an elderly patient with aspiration pneumonia and chronic obstructive pulmonary disease.
Methods: Blood cultures were carried out by BACTEC system (Becton Dickinson). The identification of microorganism was performed by standard methods and API ID 32GN system. Susceptibility testing was performed by disc diffusion method. A 85-year-old woman was admitted to hospital due to an acute onset febrile episode (39 °C) with chills, general weakness and lethargy. Her past medical history included chronic atrial fibrillation, chronic obstructive pulmonary disease, vascular encephalopathy and recent myocardial infarction. On physical examination there was a supraventricular arrhythmia, low intensity bilateral wheezes over both lungs and mid-inspiratory crackles over the right lung base. Auscultation of the lungs also revealed a uniform diminution of normal breath sounds. No other abnormal physical signs were revealed except for several superficial decubitus ulcers over the skin of her lower back. Based on clinical and radiological findings a presumptive diagnosis of aspiration pneumonia was made. Laboratory tests showed mild leukocytosis (WBCs 11800/μL with 78% neutrophils) and hyponatremia (Na = 127 mEq/L). *M. lacunata* was repeatedly isolated from blood cultures obtained on day of admission. The strain was sensitive to penicillin, cefalosporins, aminoglycosides, tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazol. The pulmonary infection was treated with intravenous administration of cefoxitin (1 mg × 3), netilmicin (150 mg × 1), metronidazole (500 mg × 3) for 11 days and ciprofloxacin (200 mg × 2) for the next 9 days. The patient had a gradual recovery and was discharged in a good general condition without fever after 19 days of hospitalization.

Conclusion: *M. lacunata* which is an unusual cause of infections may be considered as one of the rare pathogens causing bacteremia in elderly and debilitated patients with other underlying diseases such as chronic obstructive pulmonary disease.

P1533 *Brevundimonas vesicularis* bacteremia in an elderly patient with underlying diseases

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Objectives: The presentation of an unusual case of *Brevundimonas vesicularis* bacteremia in an elderly patient with underlying diseases.
Methods: Blood cultures were carried out by BACTEC system. The identification of microorganism was performed by standard methods and API ID 32GN system. Susceptibility testing was performed by disc diffusion method. A 93-year-old woman was admitted to hospital with a 5-day history of productive cough and a 2-day history of pyrexia and general malaise. Her past medical history included mild chronic renal failure, cerebrovascular disease and cardiac failure. On physical examination she was pyrexial (38 C) and tachycardic and had signs of an upper respiratory tract infection. The radiological image raised a great suspicion of infection in the area of the lower left lung. Laboratory tests at admission showed WBCs 5300/μL with 87% neutrophils, Ht 33%, platelets 65.000/μL, creatinin 1.7/dL, Na 145 mEq/L, K 5.2 mEq/L, SGOT 238 U/L, SGPT 123 U/L, LDH 642 U/L, bilirubin 2.4 mg/dL, CPK 408 U/L (CPK-MB 34 U/L). Cefuroxime (750 mg × 3, i.v.) was administered to the patient. On day 3 the clinical picture was aggravated and netilmicin 300 mg × 1 i.v. was added. Based on newer clinical and radiological findings a diagnosis of pneumonia was made. The hematological and biochemical picture were considered compatible with septicemia. *Brevundimonas vesicularis* was isolated from blood cultures obtained on day of admission. The strain was sensitive to amoxicillin/clavulanic, cefalosporines, aminoglycosides, aztreonam, tetracycline and trimethoprim/sulfamethoxazol. The patient on day 6 died because of septic shock.
Conclusions: *B. vesicularis* is an unusual pathogen, which rarely involved in human infection. This is the first case report of *B. vesicularis* fatal septicemia in Greece. *B. vesicularis* may be caused rarely fatal septicemia in elderly patients with other underlying diseases.

P1534 Bacteremia in nonagenarians

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Objectives: To evaluate the characteristics of bacteremia in nonagenarian patients.
Methods: Prospective study of all episodes of significant bacteremia in patients over 90-year-old admitted to a 320-bed general hospital from 1983 to 2000. The mortality in this group of patients was compared with the mortality in other age groups in the same period. The functional status was assessed using the Barthel index in 70 patients.
Results: We studied 87 consecutive episodes of bacteremia in 93 nonagenarian patients (31% males, 69% females). A rapidly or ultimately fatal underlying disease was present in only nine cases (10%) and five patients had a solid or hematologic malignancy. A significant cognitive deficit (Reisberg scale >4) was present in 36 cases (41%). The Barthel index was <60 in 48%. The bacteremia was hospital-acquired in 27%, nursing home-acquired in 13%, and community acquired in 60% of cases. The main sources of bacteremia were: the urinary tract (41%), the respiratory tract (22%), the biliary tract (14%) and the skin-soft tissue (9%). The portal of entry was unknown in 7% of cases. The main causative agents were: *E. coli* (45%), *S. pneumoniae* (13%), *S. aureus* (10%), *E. faecalis* ((7%), *P. mirabilis* (7%), *P. aeruginosa* (6%), and *K. pneumoniae* (5%). Thirty percent of patients presented with altered mental status, 20% with shock and in 22% of cases the axillary temperature was <37.5 °C. The crude mortality was 18% and the mortality related to bacteremia was 14%. In a multivariate analysis, the presence of acutely altered mental status, shock, and *S. aureus* were the variables independently associated with a higher mortality. When the crude and bacteremia-related mortality in this age group was compared with the mortality in other age groups the figures were as follows: <65 year: 11 and 8%; 65–80 year: 21 and 15%; 81–90 year: 24 and 19%, >90 year: 18 and 14%.
Conclusions: (1) The main sources of bacteremia in nonagenarians were the urinary tract, the respiratory tract and the biliary tract. (2) Severe underlying diseases were relatively infrequent in these patients. (3) The presence of acutely altered mental status, shock, and *S. aureus* were risk factors independently associated with a higher mortality. (4) The mortality in nonagenarian patients is not higher than the mortality in patients aged 65–90.

P1535 *Erysipelothrix rhusiopathiae* bacteremia and cellulitis in an adult

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Objective: A case of *Erysipelothrix rhusiopathiae* bacteremia and cellulitis in an adult is presented.
Methods: Blood cultures were performed by Bactec system (Becton Dickinson), the identification of Erysipelothrix by API Coryne (bioMerieux), biochemical reactions and Gram stain. The susceptibility testing was carried out by disk diffusion method. A male patient aged 18 years was admitted to the hospital because of allergy after an insect bite and fever 38 °C. The bite was on the small finger of the left hand which had a localized cellulitis developing around the bite. The lesion was violaceous and painful, indurated with edema and clearly delineated at the border. On day of admission WBC count was 28 200/μL with 92.6% neutrophils and from blood cultures *Erysipelothrix rusiopathiae* was isolated. The strain was resistant to aminoglycosides, vancomycin, rifampicin, trimethoprime/sulfamethoxazole and susceptible to penicillin, amoxicillin/clavulanic acid, cephalosporins, clindamycin, tetracycline, imipenem, chloramphenicol, erythromycin and fluorocinolones. Augmentin i.v. and antiallergic treatment were administered and the patient became afebrile. He was discharged from the hospital in good condition after 3 days of admission.
Conclusion: The disease was contracted through an insect bite on the patient's hand.

P1536 Group B streptococcal bacteremia in nonpregnant adult

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Objective: To determine the clinical characteristics, antibiotic susceptibility and evolution of group B streptococcal bacteremia (GBSB) in nonpregnant adults.
Patients and method: We retrospectively reviewed the clinical charts of patients with group B streptococcus isolated in blood cultures in a period of 105 months (January 1993–September 2001). Study protocol included demographic data, underlying comorbid conditions, associated infectious foci, in vitro susceptibility antibiotic tests, and outcome.
Results: Seventy-one GBSB were documented: 39 (55%) in early and late neonatal period, 11 (15.5%) pregnancy-related and 21 (29.5%) in nonpregnant adults. In the later group, the mean of age was 58 years (18–93), and 12 (57%) accounting in women. GBSB were community-acquired in 17 (81%) and monomicrobial in 20 (95%) cases. Underlying conditions were present in 16 (76%) patients: diabetes (38%), malignancy (30%), liver cirrhosis (15%), surgery (15%), urologic disease (5%) and HIV (5%). An associated foci of infection was present in 17 cases: skin and soft tissue infections (24%), osteomyelitis (19%), genitourinary (9.5%), pneumonia (9.5%), prosthetic (9.5%), endocarditis (5%) and arthritis (5%). Group B streptococci were uniformly susceptible in vitro to penicillin (4.2% penicillin tolerance), ampicillin, amoxicillin, cefalotin, vancomycin, clindamycin and cefotaxime. Resistance to erythromycin and gentamicin occurred in 16 and 33% of isolates, respectively. Directly related GBSB fatality occurred in one patient (5%) and GBSB was a contributor condition in two additional subjects (9.5%).
Conclusion: Group B streptococcal bacteremia in nonpregnant adults is frequently associated to underlying conditions and concomitant infectious foci, mostly cutaneous and osteoarticular. Penicillin continues to be the treatment of choice.

P1537 Bacteremia due to β-hemolytic streptococcus group G: increasing incidence and clinical characteristics of patients

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Objectives: To describe the epidemiology and features of patients diagnosed with bacteremia due to group G *Streptococcus* (GGS) during one decade (1990–1999).
Methods: Retrospective review of patients with bacteremia due to GGS and group A *Streptococcus* (GAS) during 10 years, with calculation of annual rates of bacteremias due to these organisms, expressed as percentage of all positive patient-specific blood cultures (excluding obvious contaminants) and as ratios/1000 admissions. Records of patients with GGS and GAS were retrieved and clinical data compared.
Results: During the decade 1990–1999, 741 ± 228 blood cultures were annually positive, for a total of 7415 organisms. Of these, 327 (4.4%) were β-hemolytic streptococci, including 109 (33%) group A, 99 (30%) group B, 49 (15%) group G, and 70 (22%) other types. The annual rates per 1000 admissions showed a relatively stable incidence of GAS bacteremia, and a significant, recent increase of that of GGS. The respective annual incidences of GGS and GAS bacteremias have converged to ±0.35 cases/1000 admissions in 1999; each currently constitutes ±1.2% of all positive blood cultures. Of 49 patients with GGS, 40 records were retrievable and compared with those of a random sample of 46 patients with GAS. Of patients with GGS, 62.5% were >75 year, compared to 30% of patients with GAS ($P < 0.01$); 80% of patients with GGS were male, compared to 39% of patients with GAS ($P < 0.001$). Alcoholism and malignancy were not risk factors for GGS; however, 40% of patients with GGS had peripheral vascular disease, compared to 4% only in the GAS group ($P < 0.001$). The source of GGS bacteremia was a skin infection in 77.5% of cases, compared to 46% in patients with GAS ($P < 0.001$). The mortality of the cohorts was 15% and 19.5%, respectively (NS).
Conclusions: GGS bacteremia has occurred with increasing frequency in the last decade in our hospital, currently surpassing GAS. If confirmed by others, this could indicate a change in epidemiology of serious infections due to β-hemolytic streptococci. Elderly patients, admitted because of community-acquired skin infections and diagnosed with GGS bacteremia should be treated with penicillin without need for additional imaging unless clinical features suggest malignancy or localized infection.

P1538 Acute nonrheumatic perimyocarditis associated with group A streptococcal tonsillitis

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Introduction: Acute rheumatic hearth disease is a well-known immune mediated complication of a tonsillar infection with group A β-hemolytic *Streptococci* (GAS) and usually occurs some weeks after the infection in the context of rheumatic fever. Less recognized is the presentation of acute perimyocarditis, which occurs 2–7 days after the onset of infection.
Case report: A 45-year-old man presented to the emergency room because of intermitting left-sided chest pain radiating to the left shoulder. Three days before admission because of fever, sore throat and a positive rapid streptococcus test (Strept-*A*-test) the diagnosis of streptococcal tonsillitis was made and oral cefpodoxim was started. On admission he was afebrile. Exudative tonsillitis was present. Examination of the heart was normal. ECG showed ST elevation in I, II, aVL, V3-V6 without Q waves. Myocardial necrosis was documented by the presence of elevated enzymes (CK 424 U/L, CK-MB 97 U/L). Inflammatory markers were also elevated (CRP 234 mg/L). He was treated for a (sub)acute non-Q myocardial infarction and a coronary angiogram was performed 3 days later, which showed normal coronary vessels. We revised the diagnosis of acute nonrheumatic streptococcal perimyocarditis. GAS infection was also documented by an increase of Anti-streptolysin-O-titer 4 weeks later.
Discussion: Some weeks after a GAS pharyngitis, immunological mechanisms cause the clinical picture of rheumatic fever. Multiple organs are damaged and the entire heart is involved giving rise to a pancarditis. There is a small number of reports of a less known acute nonrheumatic perimyocarditis that occurs 2–7 days after the acute tonsillitis. As in viral perimyocarditis the clinical presentation is nonspecific and can range from asymptomatic ECG changes to acute coronary syndrome mimicking myocardial infarction like in our patient. The pathogenesis is not clear but it is supposed to be toxin-mediated, some bacterial exotoxins and cellular components of GAS have toxic properties. Mononuclear infiltrates predominate histologically like in viral infection. It has typically a benign course and prognosis and most of reported patients recover completely.
Conclusion: Pericarditis can give the picture of an acute coronary syndrome with ST elevation and in face of a concomitant GAS pharyngitis, which can be easily demonstrated, it's important to consider the presence of an acute nonrheumatic streptococcal perimyocarditis.

P1539 Infective endocarditis: report of cases due to unusual community-acquired pathogens

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Of the group of 117 patients suffering from community-acquired infective endocarditis (IE) who were hospitalized in our department during years 1990–2000, several cases were caused by unusual pathogens:

1 A 59-year-old woman developed mitral IE due to *Streptococus pyogenes* A after taking care of a child with scarlet fever. The disease was complicated with early embolism to the left retinal artery leading to the loss of the eye.
2 A 69-year-old woman with a mitral bioprosthesis developed endocarditis due to *Listeria monocytogenes*. Because of penicillin allergy and renal failure, she could not be given standard therapy. She was cured with teicoplanin + rifampicin + cotrimoxasol. No immunosuppressive condition was found in this patient.
3 *Gemella morbillorum* endocarditis was diagnosed in a 31-year-old man with Fallot's congenital cardiac defect. The patient had undergone *S. mitis* endocarditis 4 months ago.
4 A 37-year-old man having suffered from fever and urticarial exanthema was diagnosed as IE due to *Neisseria meningitidis* B; predisposing condition was bicuspid aortic valve.
5 A 84-year-old man developed *Neisseria meningitidis* B endocarditis localized on the sclerotic aortic valve. The disease was complicated with shock and multiple organ system failure (MOSF) and he died 15 h after admission.
6 A 24-year-old man with a bicuspid aortic valve who arrived from Belarus developed pseudomembranous tonsillitis caused by *Corynebacterium diphtheriae*. The disease was complicated with IE involving all cardiac valves and MOSF. Despite intensive therapy he died 6 weeks after the onset of the disease.
7 A 48-year-old man with a bicuspid aortic valve developed endocarditis due to *Haemophilus influenzae* b. The disease was complicated with early onset MOSF and subvalvular abscess formation. The patient died despite intensive therapy including cardiac surgery.

P1540 Infective endocarditis cases in a general hospital, Milan, in the years 1999–2000

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Objective: Analysis of Infective Endocarditis (IE) incidence in our Hospital by means of collectable data.
Methods: We studied the record-card of 56 patients (14 in 1999, 42 in 2000) for which IE was diagnosed in accordance to the Durack's criteria modified per the latest Duke University indications. Beyond age and sex, we collected information of each patient, about predisposing heart condition, etiology, echocardiographics evaluation, localization, particular clinical scene and mortality.
Results: We observed a trend towards a higher age (60.7% over age 50 year) and prevalence of male (34 patients). Thirty-two percent of IE occurs without known cardiopathy, 20% in patients with prosthetic valve, 12% in patients with various types of cardiopathy; in 36% of the patients a previous valvular heart disease on the native valve (mainly mitral – 12 patients) was identified. The etiology was identified only in 36 patients; of the remaining ones, 13 had negative blood culture because of previous antibiotics therapy. In 17 patients, IE was caused by *Streptococcus* spp. (four oral, eight intestinal, five others), in 15 by *Staphilococcus* spp. (five aureus, seven coagulase-negative, three others), in four by other microorganisms. Transthoracic echocardiography was performed in 51 patients, with positive result in 44 (86%); transesophageal

echocardiography was performed in 31, with positive result in 30 (97%). In 80%, IE occurred on a native valve; in 51 patients the localization was on a single valve (25 mitral, 19 aortal, six tricuspid, one pulmonar), in 5 on two valves. In 47% of single valve IE and in 60% of two-valve cases, patients underwent surgery within 30 days from the diagnosis. Although the belief that a higher risk of embolus derives from high volume or mobile or increasing volume vegetation, particularly if mitral, of the 15 patients with mobile vegetation of more than 10 mm in diameter, six underwent embolus and five of these had a mitral valve-originated embolus. Several were the 'special cases': 24 aged people, two children, three drug addicts, nine nosocomial IE and 10 "slight" patients. Mortality was 14%.
Conclusions: IE incidence did not decrease in the last years, its mortality rate approaches 15% and all this despite the significant progresses in the diagnostic and therapeutic fields. This failure should foster a closer cooperation among infectious diseases clinician, microbiologist, cardiosurgeon, echography clinician and pathologist, in order to optimize the diagnostic strategy.

P1541 Pulmonic valve endocarditis: case report and review of the literature

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Objectives: To present a case of pulmonic valve infective endocarditis involving a structurally normal heart, and review the epidemiologic, clinical, and laboratory features of this rare condition.
Methods: Case report and review of the English language literature from 1960 to 2000.
Results: We identified only 38 cases of isolated pulmonic valve endocarditis occurring on structurally normal hearts. Patients with pulmonic valve infection tended to be young and male, and the vast majority of infections were community-acquired. Predisposing factors included iv drug abuse (28%), alcoholism (13%), sepsis (7%), central line (7%) or other catheter-related (5%) infection, and dental extraction, bowel surgery, liver or renal transplantation, and colonic angiodysplasia (2.6% each). No predisposing factor was identified in 28% of cases. Fever, shortness of breath, and pleuritic chest pain predominated and radiographic and laboratory evidence frequently demonstrated multiple pulmonary emboli. Pulmonic regurgitant murmurs were present in about one-half of subjects. Causative organisms included *Staphylococcus aureus* (44%), *Streptococcus species* (13%), *S. bovis* (5%), *Pseudomonas aeruginosa* (5%), *E. coli* (5%), *Candida albicans* (5%), gonococcus (5%), and *Bacteroides fragilis, H. influenzae,* and *Enterococcus faecalis* (2.6% each). Transthoracic echocardigraphy detected pulmonic valve vegetation in 29 of 38 (76%) cases. Twenty-five patients were treated medically with antibiotics alone (seven died) and 13 required surgical intervention (zero deaths) ($P = 0.07$; 95% CI, 0.1–18).
Discussion: Pulmonic valve endocarditis is an extremely rare infection that shares epidemiologic, clinical, radiologic, microbiologic, and prognostic features with tricuspid valve endocarditis.

P1542 Changing patterns of infective endocarditis (IE) in a tertiary care teaching hospital

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Aim of the study: To evaluate some current epidemiologic, microbiologic and diagnostic aspects of IE in a tertiary care teaching hospital in comparison with the data obtained from a similar study performed in the same institution during the 1982–1988 period.
Methods: All cases of IE occurred at Pisa General Hospital in the 1996–2000 period were retrospectively investigated. Patient medical charts and microbiologic records were evaluated.
Results: Sixty-nine patients with IE were recognized accounting for 0.26% of all patients admitted to the Pisa hospital. In according with the Duke criteria 58 cases of IE were definite and 11 possible; 67% of the patients were males; mean age was 60.9 + 17 year (range 19–86). The site of IE was as follows: pacemaker in 26% of patients; native valve in 62.3%; prosthetic valve in 10.2%; native and prosthetic valve 1.5%. An history of i.v. drug abuse was present in six patients; 28 patients (40.6%) had no previously known heart disease. Blood cultures were negative in 10 patients (14.2%) or not available in seven patients. The remaining causative microorganisms were distributed as follows: staphylococci 42% (*S. aureus* 11, CNS 18) streptococci 24.5% (*S. bovis* 6), enterococci 4.5%; other pathogens 4.5% (including two Gram-negative rods). Embolic complications occurred in 19 patients (27.5%). A positive echocardiographic criterion of IE was present in 60 out off 69 patients. Transoesophageal approach was more sensitive than transthoracic in detecting the site of IE, especially in patients with pacemaker IE. Valve surgery or removal of infected pacemaker was performed in 36 patients (47.8%). In hospital, mortality was 17%.
Conclusion: As compared to the previous study performed in the same institution, several new trends were recognized: (1) increased mean age of patients with IE; (2) increased frequency of pacemaker IE; (3) increased frequency of IE caused by staphylococci and *S. bovis*; (4) increased number of patients with IE undergoing valve surgery or removal of infected pacemaker.

CNS infections

P1543 Antibiotic-resistant *Haemophilus influenzae* meningitis: patient characteristics and clinical outcome during a 4-year period that included the introduction of *H. influenzae* type B conjugate vaccine

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Objective: To determine the characteristics of children less than 5 years of age admitted to hospitals in the West Midlands region of the UK with *Haemophilus influenzae* (HI) meningitis resistant to antibiotics, and to examine whether meningitis due to antibiotic-resistant HI resulted in different clinical outcomes compared to nonantibiotic-resistant HI.
Methods: Cases were identified over a 4-year period using a passive laboratory-based surveillance system supported by several other overlapping surveillance systems. During the final 2 years of surveillance, HI type B conjugate vaccine was introduced into the UK immunization schedule. Case notes were reviewed to obtain sociodemographic, clinical and microbiologic data. Cases were included if HI had been isolated from a sterile site.
Results: During the study period, 165 cases of HI meningitis were identified and 147 clinical isolates were analyzed. Thirty of the 147 (20%) isolates were found to be ampicillin or amoxycillin resistant, 24/30 (80%) antibiotic-resistant isolates were identified by the passive surveillance system alone. Resistance to either ampicillin or amoxycillin or both was associated with nontypable HI organisms ($P = 0.044$), being the youngest sibling ($P = 0.042$), being aged less than 18 months ($P = 0.004$) and having postmeningitis sensorineural hearing loss ($P = 0.033$). Significantly more ($P = 0.036$) children from socially deprived areas with antibiotic-resistant organisms had sensorineural hearing loss, despite children from more affluent areas having a greater proportion of antibiotic-resistant organisms. Duration of hospital stay, previous hospital admission and mortality were not associated with antibiotic resistance. There was a nonsignificant increase in the proportion of isolates that were antibiotic resistant from 19% (22/117) in the first 2 years of the study to 23% (7/30) in the last 2 years, the period that conjugate vaccine was introduced.
Conclusions: HI meningitis infections resistant to antibiotics are associated with higher morbidity than infections with antibiotic-susceptible strains. Children from deprived areas and young infants with siblings are at greatest risk. These results may have implications for antibiotic therapy in these key

patient groups, particularly with regard to empirical antibiotic therapy and the public health management of this disease.

P1544 Epidemiologic features of pneumococcal meningitis

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Objectives: To identify the epidemiologic characteristics of pneumococcal meningitis in our hospital that is a 800-bed, primary and tertiary care teaching hospital that provides care to urban area of Bilbao.
Methods: A retrospective study of pneumococcal meningitis cases was conducted during a 6-year period, from 1995 to 2000.
Results: The microorganism was isolated from 23 CSF with the following distribution along years: 1995 (three), 1996 (four), 1997 (six), 1998 (four), 1999 (two), 2000 (four). The highest age-specific incidence of pneumococcal meningitis was observed in children <5 years of age and in elderly of >55 years of age. There were no HIV subjects. There were eight cases in children, four males and four females (6 months–7 years). *S. pneumoniae* was recovered from blood cultures in six (75%) patients, and three showed otitis media. The treatment was started with a third-generation cephalosporin and vancomycin (62.50%) and dexamethasone (75%). The elderly group had 15 subjects (55–91 years), seven males and eight females, the organism was present in blood cultures in nine (60%) patients, and in otic exudate in three (20%), although three more patients presented otitis media. Mortality until discharge was 20%. The treatment was started only with a third-generation cephalosporin in all of the patients, except in one in whom imipenem and vancomycin was aggregated. Dexamethasone treatment was not used as primary therapeutic measure.
In both groups: Gram stain showed Gram-positive diplococci in 11 (47%). The peripheral WBC count was increased in 47%, only one showed leukopenia and died. CSF glucose, protein and white blood cell count were consistent with bacterial meningitis. Global penicillin resistance was 30% (26% with MIC between 0.1 and 1 μg/mL and 4% with MIC > 1 μg/mL). Cefotaxime MIC ≥ 1 μg/mL was found in 2 (15%) of 13 tested patients.
Conclusions: (1) *S. pneumoniae* meningitis is greatest among the very young and the very old. (2) The high fatality in our patients is in elderly. (3) Higher penicillin-resistance rate was in pediatrics patients 50% versus 20%.

P1545 Bacterial meningitis in Poland from 1997 to 2001

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Objectives: The aim of the study was to characterize major etiologic agents of bacterial meningitis in Poland during 5-year activity of the National Reference Centre for bacterial meningitis.
Methods: Strains isolated from cerebrospinal fluid or/and blood from patients with meningitis or meningococcal septicemia were identified by standard methods. Meningococcal serogroups and *H. influenzae* serotypes were determined by slide agglutination, whereas meningococcal serotypes and serosubtypes by whole-cell ELISA method. Serotypes of *S. pneumoniae* were settled by the Quellung reaction. Susceptibility testing was performed for *N. meningitidis*, *H. influenzae* and *S. pneumoniae* according to the NCCLS guidelines.
Results: During 1997–2000, altogether 460 strains were collected. The most frequently identified was *N. meningitidis* ($n = 189$, 41.1%), followed by *H. influenzae* ($n = 113$, 24.6%) and *S. pneumoniae* ($n = 104$, 22.6%). Other species were isolated sporadically. Most of the *N. meningitidis* and *H. influenzae* strains were isolated from children below 5 years of life, with the highest incidence under the age of two. *S. pneumoniae* was responsible for cases in all the age groups. Out of meningococcal strains, almost 85% belonged to serogroup B, 11.6% to serogroup C and 3.7% to W135. The most frequent serotype was 22. Most meningococci were highly sensitive to penicillin, only 5.8% of them showed decreased susceptibility to this antibiotic. More than 93% of *H. influenzae* isolates belonged to serotype b (Hib). The seven remaining isolates were noncapsulated. A total of 11.5% strains were resistant to ampicillin via production of β-lactamases. Broad distribution of serotypes was found among pneumococcal strains of which the most common were serotypes 3, 8 and 19F. Penicillin-nonsusceptible strains constituted 10.6% of all pneumococcal isolates. Results from 2001 will be added at the end of this year.
Conclusions: The most common etiologic agent of laboratory-confirmed bacterial meningitis was *N. meningitidis*, mostly serogroup B, being responsible for more than 40% of cases. High percentage of meningitis in Poland caused by *H. influenzae* strains, mostly serotype b, results from the lack of mass vaccination against Hib.

P1546 Investigation of preadmission antibiotic treatment in patients with meningococcal disease

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Objectives: Early recognition, immediate antibiotic treatment and aggressive treatment of septic shock can reduce mortality of meningococcal disease.
Methods: Investigation of preadmission antibiotic treatment was performed in 146 patients (65 females, 81 males) with invasive meningococcal disease, who were treated at five university hospitals in the Czech Republic from January 1996 to November 2001. *Meningococcal meningitis* was proved in 30 patients, septicemia in 42 patients and meningitis/septicemia in 74 patients. Twelve patients died, nine of them suffered by septicemia and three of them by meningitis/septicemia. The highest morbidity and mortality were in the age groups 0–4 years (55 children, six deaths) and 15–19 years (43 patients, five deaths). The serogroups C and B were absolutely predominant, new emerging clone *Neisseria meningitidis* C:2a:P1.2, P1.5, ET-15/37 was the most frequent strain. Statistical analysis was performed using Fisher's exact test.
Results: Preadmission antibiotic treatment was used in 106 patients before transfer to special intensive care units, only seven of them (6.6%) died. Forty patients were not treated before admission to special intensive care units and five of them (12.5%) died. Preadmission treatment was used in 26 of the 42 patients with septicemia (six treated patients of nine deaths), in 60 of the 74 patients with meningitis/septicemia (one treated patient of three deaths) and in 20 of the 30 patients with meningitis (zero death in this group). The mortality of patients with and without preadmission antibiotic treatment were 1.7 and 14.3% for meningitis/septicemia, identically 23.1% for septicemia and 0% for meningitis. The differences were statistically insignificant for all patients ($P = 0.202$) and also for patients with sepsis/meningitis ($P = 0.090$). The antibiotic therapy was started usually in local hospitals before patient's transport to special intensive care units. General practitioners and physicians of out-patients departments started antibiotic therapy sporadically, only in 14 patients.
Conclusions: Approximately three quarters of patients with meningococcal disease in the Czech Republic received preadmission antibiotic treatment. The mortality of patients with preadmission antibiotic treatment was almost twice lower then in patients without treatment (6.6% vs. 12.5%), but statistically significant differences were not proved.
Grant support: Grant Agency Czech Republic No. 310/96/K102.

P1547 Bacterial meningitis in children: a 10-year study

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Objectives: To show the epidemiologic data and trends of pediatric bacterial meningitis during the study period, and to compare the outcome of meningitis before and after the use of third generation of cephalosporins as routine treatment of infection.
Methods: The study is based on retrospective analysis of medical charts of 403 children with bacterial meningitis, aged 1 month–14 years, hospitalized to the Pediatric Department of University Hospital Center of Tirana, during the period June 1990–June 2000. The diagnosis of bacterial meningitis was established on cytochemical characteristics of CSF and positive Gram stain and/or CSF blood cultures. The epidemiologic data included in the study were: age, sex, origin, sex seasonality, complications and case fatality rate. The etiologic data and antibiotic combinations used were taken into consideration.
Results: During the last 10 years, 403 children with bacterial meningitis were admitted to our clinic. The distribution of the cases had approximate values except during 1997–1998, when a slight elevation of incidence was notified. The increase was related with the social events and the demographic

movements towards Tirana, in this period. Fifty-four percent of the cases had urban origin and male patients dominated with a rate of 1.4 times more. The most affected age was 0–1 year, which included 46.5% of the cases towards 26.5 and 27%, which belonged, respectively, to the age groups 1–4 and 5–14 years. The infection was more frequent at the first and second trimester of the year. The positivity of bacterial cultures resulted at 67%. The bacteriologic agents according to the frequency were: *N. meningitides*: 36%; *S. pneumoniae*: 34%; *H. influenzae*: 20.4%; enterobacter: 2.6%; others: 7%. During the period 1990–1995, 67% of the cases were treated by the combination of ampicillin + chloramphenicol. The complications such as hydrocephalus, mental retardation, epilepsy, and deafness were noticed in a value of 13.7% and case fatality rate was 7.4% for this group. At the second quinquennial, 61% of the patients were treated by a cephalosporin (cefotaxime or ceftriaxone), where the case fatality rate and complications were found much lower, respectively, 2 and 7.4% ($P < 0.002$).

Conclusions: Bacterial meningitis still remains a serious infection of pediatric age. Cephalosporins of third generation had decreased the mortality and improved the course of the disease.

P1548 Latex agglutination for bacterial antigens and meningococcus PCR: two useful tools in legal autopsies

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Objectives: In legal autopsies, microbiology is sometimes needed as a complementary tool for pathologists. However, postmortem bacterial cultures are often contaminated or difficult to interpret. The aim of this study was to assess the usefulness of latex agglutination test for bacterial antigens in postmortem samples from legal autopsies in which an infectious disease had to be ruled out or established as a cause of death. Moreover, since 2000, specific meningococcus PCR was performed when meningococcal septic shock was strongly suspected.

Methods: Bacterial antigen detection of *Neisseria meningitidis* (serogroups A, B, C, Y and W135), *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and group B *Streptococcus* was performed with the Directigen Meningitis Combo Test (Becton Dickinson Microbiology Systems) in 34 samples (28 sera and six CSF) according to instructions of the purchaser. Detection of genetic material from *N. meningitidis* by PCR with primers designed to amplify the specific regions of the meningococcus *ctrA* and *crgA* genes was performed in three sera. Posterior serogrouping was done by amplification of the group-specific *syaD, B, C, Y* and *W135* genes.

Results: Directigen-positive results were obtained in a 20.6% (7/34) of the samples. Latex agglutination was positive for *N. meningitidis* in six sera (five serogroup C and one serogroup B) and one serum was positive for *H. influenzae* type b. The six CSF analyzed were negative. Two of the *N. meningitidis* serogroup C-positive sera were also positive for specific group C PCR. On the other hand, one serum in which the latex agglutination could not be performed, yielded a positive PCR for meningococcus. These results allowed us to confirm the suspicion of meningococcemia in eight cases of sudden death. Pathologic findings and clinical history were always consistent with positive results.

Conclusions: In postmortem samples in which a bacterial culture is not recommended, antigen-detection tests can be a useful strategy when a fulminant infection by *N. meningitidis* or *H. influenzae* is suspected as a cause of death. Positive results provided a rapid, presumptive diagnosis that permitted early prophylaxis of contacts. In our opinion, this technique should always be run in conjunction with meningococcus PCR to confirm meningococcemia.

P1549 Reemergence of meningococcal disease in Taiwan: identification of a major clone serogroup W135

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Objectives: To determine the epidemiology of meningococcal disease involving the 2001 outbreak in Taiwan.

Methods: Antimicrobial susceptibilities, serogroups and genotypes of isolates recovered from the 2001 outbreak were determined.

Results: The annual incidence rate (per 100 000) of meningococcal disease (meningitis and septicemia) in Taiwan was 0.63 in 1964 (78 cases), declined to 0.01 in 1986 and to 0.001 in 1990, and was zero during the period from 1991 to 1995. It reemerged with a 0.075 incidence rate in 1996 and a 0.068 rate in 2000. From January 2001 to June 2001, a remarkable increase in the number of cases of meningococcal disease (31 cases, 0.14/100 000) was noted. Among the 71 patients with a diagnosis of meningococcal disease during the period from January 1998 to June 2001, 12 patients (16.9%) died. During this period, the disease occurred most commonly during February–May and predominantly in patients aged less than 1-year-old and from 11–30-year-old. The majority of the 71 isolates recovered from the 71 patients belonged to serogroups B (42.3%) and W135 (32.4%). In 1999, serogroup B isolates accounted for 84.6% of all isolates (11 out of 13 isolates), but during the period from January to June 2001 this prevalence decreased remarkably (22.6%), while the prevalence of serogroup W135 (35.5%) increased. Agar dilution susceptibility of 32 preserved isolates of *Neisseria meningitidis* (31 from the 31 patients treated from January to June 2001, and one in 1998) to 15 antimicrobial agents showed that one isolate (3.2%) had intermediate susceptibility to penicillin (MIC, 0.5 Ýmg/L) and was β-lactamase negative. All isolates were susceptible to rifampin, fluorquinolones and macrolides, and all were resistant to trimethoprim-sulfamethoxazole (MICs, d4/76 Ýmg/L). Molecular typing of the 32 isolates by arbitrarily primed PCR using three random oligonucleotide primers revealed 23 clones with a major clone (clone IV, serogroup W135) which was isolated from six patients from four different regions of Taiwan.

Conclusions: The recent reemergence of meningococcal disease in Taiwan may be partly due to the wide dissemination of several clones of *N. meningitidis*. Trimethoprim-sulfamethoxazole should not be considered as the drug of choice for management for meningococcal disease or carriage in Taiwan.

P1550 Diversification in *Neisseria meningitidis* genome correlates with transition from carrier state to invasive disease

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Objective: The aim of the study was to investigate epidemiologic situation within a family where two cases of invasive meningococcal disease were diagnosed.

Methods: Meningococcal isolates were sent to the National Reference Centre for Bacterial Meningitis (NRCBM), where they were reidentified and serotyped by whole-cell ELISA method. MICs of the following antimicrobial agents were evaluated by E tests: penicillin, ceftriaxone, cefotaxime, rifampin and ciprofloxacin. Molecular typing was performed by randomly amplified polymorphic DNA method (RAPD) with three different primers, pulsed-field gel electrophoresis (PFGE) of bacterial chromosomal DNA digested with BglII, NotI and SpeI restriction enzymes and multilocus sequence typing (MLST).

Results: In June 2001, 5.5-year-old boy was admitted to a hospital with severe symptoms of bacterial meningitis without any skin changes. Next day, his 8-year-old brother reached the hospital with symptoms of septicemia (high fever, septic shock and purpura fulminans). *N. meningitidis* was isolated from cerebrospinal fluid (CSF) of the first boy. Blood culture of the second boy gave negative results, but Wellcogen latex test was positive for antigens of *N. meningitidis* A, C, Y, W135. The first boy was treated with cefotaxime and the second one with ampicillin and amikacin. After recovery, nasopharyngeal swabs were taken from the whole family, including the youngest 4.5-year-old brother and parents. Meningococci were isolated from the first boy and parents. All four meningococcal isolates were susceptible to antimicrobial agents tested. They belonged to serogroup C, serotype 2b and serosubtype P1.2.5. Molecular typing by RAPD and PFGE using NotI and SpeI showed identity of the isolates. On the contrary, PFGE performed with BglII restrictase differentiated the isolate from CSF by three bands from those from nasopharynx. MLST identified all four isolates as representatives of sequence type 66 (ST 66) according to the Oxford MLST database (http://www.mlst.net).

Conclusions: By using a set of molecular typing techniques, we were able to detect differences between the meningococcal isolate that caused meningitis and those involved in carriage in the same patient and his family. Such changes reflect the rapid diversification of *N. meningitidis* genome and may be connected with a shift in biologic properties.

P1551 Development of venous sinus thrombosis and *Clostridium* abscess after *Proteus vulgaris* meningitis: a case report

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Gram-negative bacilli are rare cause of bacterial meningitis in adults, but they have become particularly important in patients with chronic mastoiditis, otitis media, head trauma, neurosurgical operations, and impaired host defenses. A 19-year-old woman was admitted to the hospital with headache, fever, vomiting, aural pain. She had a history of previously chronic otitis media. The cerebrospinal fluid evaluation demonstrated purulent meningitis caused by *Proteus vulgaris*. She was treated with 6 g/day intravenous ceftazidime and 1 g/day intravenous amikacin. Initial cranial magnetic resonance imaging (MRI) scanning showed meningeal contrast enhancement, mastoiditis and otitis media at the right side and sigmoid-transverse sinus thrombosis secondarily to local dissemination of infection. Mastoidectomy was performed on the patient in the department of otolaryngology. Five days later, control cranial MRI demonstrated abscess formation along the trombosed venous sinuses. The abscess evacuation was performed in the department of neurosurgery. *Clostridium* spp. has been isolated from intraoperative culture. An amount of 2 g/day metronidazole was added to antibiotic treatment and it was continued for 6 weeks. She recovered with medical and surgical therapy. As a conclusion, persistent infection and local complications may occur in Gram-negative bacilli meningitis. When severe complications develop, surgery should be performed in addition to the medical treatment, if necessary.

P1552 Infectious meningitis: 5 years of experience in a general hospital

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Objectives: To study the clinical and epidemiologic characteristics of the infectious meningitis (IM) in our area.
Methods: This is a retrospective study which described all the cases of IM diagnosed in the Costa del Sol Hospital from January 1994 to December 1999. Our center is a general hospital attending to an area of 250 000 habitants in the south of Spain.
Results: Total admissions during this time were 59 329; 77 patients were diagnosed with IM: 43 males (55.8%) and 34 females (44.2%). The mean age was 22 ± 23 years (1–78); 17 patients (22%) were less than 1-year-old; between 1- and 14-year-old in 27 cases (35%); and more than 14 years in 33 cases (43%). Patients were admitted in the following units: Pediatric Unit: 43 cases; Internal Medicine (M): 12 cases; Intensive Care Unit (ICU): four cases; Otorhinolaryngology Unit: one case; ICU + M: 16 cases; and ICU + Pediatrics: one case. In 10 patients, *Haemophilus influenzae* was isolated (13%), in seven *Streptococcus pneumoniae* (9%), two cases of *Listeria monocytogenes*, *Criptococcus neoformans*, *Staphylococcus aureus* and *Streptococcus* group C, and one case of *S. agalactiae*, *S. epidermidis*, *Escherichia coli*, *Mycobacterium tuberculosis* and *Rickettsia conorii*. The causal agent was not determined in 47 cases (61%), in those patients CSF has lymphocyte prevalence in 17 (36%). Our patients suffer from associated problems in 27 cases (35%): otorhinolaryngologic diseases (seven cases), HIV infection (four cases) and in smaller number of cases hepatopathy, small-for-date baby, diabetes, hematologic diseases, neoplasm and previous IM. Sequels were developed in 11 cases (14%): epilepsy in three cases, permanent dysfunction of the central nervous system in three cases, hydrocephalus in two cases and one case was diagnosed of chronic migraine post-IM. About the outcome: four patients died (5.2%), three adults and one child. The length of stay was 10 days, 6.61 days in the pediatric patients (<14 years) and 14.52 days in adults.
Conclusions: In our area, IM is present in more than two-third parts in patients smaller than 14 years. The causal microorganism is not isolated in more than half of the cases neither by culture nor serology. Our cases are predominately patients without predispose factors; this rules out preventive sanitary actuation over the population. In adults, this infection causes an elevation in the length of stay and admissions in ICU, and an important rate suffer from serious sequels.

P1553 Meningitis by *Gemella morbillorum* with associated pituitary apoplexy

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Gemella morbillorum is a commensal microorganism of the human gastrointestinal, respiratory, and genitourinary tracts. The spectrum of disease is very similar to that caused by *S. viridans*, although the microorganism seems to be rarely associated with infections other than infective endocarditis and bacteremia. We report a case of meningitis due to *G. morbillorum* followed by hypophyseal hematoma with pituitary apoplexy and secondary panhypopituitarism. A 56-year-old man was admitted to our hospital with a 5-day history of severe headache and vomiting. Two days before admission, he developed fever (39–40 °C) and malaise. The initial exploration showed very bad status, slight confusion and nucheal rigidity. A cranial CT scan was normal. Cerebrospinal fluid (CSF) analysis showed pleocytosis, hyperproteinorraquia and normal level of glucose. The Gram stain was negative. Intravenous therapy with cefotaxime was started. A week after admission, the patient acutely developed an impairment of headache and clinical status, with left hemianopsia. The MRI showed acute hypophyseal hematoma, with optic chiasm compression and lateralization, compression of cavernous sinus, and maxillary, sphenoid, ethmoid and frontal sinus occupation, suggesting the diagnosis of pansinusitis. Laboratory tests confirmed a panhypopituitarism and therapy with dexametasone was started. CSF culture revealed growth of *G. morbillorum* and antimicrobial treatment was changed to benzilpenicillin, ceftazidime and clindamycin. After 2 days, the general condition and neurologic status improved greatly. Antibiotics were administered for 15 days and the patient was discharged in good condition. No relapse was observed. Human infections caused by *G. morbillorum* are extremely rare. In fact, only two previous cases of meningitis by this microorganism are reported in the literature. Furthermore, our patient suffered a pituitary apoplexy, a very unusual complication of sepsis and meningoencephalitis. Despite its rarity, our case suggests that the pathogenicity of *G. morbillorum* should not be underestimated.

P1554 Acute bacterial meningitis in elders: risk factors concerning mortality

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Objectives: The purpose of the study is to point out the risk factors leading to death in acute bacterial meningitis in elders.
Material and methods: A retrospective study was conducted on 73 elderly patients (54.8% male and 45.2% female) admitted in our clinic during a 7-year period (1994–2000).
Results: The organisms isolated were *Streptococcus pneumoniae* in 21.9%, *Neisseria meningitidis* in 8.2%, *Listeria monocytogenes* in 5.5%, *Staphylococcus aureus* in 4.1% and unknown etiology in 60.3% of cases. In 20 cases (27.39%), bacterial meningitis was nosocomially acquired (postneurosurgical procedures). The overall case fatality was 26%. The factors related to mortality were male gender (68.42% of death cases), evolution time before admission >48 h (death in 43% of cases), coma (death in 100% of cases), seizures (death in 62% of cases), altered immune states (death in 56% of cases), causative organisms (mortality rate was 56.25% for pneumococcus, 33.33% for staphylococcus and 20.45% for unknown etiology), pleyocitosis <1000 cell/mmc (death in 100% of cases), high albumin CSF level (death in 84.2% of cases), nosocomial acquisition (47.36%).
Conclusions: Bacterial meningitis has a severe evolution in elderly patients with late admission, neurologic impairment, nosocomial acquisition, altered immune states, low pleyocitosis, increased albumin values in CSF, pneumococcal and staphylococcal etiology associated to high mortality.

P1555 *Listeria monocytogenes* meningitis complicated by granuloma formation in cerebellar pedincule

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Meningitis caused by *Listeria monocytogenes* is a rare disorder, especially in an immunocompetent adult. A 41-year-old woman was admitted with the complaints of high fever, severe headache and nausea and vomiting. She was mentally alert but her body temperature was 38.7 °C and also had a stiff neck. Lumbar puncture revealed increased CSF pressure, purulent appearance and leukocytes 1200/mm^3 (75% PNL and 25% lymphocytes), protein 269 mg/dL, glucose 55 mg/dL. Gram stain of CSF was negative. Ceftriaxon 4 g/day was initiated empirically however, lack of clinical improvement prompted a control LP at the 48th hour of the treatment and revealed the following CSF findings; 2200 leukocytes/mm^3 (80% lymphocytes and 20% PNL), protein 456 mg/dL, glucose 71 mg/dL. CSF culture yielded diphteroids which arouse a suspicion of *L. monocytogenes* as the probable etiologic agent. Motility test, catalase test and animal inoculation tests all confirmed *L. monocytogenes*. Antibiotherapy was altered to ampicillin + gentamycin. On the third week of the treatment, the patient developed diplopia, severe headache and ptosis of the left eye, dysartria and somnolance. MRI scans showed minimal signs of hydrocephalus. In addition, in the left cerebellar peduncle a contrast-enhanced area with 8 mm diameter was diagnosed as granuloma formation. At the end of 6 weeks of antibiotic treatment, the control MRI scans showed disappearance of the granuloma. The patient was discharged and in the follow-up period of 6 months after the discharge, the patient was normal.

P1556 Epidemiology of *Listeria monocytogenes* and *L. innocua* using multilocus enzyme electrophoresis

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Objectives: *Listeria monocytogenes* is considered an ubiquitous food-borne pathogen which can lead to serious infections, especially in newborns and elderly, pregnant, and immunocompromised people. *L. innocua* is a closely related nonpathogenic species of *Listeria*, which has been suggested as an appropriate epidemiologic marker for *L. monocytogenes*. Little information is available on the comparative epidemiology and molecular biology of *L. monocytogenes* and *L. innocua*. The purposes of the present study were to employ MEE: (1) to determine the genetic diversity amongst isolates of *L. innocua* and *L. monocytogenes* from a number of Australian sources; (2) to employ information on that diversity to: (a) increase understanding of the origins of and means of transmission of *Listeria* via poultry as human foods, (b) and contrast poultry isolates to clinical and other isolates.
Methods: United States Department of Agriculture method of isolation used to isolate 168 strains of *Listeria* mainly from poultry production environments, foods and human cases. Multilocus enzyme electrophoresis was applied to analyze ancollected strains.
Results: It was shown that *L. innocua* maintains 1.9 alleles per polymorphic loci and a genetic diversity of 0.12 compared with 2.9 alleles and a genetic diversity of 0.48 in *L. monocytogenes*. The number of electrophoretic type (ET) found in isolates obtained from two poultry production plants in Sydney, Australia also varied with 54 ETs in 73 isolates of *L. monocytogenes* and 10 ETs in 95 isolates of *L. innocua*. There was one common ET (67%) in *L. innocua* but no common ET in *L. monocytogenes*, where 79% of ETs were represented by a single isolate. Nine isolates obtained from episodic human cases of listeriosis in Australia represented nine different ETs and there was no evidence of a disease-specific strain.
Conclusions: The marked difference in the level and distribution of genetic variation in *L. innocua* compared to *L. monocytogenes* raises serious doubts as to the validity of using it as a marker surrogate for *L. monocytogenes*.

P1557 Cerebrospinal fluid ferritin: a new diagnostic tool in CNS infections?

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Differential diagnosis of CNS infections becomes often troublesome, necessitating more laboratory support. As such ferritin could be used as an acute phase protein measured in the CSF. The objectives of this paper were the appraisal of CSF ferritin in the differentiation of CNS infections, since relevant reports are so far scarce in the literature.
Methods: Seventy-six adult patients with miscellaneous CNS infections, as well as 20 healthy subjects (control group) were included in the study. Ferritin levels in the serum and the CSF specimens, as well as CSF leukocyte count, protein, glucose and lactate values were assessed and interrelated. For the measurement of ferritin levels, a microparticle enzyme immunoassay (IMx-Abbott) was used.
Results: CSF ferritin values (mean ± SE) were 7.5 ± 1.9 ng/mL in the controls, 10.85 ± 1.81 in the 42 persons with aseptic meningitis, 38.5 ± 32.14 in the six patients with encephalitis, 40.43 ± 8.87 in the seven with meningitis of specific etiology (TBC, brucella, mycoses), and 72.14 ± 16.23 ng/mL in the 21 individuals with bacterial meningitis. There was a statistically significant difference between: (a) the controls and the patients with bacterial meningitis and specific infections ($P = 0.000$); (b) aseptic meningitis and all the other infections ($P = 0.000$ for the bacterial and the specific meningitis, and $P = 0.029$ for the encephalitis). On the contrary, between bacterial meningitis on one hand and specific meningitis or encephalitis on the other, as well as between encephalitis and specific infections, no difference was observed. CSF ferritin values correlated significantly: in the sum of patients with CNS infection with their corresponding serum values ($r = 0.316$, $P = 0.005$) and in addition with all inflammatory CSF parameters: cells ($r = 0.315$, $P = 0.006$), protein ($r = 0.573$, $P = 0.000$), glucose ($r = 0.344$, $P = 0.002$), and lactate ($r = 0.616$, $P = 0.000$); in the bacterial meningitis group, values were in correlation with CSF protein ($r = 0.510$, $P = 0.018$), glucose ($r = -0.406$, $P = 0.068$), as well as with lactate ($r = 0.507$, $P = 0.023$), whereas in the other cases of meningitis, no such correlation was found.
Conclusion: Measurement of CSF ferritin may contribute to the differential diagnosis of the various CNS infections, mostly in the distinction of aseptic from bacterial or specific infections. There is, undoubtedly, need for further studies in which a higher number of patients, chiefly with specific infections and encephalitis, have to be included.

P1558 Performance of the Gram stain in the bacterial meningitis diagnosis

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Objectives: To evaluate the performance of the Gram stain in the diagnosis of the cerebrospinal fluid (CSF) infection.
Methods: During 2000 and 2001, we analyzed 626 CSF. We processed the Gram stain following an established protocol and using as a culture an enriched chocolate agar plate and a thioglycolate broth. The culture has been considered as the gold standard and we compared the Gram-stain result to the culture result. In case of disagreement, we checked to see if the patient had received antimicrobial therapy prior to lumbar puncture, the clinical diagnosis, and the result of the blood culture. The established protocols were: (1) leukocytes count of at least 10/mm^3 with predominant polymorphonuclear; (2) predominant lymphocytes with glucose below 40 mg/dL and protein above 50 mg/dL; (3) suspicion of sepsis or meningitis; (4) immunodeficiency; (5) when the physician asked for it. The data were analyzed with the statistical program SPSS 10.0. We calculated the sensitivity (S), the specificity (SP), the positive predictive value (PPV), and the negative predictive value (NPV).
Results: We analyzed 626 CSF. Only 190 (30.4%) reached the established protocol for realizing the Gram stain. We obtained 23 (12.1%) CSF with

culture positive: 10 (43.4%) *Neisseria meningitidis*, 10 (43.4%) *Streptococcus pneumoniae*, 1 (4.4%) *S. agalactiae*, 1 (4.4%) *Haemophilus influenzae*, and 1 (4.4%) *Listeria monocytogenes*. We found S = 87%, SP = 98.8%, PPV = 91%, and NPV = 98.2%.
Conclusions: The Gram stain is a simple, rapid, accurate and inexpensive method to find out the etiologic agent in approximately 80% of the bacterial meningitis. We have found good S, SP, PPV and NPV in comparison to the bibliography.

P1559 Bilateral thalamic abscesses: a case with full recovery

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Objectives: Cerebral abscesses may arise from cerebral or facial infections or trauma or bacteremia. Here we describe a peculiar case of bilateral thalamic abscesses in an immunocompetent boy.
The case: A healthy Caucasian male at the age of 14 years developed a pharyngitis, with fever (T: 39 °C), chills and severe asthenia, that resolved after 5 days of a first-generation kephalosporin. Fifteen days later, fever reappeared with headache and asthenia. After another week of antibiotic treatment, a confusional state developed, associated with signs of meningitis, isocoric mydriatic pupillae, and normal fundus oculi. CT and MR scans showed two thalamic, bilateral lesions 4–5 cm of diameter. The analysis of CSF showed 6800 cells/mL, mainly granulocytes, glucose: 43 mg/dL, proteins: 170 mg/dL, Cl: 123 meq/L. CSF cultures and coagglutination assay were negative. The patient was treated with meropenem (120 mg/kg/die), vancomicin (40 mg/kg), fluconazole (8 mg/kg/die), and desamethazon (0.8 mg/kg/die). There was a progressive improvement on clinical and TC controls. Twelve days later, apparently after missing one single dose of meropenem, a second-stage coma arose: on TC scan the abscesses showed a new significant enlargement. They were drained and a *Gemella* spp. grew on the cultures of the brownish, thick, purulent-like material. Based on the sensitivity tests, meropenem was kept, for 2 months, associated with vancomicin and then chloramphenicol for 40 days. A new progressive clinical and CT improvement followed. Two months later, the general condition of the patient was good, neurologic signs had disappeared apart from a slow pupillar reflex left, a CT scan showed two hyperdense scars in place of the abscesses. Meropenem was stopped. The boy was sent home and treated with i.v. teicoplanin for 25 days, followed by oral chloramphenicol for 25 more days. He is at the moment in good condition, apparently fully recovered.
Conclusion: This case indicates that in the deep cerebral abscesses surgical drainage plus prolonged specific antibiotic treatment are necessary. By the way, bilateral thalamic abscesses due to an opportunistic agent in an immunocompetent patient are a very rare event.

Brucellosis

P1560 Discovertebral infection in brucellosis

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Diskovertebral involvement in brucellosis occurs approximately 20–50% of cases. In this report discovertebral infection in 11 of 47 patients with brucellosis were presented. The age of the patients were between 29 and 73 (average 55). Eight of the patients had intervertebral disc bulging symptoms. Diskovertebral infection and paravertebral abscess was detected in one patient at the same time. Infection was localized at C3–C4 level in one case, L5–S1 level in two cases, L3–L4 level in three cases, L2–L3 level in two cases, L4–L5 level in two cases and T12–L1 level in one case. *Brucella* spp. was yielded from the blood cultures of the eight patients. Wright agglutination test was positive (>1/800) in all of the cases. Diskovertebral infection was showed in magnetic resonance imaging. No pathologic finding was detected on X-ray. Eight patients were treated with streptomycin (20–40 g), doxycycline and rifampin. One patient did not tolerate doxycycline and instead they were given trimethoprim-sulfamethaxazole. One patient developed allergic reaction to all of the antibiotics used and treatment had to be stopped at the end of the fifth week. Antimicrobial therapies were continued for 4–6 months except this one patient. At 2–10 month follow-ups, no recurrence was observed at any patient and surgical intervention were not needed in any case.

P1561 Osteoarticular changes in patients with human brucellosis

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Objectives: To evaluate features of osteoarticular involvement in patients with human brucellosis: rates, types and numbers.
Materials and methods: A total of 768 new patients with human brucellosis between 01.1991 and 11.2001. Four hundred forty-six of them, 58%, were with some osteoarticular changes. Diagnosis was made using standard clinical, biochemical, serological (BAB, Wright, COOMB, S, RVK, 2-mercaptoethanol, ELISA IgM and IgG) analysis. Also, we made standard radiographs.
Results: Main changes in all patients were: high temperature in 96%, swelling in 83%, osteoarticular changes in 58% (307 males and 139 females). In 69 patients, osteoarticular changes were the only sign of the disease. Sacroileitis was found in 263 patients (179 bilateral), peripheral arthritis in 145 and spondylitis in 38. Polyarticular changes were in 62.1% and monoarticular in 37.9%. In treatment, we included SMC, cotrimoxasol and doxyciclin or rifampicin, cotrimoxasol and oxytetraciclin plus diclofenak in all of them. In 72 patients disease relapsed.
Conclusion: Osteoarticular changes are frequent in patients with human brucellosis and could be diagnostic dilemma when are presented as the only sign of the disease, especially out of the endemic regions.

P1562 Detection of *Brucella* spp. in a tertiary Greek hospital

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Objectives: Brucellosis remains a common infectious disease in many parts of the world, notably in Mediterranean countries and the Middle East. In this study, we present the laboratory detection of *Brucella* spp. in our tertiary hospital between 1994 and 2000.
Methods: The samples included blood and bone marrow for culture and serum for detection of *Brucella* IgG, IgM and IgA antibodies by immunofluorescent assay, Wright tube test and Rose Begal test. BACTEC NR 660 and recently BACTEC NR 9240 bottles were incubated for 20 days from patients with suspicion of clinical brucellosis. Growth time of *Brucella* was approximately within the second to fourth day (range 36 h–14 days).
Results: We found 66 patients, 40 males and 29 females, mean age 57 ± 2 with positive blood cultures. The yearly distribution of *Brucella* spp. between 1994 and 2000 was 6, 4, 8, 9, 5, 13, 21 cases, respectively. In two cases *Brucella* spp. was also isolated from bone marrow culture and in one case in synovial fluid. Positive titers of *Brucella* antibodies, Wright tube and Rose Begal test were present in 59 tested out of 66 blood culture positive cases. All but one patients were cured with antimicrobial treatment (mainly rifampicin plus tetracycline or quinolone plus rifampicin).
Conclusion: Brucellosis remains a public health problem in Greece. Blood culture system (BACTEC) is as effective as serologic routine tests. The prevention of the disease requires animal testing and education of high risk population.

P1563 *Brucellar sacroliitis*: evaluation of 41 cases

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Objectives: Sacroiliitis, a common involvement of brucellosis, is the main cause of the back pain. In this study, clinical and laboratory characteristics of patients with brucellosis were retrospectively evaluated.
Methods: Clinical features and laboratory findings of 41 patients with sacroiliitis due to brucellosis followed and treated at Infectious Diseases Department were investigated. Diagnosis of brucellosis was made consistent symptoms and on serum titers >1/40 standard agglutination test and/or isolation of *Brucella melitensis*. Sacroiliitis was investigated by spot X-ray, Tc99 scintigraphy or magnetic resonance imaging (MRI).
Results: Forty-one patients whose mean age was 38.8 ± 18.3 years were evaluated. Thirteen (31.7%) of those were male. Sacroiliitis was detected by X-ray in 27 (65.8%), scintigraphy in 8 (19.5%), X-ray + scintigraphy in 4 (9.8%), scintigraphy + MR in 1 (2.4%), and MR in 1 (2.4%). Sacroiliac joint has been involved unilaterally in 21 (51.2%) and bilaterally in 20 (48.8%). Symptoms and clinical examination findings of patients were as follows: arthromyalgia ($n=36$; 87.8%), fever ($n=34$, 82.9%), chills ($n=25$, 61.0%), weight loss ($n=2$, 4.9%), diarrhea ($n=1$, 2.4%), hepatomegaly ($n=14$, 34.1%), splenomegaly ($n=7$, 17.1%), spondylitis ($n=7$, 17.1%), arthritis ($n=7$, 17.1%), lymphadenopathy ($n=3$, 7.3%), orchitis–oophoritis ($n=2$, 4.9%), osteitis ($n=1$, 2.4%). On laboratory investigations, erythrocyte sedimentation rates (ESR) were found high levels, but 9 (27.5%). Mean value of ESR was 45 ± 29. Titers of SAT were ^{3}1/160 in 39 (92.7%) patients. All patient were given as combination of at least two antibiotics. Cure rate was 97.6% by minimum 6-week therapy. The only case whose complaints were not lessened despite 6-week therapy, was determined he had also spondylodisciitis by MR and treated for 6 months.
Conclusions: In this study majority of patients with sacroiliitis were women. The considerable point is almost equal rates of unilateral and bilateral involvement of sacroiliitis in brucellosis.

P1564 Chronic hepatosplenic abscesses in brucellosis: clinico-therapeutic features and molecular diagnostic approach

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Objectives: To describe clinical, radiological and histological features of chronic hepatosplenic abscesses (CHSA), and define the contribution of new molecular techniques for diagnosis, and our experience with treatment of this unusual complication of brucellosis.
Methods: From January 1982 to July 2001 we studied prospectively 832 patients with brucellosis. Diagnosis was established according to isolation of *Brucella* spp. in blood or other body samples or a compatible clinical picture with specific antibodies at significant titers or seroconversion. Liver and splenic samples of patients with CHSA were studied by a PCR technique consisting of the amplification of a 223-bp fragment from the gene coding for the synthesis of a membrane protein of *B. abortus* (BCSP31). All patients were treated and followed homogenously.
Results: Eight patients (0.96%) had CHSA, six men and two women, aged 57.6 + 16.6 years (range: 39–80). Their localization was hepatic in five cases and splenic in three. The duration of the symptoms was 6.7 + 9.2 months (range: 1–24). Blood cultures were negative in seven cases, SAT titers were diagnostic in three (37.5%) and Coombs' in seven (87.5%). Culture of the samples of tissue or pus was positive in one (16.6%) of the six cases in which it was performed, whereas the PCR assay was clearly positive in all these samples. Abdominal CT showed poorly defined heterogeneous lesions with large central calcifications, surrounded by a hypointense area. Conservative therapy with or without percutaneous drainage failed in all six cases attempted. One patient received surgical treatment initially, and one patient refused splenectomy. All excised pieces showed the presence of necrotizing granulomas. After a followup of 35.8 + 14.4 months (range: 14–61) six of the surgically treated patients remain asymptomatic.
Conclusions: (1) CHSA are a rare but severe complication of brucellosis. (2) Their diagnosis is difficult with conventional microbiological techniques. (3) Molecular techniques appear to be a very powerful diagnostic tool. (4) Combined medical and surgical therapy should be recommended.

P1565 *Brucella endocarditis*: clinical experience with 11 cases

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Background: *Brucella endocarditis* (BE) is a rare complication of *Brucella* infection but nevertheless responsible for the majority of deaths related to this illness.
Methods: Descriptive study of BE in a prospective cohort of 1000 patients with brucellosis diagnosed between January 1985 and December 2000, in two tertiary hospitals in the South of Spain. BE cases were defined according to the Duke criteria.
Results: We observed 11 cases of BE which represents the 1.1% of all cases of brucellosis, and the 3.5% of the left-sided infective endocarditis diagnosed in the period of study. Ten patients were male and the mean age was 42 ± 14 years (range: 21–69). The average time to diagnosis was 142 days (range: 15–365). A pre-existent valvular disease was present in 5 (45%) patients: rheumatic valvular disease in four and one patient with bicuspid aortic valve. The aortic valve was infected in nine (82%) patients and the mitral valve in two (18%). Ten (91%) patients developed left ventricular failure (LVF) and two patients had severe embolic complications. The echocardiographic study showed vegetations in all cases. Blood cultures were positive in seven (65%) patients. Cardiac surgery was performed during the active endocarditis in eight (72%) patients with aortic valve infection because of hemodynamic instability, and annulus abscess was observed in three of them. Antimicrobial treatment given was doxycycline plus rifampin for 3 months plus streptomycin the three first weeks. One patient (9%) died and the others were asymptomatic and without relapses after 2 years of follow-up.
Conclusions: (1) The incidence of BE remains a life-threaning complication of brucellosis, even in the last years; (2) the diagnostic delay would enhance the valve damage; (3) cardiac failure is the major reason for valvular replacement; (4) combined medical and surgical treatment is still necessary in most of cases.

P1566 Epididymo–orchitis and spondylitis due to *Brucella mellitensis*

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A 65-year-old Greek farmer was admitted to the hospital because of painful scrotal swelling, hepatosplenomegaly, lumbar pain that was radiated to the lower limps and low-grade fever accompanied by profuse sweating. His medical records were unremarkable while his life style included occupational animal exposure ingestion of raw milk and milk products. The leading symptoms were vertebral pain that was started 6 months ago and gradually impaired his functional status requiring bed rest and analgesics, along with the scrotal pain. No neurological deficits were noticed. The liver function tests, the hemoglobin level, the ESR and the rest of the laboratory data were within the normal ranges. Focal hypoechoic right testicular lesions, swelling of the concurrent epididimis along with an increase in the vascularity of the right testis were seen on a Doppler examination. These findings were consisting in unilateral epididymo–orchitis. A CT scan of the lumbar spine area was also performed and showed a decrease of the signal intensity localized in the anterior aspect of L5 vertebral body at the discovertebral junction involving the subchondrial parts of the L5 and S1 vertebrae. These focal spondylitis lesions were also demonstrated on the technetium bone scanning (increase uptake at the affected area). Standard tube agglutination test was positive for antibodies to *Brucella mellitensis* (titer >1/1280) and the Coomb's test revealed titers of antibody to *Brucella* of >1/320. Cultures of blood specimens were positive for *Brucella mellitensis*. The patient was placed on a combination of antibiotics with doxycycline, streptomycin and rifampin. A remarkable improvement of his clinical condition was showed 2 weeks later. This case illustrates the following points: In areas in which brucellosis is endemic when scrotal abnormalities are seen the possibility of genitourinary tract complications of *Brucella* should be considered. Epididymoorchitis is a focal form of human brucellosis described in 2–20% of patients with *Brucella*. Therefore,

Brucella must be placed in the differential diagnosis of orchitis. Focal spondylitis may coexist and the patient evolved to diffuse disease. A high index of suspicion along with the proper medical treatment is recommended to complete resolution.

P1567 Relapsing *Brucella abortus* epididymo-orchitis

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Brucella abortus rarely causes epididymo-orchitis. In this report a case of *Brucella abortus* epididymo-orchitis relapsing 4 months later was presented. A 47-year-old male was admitted to the hospital with fever and right testicular pain and swelling. Four months earlier, he had been hospitalized in our clinic and treated for brucellosis associated with left epididymo-orchitis. He had taken rifampin plus doxycycline for 6 weeks and completely recovered. None of the risk factors for reinfection was detected in this case. *Brucella abortus* was yielded from his blood cultures. Testicular ultrasonography showed right epididymo-orchitis. He was given streptomycin plus doxycycline and non-steroidal anti-inflammatory drug. At the fourth day of the treatment fever, testicular edema and pain were disappeared; but aspermia was detected in his spermogram. Antimicrobial treatment was planned to be continued for 8 weeks and he was discharged. What interesting for this case was relaps of *Brucella* epididymo-orchitis and involvement of the both testisses.

P1568 A case of brucellosis presenting with severe thrombocytopenia

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Thrombocytopenia is one of the rare hematologic complication of brucellosis. Herein a case of brucellosis with severe thrombocytopenia was reported. The patient was admitted to hematology service with epistaxis, ecchymoses, fever, thrombocytopenia (0.6×10^3/L) and anemia. His initial diagnosis was idiopathic thrombocytopenic purpura. Corticosteroid treatment was started and erythrocyte and thrombocyte suspensions were transfused. Despite this treatment clinical symptoms and laboratory disorders were not improved. No significant pathology was detected in the examination of bone marrow aspiration. On the seventh day, *Brucella abortus* was yielded from his blood cultures. Steroid was stopped and rifampicin plus doxycycline started. His clinical symptoms were disappeared and laboratory findings improved (thrombocyte count: 205×10^3/L) at the second week of the antibiotic treatment. It was thought that thrombocytopenia was related to immune destruction of thrombocyte in the peripheral blood. This case was interesting showing us that thrombocyte count might decrease to very low levels in brucellosis. So that, especially in the endemic areas, brucellosis should be kept in mind for the etiology of fever and thrombocytopenia.

P1569 Breast abscess caused by *Brucella melitensis*

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Brucellosis has been significantly decreased worldwide, but still remains a problem in some regions of Greece. Early diagnosis of focal forms of brucellosis is especially important for the prognosis and treatment of the disease. Cutaneous and soft tissue lesions are uncommon manifestations of brucellosis. Though breast involvement in animal brucellosis is not uncommon, involvement of the breast in human brucellosis is very rare. We report a case of breast abscess in a 55-year-old female caused by *B. melitensis*. The patient was admitted to the internal medicine department of our hospital, with fever, chills, malaise and weight loss. During physical examination a palpable mass was found in the left breast. Ultrasonography and mammography revealed findings suggestive of abscess. *B. melitensis* biotype II was isolated from blood and soft tissue culture. Rose Bengal and Wright were also positive. Surgical drainage of the abscess was performed. Combination of rifampicin and doxycycline for 3 months resulted in clinical cure. In conclusion, although breast involvement in human brucellosis is extremely rare, one should be aware of the diagnosis, especially where this zoonosis is endemic.

P1570 Hematological manifestations of brucellosis

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Objectives: To determine the hematological manifestations of brucellosis observed during the course of active infection.
Methods: One hundred and thirty-two patients who were followed-up with brucellosis in our clinic between February 1999 and October 2001 were reviewed retrospectively. The diagnosis was based on clinical findings and serologic results and/or blood culture confirmations. Duration of complaints physical examination and laboratory findings were noted. All these 132 patients were divided in three groups due to the duration of their complaint (acute, <8 weeks; subacute, 8–52 weeks; chronic, >52 weeks) to assess the rate of their hematological complications.
Results: The mean age of patients was 42.2 years. The female and male ratio were 57/75 (43.2–56.8%, respectively). Anemia was detected in 20/132 (15.2%) patients. Thrombocytopenia was found in 8/132 (6.1%) patients. A mild thrombocytopenia ($(100–150) \times 10^9$/L) was detected in four of them where as a severe ($(5–90) \times 10^9$/L) form noted in others Leukopenia was seen in 6/132 (4.6%) patients. Pancytopenia occurred in 2.3% (3/132) of cases. One of the pancytopenic patient had deep thrombocytopenia (5×10^9/L) and leukopenia (1.1×10^9/L). No clinically detectable bleeding occurred. Hematological complications of brucellosis observed mostly in acute brucellosis cases. Thrombocytopenia and leukopenia were resolved rapidly with treatment of the *Brucella* spp. infection.
Conclusions: Patients with *Brucella* spp. infection occasionally manifest hematological abnormalities including Pancytopenia especially in acute cases. Though these hematological complications might be severe, all could be disappeared following successful antimicrobial therapy. Brucellosis may be considered in patients whose blood picture reveals leukopenia, trombocytopenia or Pancytopenia particularly when the disease is epidemiologically suspected.

P1571 Brucellar orchiepididymitis as a clinical manifestation of human brucellosis

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Objectives: Human brucellosis is endo-epidemic disease in our country among the cattle and people. In our study we present our experience with brucellar orchiepididymitis in our region during a period from 1980 until 2000 as a part or the only clinical manifestation of brucellosis.
Material and methods: During the period of 20 years, 31 patients were treated of unilateral or bilateral orchiepididymitis out of total number (502) patients infected with brucellosis meaning that 6,1% of the patients had genital involvement. Ages were among 13–59 years. Diagnosis was obtained using standard clinical, biochemical and serological (Rose-Bengal, Wright, Coombs, RVK, ELISA) investigations.
Results: Our patients were distributed in two groups according to living place: 19 patient lived in surrounding villages and other 12 patients lived in towns. The predominant symptoms were: fever, malaise, osteoarticular involvement, enlarged lymph nodes, enlarged liver and spleen. Twenty-three patients had orchiepididymitis as a part of brucellosis, and the other eight patients had orchiepididymitis as the only clinical manifestation. Twenty-five patients had bilateral, and the other six had unilateral orchiepididymitis. The affected testes were very painful, warm and swollen. The treatment consisted bed rest with immobilization of the scrotum, corticosteroides in the most refractory cases for a 7-days and SMC, cotrimoxasol, and doxycyclyne or rifampicin cotrimoxazole and oxytetracycline. All patients responded well to the treatment with completely resolution. In five patients (16.2%) we had relapse which consequently responded well to the additional therapy.
Conclusions: The study is going on to determine whether the infection would have any adverse effect on the fertility in bilateral involvement. We confirm that with the fact that two our patients who had bilateral brucellar orchiepididymitis are being treated from infertility for more than 5 years. We added that human brucellosis is a significant health and socioeconomic

problem in Macedonia. Adequate medical, veterinary and sanitary measures and care should be exercised in order to put the infection under control.

P1572 *In vitro* activities of antimicrobials against *Brucella melitensis* isolates

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Objectives: To test the antimicrobial susceptibility of *Brucella melitensis* isolates to various antimicrobial agents by using Sceptor automatic system.
Methods: The 21 *B. melitensis* isolates were collected between 1 January 1998 and 28 February 2001 from blood cultures of individual inpatients with acute brucellosis at Cumhuriyet University Hospital. Ten milliliters of blood were inoculated into a Bactec Plus aerobic/F bottle for adult patients and three milliliters inoculated into a Bactec Peds Plus aerobic/F bottle for pediatric patients. These inoculated bottles were incubated at 35 °C and were monitored in Bactec 9050 (Becton Dickinson Diagnostic Instrument systems, Towson, MD) automatic system. The bottles were kept in incubation during 21 days, and they were subcultured when the machine detected its growth; if not, a blind subculture was performed after 21 days. The isolates were identified to the species level by conventional methods, on the basis of not requiring CO_2 and not producing H_2S. All microorganisms isolated were *B. melitensis*. The in vitro antimicrobial susceptibilities of the 21 clinical isolates of *B. melitensis* to tetracycline, streptomycin, cotrimoxazole, rifampicin, ciprofloxacin and some of the third generation of cephalosporins were determined by using Sceptor (Becton Dickinson Diagnostic Instrument systems, Towson, MD) automatic system.
Results: Antimicrobial susceptibility of *B. melitensis* isolates was: cotrimoxazole, 100%; rifampicin, 81%; streptomycin, 86%; and tetracycline, 100%; cefotaxime, 100%; cefoperazone, 67%; ceftriaxone, 100%; ciprofloxacin, 100%.
Conclusion: *B. anthracis* isolates are generally susceptible to antimicrobials suggested the treatment of brucellosis especially tetracycline and cotrimoxazole in our region (Sivas city, which is located in the Middle Anatolian of Turkey).

P1573 Detection of Brucella DNA in serum specimens from patients with suspected brucellosis

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Objectives and methods: *Brucella* organisms are intracellular pathogens that have the capacity to survive and multiply within the phagocytes of the host. Different methods have been described for diagnosis of human brucellosis. Among them, peripheral blood PCR assay seems to be a very promising technique not only for the initial diagnosis of the disease, but also for the post-treatment followup and the early detection of relapses. Recently, PCR has been applied to detect *Brucella* DNA in serum of patients with brucellosis, and serum has been proposed as the optimal specimen for the diagnosis of brucellosis. In the present study the PCR technique with primers that amplify a 223-bp DNA sequence, present in a gene encoding a 31-kDa *Brucella abortus* antigen, was used to detect the DNA of this bacterium in serum specimens from patients with suspected brucellosis. Sixty-four serum specimens from equal number of patients referred as probable cases of brucellosis and 50 serum specimens from healthy individuals, as negative controls, were examined. All serum samples were also examined using the Rose Bengal Plate test (RBPT), the wright test and an enzyme linked immunoassay (Serion ELISA) able to detect specific IgG/IgM/IgA antibodies.
Results: From 64 sera, *Brucella* DNA was detected in 25 serum specimens (39%). These specimens, except two sera that developed specific antibodies 2 weeks later, revealed positive RBPT, Wright test and ELISA for one or more antibody classes. The remaining 39 *Brucella* negative DNA sera were RBPT, Wright test and ELISA negative. All the control sera were negative for *Brucella* DNA and antibody detection.
Conclusions: Our results suggest that PCR assay of serum specimens can contribute to the rapid and accurate diagnosis of brucellosis. However, it remains to be clarified whether positive PCR results are due to *Brucella bacteremia* or to breakdown products that are present during the infection and whether there is correlation between the DNA detection and the presence of specific antibodies.

P1574 Differences in acute phase reactants and blood count in brucellosis

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Objectives: The aim of this study is to determine increase of acute phase reactants and changes of blood count in brucellosis. Acute phase reactants are a group of protein those are synthesized by liver as a response to infection, including haptoglobulin, some protease inhibitors, components of complement system, seruloplasmin and fibrinogen. These are early response to infection. After days some other changes, including increase in erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), decrease in blood iron and albumin levels occurs. These are late acute phase reactants and occur as response to some cytokin related changes in vivo. In brucellosis diagnosis is based primarily on semptoms of patient, clinical findings and seropositivity with Wright's agglutination test in routine practice. Acute phase response may be used to confirm the diagnosis of brucellosis either is acute or chronice illness.
Methods: Fourty-four patients diagnosed as acute brucellosis were chosen and their blood levels of ESR, CRP, antistreptolysin O (ASO), rheumatoid factor (RF), aminotransferases (AST, ALT), alkaline phosphatase (ALP), serum iron, albumin are compared with patients seronegative to brucellosis as a control group. Also total blood count of two groups have been compared prospectively.
Results: The increase in blood levels of ESR, CRP, AST, ALT and ALP were found significant compared to control group ($P = 0.000$ and 0.001). Although no differences were found in blood levels of ASO and RF in both groups. Decrease in serum iron and albumin levels were more significiant than control group ($P = 0.001$). According to comparison of blood counts of two groups, decrease in white blood cell counts (WBC) and hemoglobin levels were significiant. The platelet count was found insignificant compared with control group. All findings were proven by statistically with using Student's *t*-test.
Conclusions: *Brucella* species are an intracelluler organisms so the treatment of brucellosis is long lasting and struggling for clinicians. Especially chronic patients with relapses have to been treated for longer periods than the acute ones. So the determination of the course of illness is a milestone in effective treatment. We propose to acute phase reactants especially increased ESR and CRP levels as a cooperative tests for a clinician to decide whether the illness is acute or not.

P1575 Thrombocytopenia in acute Brucellosis

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Brucellosis is an intracellular zoonotic disease caused by *Brucella* spp. which involves many systems and organs lead to serious complications in organs involved. Different symptoms and clinical findings makes disease to be confused with several other diseases such as; rheumatoid, cardiac, dermatological, neuological and hematological diseases. The hematologic manifestations of brucellosis include anemia, leukopenia, thrombocytopenia and clotting disorders. Two cases of brucellosis with thrombocytopenia are reported here. With the appropriate treatment of brucellosis thrombocytopenia was fully recovered. Brucellosis is an endemic infection in our country. Sometimes, rare complications may be prominent as in our cases. In cases going with thrombocytopenia and Pancytopenia brucellosis should be included in differential diagnosis.

P1576 A case of primary psoas abscess due to brucellosis

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Objective: Skeletal system involment is relatively common complication of human brucellosis. But rarely reported muscular involvement and particularly psoas abscess is always secondary to spondylitis.
Case: A 70-years-old female patient admitted to our clinic with the complaints of fever, night sweats, pain in the joints of his back and leg for 3 weeks. Physical examination revealed a body temperature of 37 °C and pulse rate of 70 beats/min. Tenderness on hip and knee joint was present. Joint movements was limited due to pain. Complete blood count showed a 7000/mm^3 white blood cell count with 59% polymorphonuclear leukocyte and 38% lymphocyte. Erythrocyte sedimentation rate was 12 mm/h and rheumatoid factor was 30 (normal range 0–20). Lumbosacral X-ray showed osteoporosis and osteophyte formation in L3–L4 vertebral level. Nuclear magnetic resonance (NMR) imaging of the lumbosacral region showed psoas abscess and spondylitis on vertebral corpus. Wright Agglutination test was positive with 1/1280 titer. Blood culture for *Brucella* species yielded negative result. Doxycyline 200 mg/day and rifampin 600 mg/day therapy together with NSAID is started. Antibiotic therapy continued for 6 weeks. Patient was fully recovered both clinically and radiologically after 6 weeks treatment.
Conclusion: Rare complications of brucellosis should be always on mind of physicians, since small portion of brucellosis cases are capable of developing unexpected complications in unusual organs.

P1577 Epididymo-orchitis due to *Brucella* species: report of four cases

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Objective: Brucellosis is a zoonotic infection involving many organ systems. Involment of genitourinary system especially in young man is a rare complication with incidence ranging between 2 and 10% of all *Brucella* cases.
Methods: Between 1996 and 2001 years 120 (78 male, 42 female) patients hospitalized for brucellosis were analyzed retrospectively for clinical and laboratory findings together with complications of brucellosis.
Results: The most common symptom of all patients were fever, arthralgia, and night sweats on admission. Besides clinical symptoms, laboratory findings included mostly leukocytosis, elevated erythrocyte sedimentation rates and positive serological tests for brucellosis. Four of 76 (%5, 12) male patients admitted with the signs and symptoms of testicular involvement. These were tenderness, swelling, calor and rubor of the unilateral scrotum. Diagnosis is corrected by serological tests in both patients. Only two patients' blood culture yielded positive result for *Brucella* spp. Ultrasonographic findings of the scrotum corrected our diagnosis as epididymo-orchitis. Both patients responded to the medical therapy and no surgical procedure is needed. No genitourinary complication was observed among female patients.
Conclusion: Epididymo-orchitis due to brucellosis is a rare complication of the disease. Testicular involvement is usually unilateral as it is in our cases. Physicians who evaluated a patient as epididiymo-orchitis especially in brucellosis endemic areas should include the disease in different diagnosis.

P1578 Isolation and typing of *Brucella* from blood culture of human brucellosis and its determinants in a region of Iran

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Objectives: Brucellosis remains a major zoonosis worldwide. As bacteriological aspects of disease is the basic knowledge for control and elimination of brucellosis, this study was carried out on human brucellosis in hospitals and clinics of the province in 1999. However, in Iran with reporting at least 15000 cases in each year (incidence rate = 25/100 000) research on biological and biochemical characteristics of the causative organisms is very important.
Methods: A total of 101 cases with clinical and serological diagnosis of brucellosis and any use of antibiotics have answered to 44 questions. The blood sample, approximately 7–10 mL, was taken from all of them and transferred aseptically to a *Brucella* medium, Castanida. If the blood culture was positive, typing of isolated *Brucella* was perfumed by biotype classification, using the technique recommended by WHO (Alton et al. 1975, Corbel et al. 1978) in Razi Institute.
Results: A total of 52 persons of patients were the males and others were females. From blood culture of 34 (33/6%) patients *Brucella melitensis* biotype 1 was isolated and sex ratio (male/female) was 20/14. Positive blood culture patients (first group) are significantly younger than negative blood culture patients (second group) {25 (SD = 15/9) and 35/3 (SD = 18/8) years old, respectively, (*P*-value < 0/01)}. From total of patients, 22 persons (21/8%) had <15-year-old and 50% of them (11 persons) were in the first group, whereas in adult patients isolation of *Brucella* was 29/1% (23 persons). History of disease (brucellosis) in patients decreases the chance of *Brucella* isolation {OR = 0/12 (0/02–0/57), *P*-value = 0/002}. Geometrical mean of seroaglutination test and 2-mercapto ethanol was not different in two groups (1/265 and 1/132, respectively, for 84 and 50 patients. Orchtic sign in the males of first group was 36/7% and in second group was 12/1% (*P*-value = 0/01). *P* revalence of exposure with animals and aborted fetus of animals in first group was higher than other group. Variation of isolation *Brucella* in five cities of province was from 11 to 50% of patients in each city that is conform with distribution of exposure with animals and aborted fetus of animals and chronic forms of disease.
Conclusion: *Brucella melitensis* biotype 1 is the main species and biotype in Iran and age, contact with animals and their aborted fetus, incubation period and history of disease are the main factors affecting isolation of *Brucella* from blood culture of patients.

P1579 Specific ELISA IgM and IgG antibodies for the detection of active brucellosis

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Objectives: In this study, we analyzed the results of classic serological tube agglutination test and IgM and IgG ELISA for diagnosis of active disease; and their variations during the clinical course of brucellosis.
Patients and methods: Eighty-six patients diagnosed with brucellosis who were admitted to our hospitals between April 1999 and August 2001 were included in the study. The diagnosis of the cases was based on clinical findings and on serological and/or blood culture positivity. The Rose Bengal, tube agglutination (STA), ELISA for specific IgM and IgG were performed for each serum samples on admission and several months after treatment. These 86 patients were divided in three groups according to the duration of their complaints (acute group <8 weeks; subacute group, 8–52 weeks and chronic group, >52 weeks), to compare the test results.
Results: The mean age of patients was 42.7 years. The female and male ratio was 38/48 (44.2 and 55.8%). Except for 30 instances involving the IgM ELISA and six instances involving the IgG ELISA, all serological tests gave positive results at admission. There were 40, 30, 16 patients in acute, subacute and chronic groups. At admission, the positivity rate for IgM antibodies were, 32/40, 16/30 and 8/16 for the groups, respectively. These rates for IgG antibodies were as follows; 37/40, 27/30 and 16/16. These tests were repeated in 60 patients who were treated for brucellosis. The positivity rate of ELISA IgM and IgG titers for acute, subacute and chronic groups were as follows; 5/22, 20/22; 3/24, 19/24, 2/14, 14/14, respectively. When ELISA IgM levels from the beginning of treatment and from several months later were compared, a statistically significant decrease was observed ($P < 0.001$). For STA titers evaluated after treatment, there was a clear decline in titers, but none of 60 patients did have a negative value. In six patient an increase in IgG level was noted but none of these six patients had relapse of the illness.
Conclusions: Our results show that ELISA IgM is a better method for diagnosis and followup of brucellosis than the other tests.

Miscellaneous community-acquired infections

P1580 Antiphospholipid syndrome as a cause of fever of unknown origin

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Antiphospholipid syndrome is defined as the occurrence of thrombosis, recurrent miscarriage, or both in association with persistent antiphospholipid antibodies. In veins, deep venous thrombosis is the most common manifestation, but involvement of visceral veins is well recognized. Due to a wide range of its clinical manifestations and laboratory studies, the diagnosis is difficult. We describe a patient presented with fever of unknown origin (FUO) and found to have the primary antiphospholipid syndrome. A 46-year-old male was admitted with abdominal pain, fatigue, anorexia, dark urine, diarrhea, and fever of 2 days. His past history was noncontributory, elder brother had a history of myocardial infarction. During admission, the temperature was 40 °C, and he had jaundice on scleras. Crackles over the left lower lung was significant. A chest X-ray revealed left lower lung atelectasis. Laboratory studies were as follows: hematocrit 36.3%, WBC 13 300/mm^3 (granulocyte 83%, lymphocytes 9%, monocytes 8%), platelets 140 000/mm^3, erythrocyte sedimentation rate 88 mm/h, CRP 219 mg/L (normal: 0–5), total protein 5.6 g/dL, albumin 3.1 g/dL, ALT 68 U/L, AST 64 U/L, γ-GT 118 U/L (7–47), ALP 297 (64–306), *T. bilirubin* 3 mg/dL with a direct fraction of 2.2 mg/dL. An abdominal US was normal. He was then prescribed ceftriaxone (1 g, twice daily) and clarithromycine (500 mg, twice daily), regarded as a community acquired pneumonia but he did not respond within 10 days. A thorax CT showed the atelectasis of posterobasal segment of left lower lobe. Bronchoscopic examination was normal and BAL fluid remained sterile. Jaundice was regressed within 1 week but fever persisted. An abdominal CT revealed portal, splenic, and superior mesenteric thrombi. ERCP, lower extremity Doppler sonography, cranial MRI, and ophthalmic angiography were negative. Lupus anticoagulant was negative and protein C level was normal; but anticardiolipin IgG was found as 22 GPL (0–8). The test repeated 10 days later resulted as 23.9 GPL. The fever persisted all 7 weeks. The patient was initiated LMW heparin and then oral coumadin. He responded well; no fever was encountered after 1 week. During follow-up of 4 months, he is doing well. This patient represent the first report of antiphospholipid syndrome presented with FUO in English-language literature. Although very rare, antiphospholipid syndrome should be considered in differential diagnosis of prolonged fever associated thrombosis.

P1581 Evaluation of 80 adult fever cases of unknown origin in Turkey

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Objective: To evaluate the fever of unknown origin (FUO) cases in terms of their diagnosis and prognosis.
Methods: A prospective evaluation was performed among the inpatients who had been admitted between 1993 and 1999 to Clinical Bacteriology and Infectious Diseases Clinic in Ibni Sina hospital with 1100 beds. Fever of unknown origin was defined as, at least 3 weeks of fever >38 °C prior to admission and at least one examination by a physician during this period.
Results: All patients were >15 years of age and 52% was female. The mean and median of the age were 43.9 and 44, respectively. Prior to admission, 33% of the patients was hospitalized in different institutions, 34% of them was evaluated in tertiary, 43% in secondary levels of health care system, and 25% applied to more than one institutions for medical evaluation. Before their admission, all patients had at least one course of antibiotic therapy and majority of them (65%) was examined by internal medicine specialist. At the end of evaluation, in 52% of he cases the etiology was infectious, in 19% immunological and in 17% oncological diseases. In 12% of the cases the reason for high fever couldn't be explained. Among the most common infectious diseases, the various forms of tuberculosis (12%), brucellosis (12%), salmonella infections (7%) and malaria (5%) were diagnosed. 41% of the patients was totally cured, 58% was transferred to other clinics of the hospital and only one of them was recorded as exitus. The mean and median of length of stay were 20.7 and 17.5, respectively. To detect the underlying diagnosis, the most useful tools were serology (19%), pathological evaluation of biopsy specimens (17), imaging techniques (14%), and detection of microbiological agents by culture (14%) or smear (9%). The consultations from various surgical and medical fields were asked for 29% of the patiens.
Conclusion: To evaluate FUO cases, we used a unique definition which was relevant for the health care system of our country. Infectious reasons were still the leading etiology among FUO cases in Turkey. Microbiological and serological techniques were found to be crucial in detection of underlying causes.

P1582 Fever of unknown origin: an evaluation of 83 cases

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Objectives: In this study, we reviewed 83 cases with fever of unknown origin (FUO) admitted to Infectious Diseases Department of Çukurova University, Medical Faculty Hospital between January 1994 and July 2001.
Methods: The criteria of Petersdorf and Beeson (Medicine 1961; 40: 1–30) were used for definition.
Results: A total of 59 (67%) were male, and 24 were female (median age 38.5, range: 14–80). Among the 83 cases included in this study 77 (93%) were determined as classic FUO whereas six of them (7%) were determined as neutropenic FUO. Infectious Diseases, *n*: 49 (59%) were the most common causes of FUO. Tuberculosis (Primary TB and miliary TB) constitutes the most common infectious reason, *n*: 11 (22%). Other infectious reasons were: infective endocarditis, *n*: 6; abdominal abcess, *n*: 5, brucellosis, *n*: 6; visceral leishmaniasis, *n*: 4, salmonellosis, *n*: 4, atypical pneumonia, *n*: 4, rhinocerebral mucomycosis, *n*: 3, cerebral toxoplasmosis, *n*: 1, Cytomegalovirus infection, *n*: 1. Non-infectious reasons of FUO were: I. Collagen vascular diseases, *n*: 13 (16%); (SLE, *n*: 5, Still's disease, *n*: 3, Behcet Disease *n*: 1) II. Neoplasms, *n*: 12 (14.4%) (Lymphomas *n*: 4); III. Miscellaneous, *n*: 3 (4%) (tiroiditis, hemosiderosis, granulomatous hepatitis); IV. Undiagnosed, *n*: 6 (7%).
Conclusions: Infectious diseases especially tuberculosis are the leading diagnostic category of FUO in Turkey. we followed undiagnosed cases with FUO (*n*: 6) during 1 year, five of them (83%) completely recovered and only one of them died.

P1583 Clinico-epidemiological study of Mediteranean fever cases hospitalized in Bucharest between 1999 and 2001

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Objectives: To describe the clinical and epidemiological features of the adult cases of Mediteranean fever (MF) treated in our hospital from 1999 to 2001.
Methods: Retrospective study of the hospital files of all adult patients discharged with the diagnosis of MF during the last 3 years.
Results: A total of 129 adult cases were recorded at our institute, with an increasing incidence over the time: 21 cases in 1999, 36 cases in 2000, 72 cases in 2001. The mean age was 49.3 years in the majority of cases (69.2%) that came from the urban area. The risk factors (owing to the dogs with ticks and/or manual tick removal) were present in 50% of cases. The mean values of the most frequent clinical and biological features in these 3 years are presented below:

- Incubation time was 6.5 days;
- Fever was present in 95% of cases;
- The 'button' rush was present in all 129 cases;
- Innoculation eschar was present in 52% of cases;
- Leucocytosis was present in 18% of cases;
- Elevated ESR was present in 76.5% of cases.

Radiographic signs of pleuropulmonary involvement were noted in 1/3 of patients. The mean duration of treatement was 7.5 days in monotherapy (87%) with tetracyclines (80%) or fluoroquinolones (7%) alone, or their association in 13% of cases. The seroconversion was documented in 68% of cases. The outcome was favorable in the vast majority of patients (98.4%) with only one lethal case (generalized vasculitis with disseminated intravascular coagulopathy) and one case healed with sequelae (cerebral vasculitis with infarction). Complications with favorable outcome were presented in 6.2% of cases.
Conclusion: We assist to a remarkable increase of MF cases requiring hospitalization during 1999–2001: 129 compared to only 23 cases between 1990 and 1997. We presume that the great number of errant dogs present in the urban area that were partially 'adopted' by the city population is the risk factor involved in this phenomenon. In 2001 we also noted the first lethal case of MF in Bucharest, 53 years after the introduction of the diseases in our geographic area.

P1584 Acute rheumatic fever (ARF) and long-term sequelae of heart disease: a 15-year experience

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Introduction: Acute rheumatic fever (ARF) is a delayed, nonsuppurative sequel of upper respiratory infection by *S. pyogenes*, characterized by inflammatory lesions involving joints, heart, skin, and subcutaneous tissues. Clinical manifestations include polyarthritis, carditis, subcutaneous nodules, erythema marginatum and Sydenham's chorea.
Objectives: Analysis of carditic manifestations and permanent effects of 27 acute rheumatic fever.
Materials and methods: 27 patients (15 women and 12 males) were evaluated from February 1986 to May 2001. Age range was 9–66 years; eight patients were aged under 15. In all patients ARF had been diagnosed according to Jones criteria (American Heart Association). In eight patients ARF was a recurrent episode. Clinical manifestations, epidemiological aspects and laboratory features of the patients were evaluated.
Results: Patients presented fever (96%), arthritis (85%), angina (70%), carditis (63%), chorea (4%), and erythema marginatum (11%). Six patients presented a first degree atrioventricular; 2 an atrial and ventricular escape. Throat culture was positive for *S. pyogenes* in 7/25 patients (28%). 24/27 patients had antistreptolysins O antibodies titers >400 UI/L. ESR was >35 mm/h in all patients (100%). 11/17 patients with carditis (65%) had mild-to-moderate heart disease: long-term sequelae with mild mitral regurgitation and no signs of heart failure (4/11); carditis (6/11); mitroaortic insufficiency requiring surgical valves replacement (5/11). 9/27 (33%) had permanent sequelae. Severe carditis and heart failure were associated to later ARF diagnosis. ARF was diagnosed in 78% of cases from October to May, and in 33% from April to May. The higher incidence was in the young people (mean = 26 years). 26/27 streptococcal pharyngitis had not been correctly treated.
Conclusions: Data confirm that rheumatic heart disease determine permanent and/or severe heart damage, potentially life-threatening requiring cardiac surgery intervention. They confirm that ARF presently affects mostly young people and that carditis is reported in 63% of cases. The absence of an adequate antistreptococcal treatment is determinant in rheumatic fever development. Reduction in ARF incidence in all western countries over the last decades has stated new issues.

P1585 The role of mycoplasmas in rheumathoid arthritis in comparison with other arthritis

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Objectives: At the rheumatoid arthritis (RA) pathogenesis, some genetic, immunological and inflammatory instances are nominated, but how these immunological and joint inflammations have come into existence is still unknown. Various infectious agents including mycoplasmas have been taken into consideration as a synovial inciter that is followed by immunological responses in chronic arthritis.
Methods: At the culture of synovial fluid for mycoplasma in PPLO media (with standard methods) and investigation of some serological tests such as C-reactive protein (CRP), rheumatoid factor (RF) and cold agglutinins from 100 patients with RA and comparing them with 40 patients with osteoarthritis and 37 patients with septic arthritis (as the control group), following results have been gained.
Results: Culture of synovial fluid of RA patients in seven cases were positive for mycoplasma. Isolated species are *M. pneumoniae* 3, *M. fermentans* 2, and *M. hominis* 2 cases. Concerning with other two groups synovial fluid culture was negative. Other serological tests showed no significant difference between these three groups.
Conclusion: It is supposed that the mycoplasma can be considered as a starter of inflamation in some chronic arthritis such as RA.

P1586 Chronic furunculosis – familiar occurrence and recurrence

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Objectives: To analyze familiar occurence of furuncles, the risk factors of infections and *Staphylococcus aureus* nasal carriage and antibiotic susceptibility results.
Methods: We observed 70 patients from 1998 to 2000, age 2–78, with chronic furunculosis. From each patient purulent discharge from furuncle, nasal and throat swabs were taken. In some cases of familiar furunculosis we took only nasal and throat swabs from all family members. *S. aureus* was identified by standard methods and disk – diffusion susceptibility testing was done according to NCCLS.
Results: We noticed familiar furunculosis in 23 cases (32.8%). 66 patients had reccurent furuncles (94.3%) (lasting several years). Local symptoms were observed in 65 (92.9%) patients, systemic (fever, discomfort, chills) in only five patients (7.1%). Among all patients, three had diabetes and one leukemia. Nasal carriage was observed in 27 patients (38.6%). Most patients were adults >21 years (41 pts, 58.6%), other adolescent 13–21 years (21 pts, 30%), children aged 5–12 years (7 pts, 10%) and only one younger than 5 years. Most of the *S. aureus* strains were resistant to penicillin (94.1%) and doxycycline (27.1%), rarely to clindamycin (4.7%), erythromycin (7.1%), trimethoprim/sulfamethoxazole (5.9%) and ciprofloxacin (1.2%).
Conclusions: Furunculosis has tendency to become recurrent and occur in families. Familial occurence is often connected with nasal *S. aureus* carriage. Not every carriers suffered from furunculosis. Most *S. aureus* in community-acquired infections are resistant to penicillin and doxycycline which one should bear in mind when one wants to prescribe antibiotics.

P1587 The correlation of shock categories with infectious nosologies

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Objectives: The recognition of the nosological–etiological structure, shock type and its mortality during infectious diseases.
Methods: The study includes 300 cases with shock manifestation in the infectious diseases during 1970–2000. The patients' age was 14–82 years.
Results: We distinguished: **1** – hypovolemic shock – 146 cases, 48,6%: 1.1 – hemorrhagic 30 cases, 10%: 13 hepatitis; 11 hemorrhagic fever, 1 flu with DIC; 2 leptospirosis; 2 typhoid fever; 1 shigelosis. 1.2 – dehydrative 116 cases, 38.6% :80 cholera; 30 gastroenteritis; 5 salmonellosis; 1 giardiasis. **2** – distributive shock – 118 cases, 39.3%: 2.1 septic 95 cases, 31.5%: 54 sepsis, 5 gastroenteritis, 3 salmonellosis, 3 hepatitis with sepsis, 1 leptospirosis, 3 leishmaniasis with sepsis, 1 typhoid fever, 1 brucellosis, 1 yersiniosis, 1 meningocoxemia, 1 staphylococcal endocarditis, 1 septic shigellosis, 4

anthrax, 2 streptococcal angina, 2 septic mononucleosis, 1 Landousy typhobacillosis, 1 hyperpiretic malaria, 2 erisipelas, 1 gangrenous orchitis, 2 necrotic fascities, 2 DIP, 1 candidiasis septicopyemia, 2 facial malignant staphylococci. 2.2 Neurogenic: 12 cases, 4%: 3 rabies, 2 tetanus, 3 poliomyelitis, 2 meningoencephalitis, 2 purulent meningitis. 2.3 microvascular failure 5 cases, 1.6%: 4 hemorrhagic fever, 1 leishmaniasis with lomidine. 2.4 anaphylactic 6 cases, 2%: 1 spontaneous rupture of hepatic echinococcosis, 5 anaphylaxia: 3 by penicillin, 1 by ampicillin, 1 by antitetanic serum. 3 – cardiogenic shock: 19 cases, 6.3%: 3.1 myocarditic 7 cases, 2.3%: 1 mononucleosis. 1 flu, 1 typhoid fever, 1 diphtheria, 1 murine typhus, 2 leishmaniasis with glucantime. 3.2 Aritmic 3 cases, 1%: 1 diphtheria, 1 leishmaniasis with glucantime, 1 tetanus. 3.3 Mechanical obstruction 12 cases, 4%: 3 hemopericardium: 1 leptospirosis, 1 flu, 1 hemorragic fever; 1 flu exudative pericarditis; 3 pulmonary thrombosis: 1 from flu, 1 from rickettsiosis, 1 from endocarditis; 2 pneumorrhagia: 1 from leptospirosis, 1 from flu. In 17 cases, 5.6%, we had so-called mixed shock. Total mortality rate was 71 cases, 23.66%.
Conclusions: We noticed shock directly related to infectious diseases on 33 such nosologies.

P1588 Danish national survey on *Moraxella (Branhamella) catarrhalis* isolated from blood cultures: report of 20 cases

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Objectives: *M. catarrhalis* seldom causes systemic infections. A nationwide survey of *M. catarrhalis* bacteremia from the years 1980–2000 was performed.
Methods: Reported cases of *M. catarrhalis* isolated from blood from Danish Departments of Clinical Microbiology were registered. Isolates were identified with either conventional tests for oxidase production, acid production from glucose, maltose and sucrose, hydrolysis of tributyrin or by Minibact-N (The Statens Serum Institut, Copenhagen, Denmark). β-lactamase production was determined. Serum, if obtainable, was examined for presence of IgG and IgM antibodies towards outer membrane proteins (OMPs) of *M. catarrhalis*.
Results: Twenty isolates from 20 patients were recorded. From four patients additional bacterial isolates were recovered; from two patients *Staphylococcus aureus* was isolated, whereas in one patient each *Haemophilus parainfluenzae* and nonhemolytic streptococci were found. Seventeen of the 20 strains produced β-lactamase. Fifteen patients were middle-aged to elderly, while five patients were 8 years of age (median age in years (range): 53 (4 months – 74)). Sixteen patients presented with febrile episodes either of unknown origin or in relation to granulocytopenia ($n = 13$) and/or with a pulmonary focus ($n = 11$); one patient each had acute otitis media, viral infection, tonsillitis with hemolytic streptococci Group A, and gastrointestinal infection. Fifteen of 20 patients had predisposing conditions, especially malignant diseases ($n = 8$) or chronic bronchitis ($n = 3$). All except three patients received antibiotics at the time of blood culturing. All patients, except one who died on the day of admittance, recovered from their infective episode. In patient sera ($n = 7$), IgG and IgM antibodies against OMPs of *M. catarrhalis* were more often present than in serum from blood donors.
Conclusion: The occurrence of *M. catarrhalis* in blood cultures plus an immunological response attest to the organism's potential of causing invasive disease.

P1589 First report on Toscana virus circulation in Umbrian Italy

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Objectives: To evaluate for the first time circulation and clinical expression of Toscana (TOS) virus in Umbria region. TOS virus, which belongs to the Bunyaviridae family, Phlebovirus genus (sandfly fever group), was isolated for the first time in Tuscany in 1971 from *Phlebotomus perniciosus*. It is a neurotropic virus which causes in man lymphocytic meningitis and meningoencephalitis during the summer, due to the higher circulation of the vectors. Originally, it was thought that the virus was limited to some parts of Tuscany, but subsequent reports from another Italian region, Marches, and also other Mediterranean Countries (Cyprus and Portugal) disproved this hypothesis. A region of central Italy, Umbria, borders with Marches on the east and with Tuscany on the west, and it is extensively visited by tourist during the summer, particularly from northern Europe and USA.
Methods: (a) We retrospectively studied all cases of aseptic meningitis and meningoencephalitis admitted in the Clinica di Malattie Infettive of Perugia Hospital from January 1989 to October 2001, with negative results concerning common neurotropic viruses. Clinical data were taken from the medical records. (b) We examined sera from 50 healthy subjects who were residents in Umbria, near Trasimeno lake, on the borders with Tuscany. We used an ELISA test for both specific IgG and IgM antibodies. A nested RT-PCR was performed at the Arbovirus Unit of the Istituto Superiore di Sanità on CSF from mengingoencephalitis.
Results: We examined sera from 92 patients. Of these, 24 (22 meningitis and 2 meningoencephalitis) were positive for specific IgM and IgG (26.1%). The presence of TOS viral genome was confirmed in CSF of 1 out 2 meningoencephalitis. Out of 50 healthy controls, 8 (16%) were positive for specific IgG. Males (m/f = 2.5) and intermediate age (18–65) were more frequently affected. The highest incidence of clinical cases was during July and August. From the clinical point of view, we emphasize that the 2 meningoencephalitis showed a particularly severe course.
Conclusions: Our study showed, for the first time, that also in Umbria the TOS virus is an important etiological agent for summary lymphocytic meningitis and acute meningoencephalitis. This virus circulates among the general population (border zone with Tuscany) and can cause asymptomatic infections. We aim at extending our seroepidemiological study to evaluate the virus circulation in general population living in other parts of Umbria.

P1590 Asymptomatic pharyngeal carriage of Lancefield's group B streptococci in medical students

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Objectives: Aim of the study was to test the asymptomatic carriage of GBS in medical students.
Methods: Pharyngeal swabs of 560 (male: 257, female: 303) healthy, volunteer medical students were tested for the presence of GBS. In comparison 30 clinical isolates of GBS from patients suffering from acute pharyngitis (male: 22, female: 8) were selected from the collection of the Institute's own diagnostic unit. β-haemolytic streptococci were subjected to sero-grouping, furthermore – if applicable – subgrouping (BI, BII, BIII) by two commercial latex agglutination tests. Each GBS has been tested for bacitracin susceptibility and by Christie–Atkins–Munch–Petersen (CAMP)-test. The cell surface hydrophobicity (CSH) was determined by standardized salt aggregation test (sSAT), and hydrophobic interactive chromatography (sHIC). Statistical analysis of the sSAT and sHIC results was carried out by using the Student's *t*-test.
Results: (see table).

	Asymptomatic carriers ($n = 32$)		Pharyngitis ($n = 30$)	
	Male ($y = 30$)	Female ($x = 2$)	Male ($y = 22$)	Female ($x = 8$)
S. agalactiae	28 (93.3%)	2 (100%)	22 (100%)	8 (100%)
S. porcinus	2 (6.6%)	0 (0%)	0 (0%)	0 (0%)
Ia, Ib, Ic	0 (0%)	0 (0%)	17 ($n = 77.3\%$)	5 (62.5%)
II	8 (26.6%)	0 (0%)	0 (0%)	0 (0%)
III	20 (26.6%)	0 (0%)	5 (22.7%)	3 (37.5%)
Untyped	4 (13.1%)	2 (100%)	0 (0%)	0 (0%)
sSAT [mean (SD)]	3.73 (0.35)	3.73 (0.35)	38.10 (4.66)	38.10 (4.66)
sHIC [mean (SD)]	3.14 (0.26)	3.14 (0.26)	58.77 (9.84)	58.77 (9.84)
Significance (*P*-value)	<0.0001	<0.0001	<0.0001	<0.0001

Conclusions: Higher rate of asymptomatic carriage of GBS in males is explained by either epidemiological or hormonal factors. Sub-group distribution in asymptomatic carriers implies no association with more virulent

Ia, Ib, and Ic strains. Cell surface hydrophobicity was significantly higher in the group of clinical subjects; therefore it can be concluded that it may play an important role in the pathomechanism of infections caused by GBS.

P1591 Is sciatica an infection?

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Objectives: (1) To determine if low virulent microorganisms including *Propionibacterium acnes and* coagulase negative staphylococci (CNS) are associated with the interveterbral tissue of patients with severe sciatica; (2) To determine the serum IgG levels to lipid S, an exocellular Gram-positive antigen, in these patients.
Methods: Two-hundred and seven patients who underwent microdiscectomy for unremitting pain, and 27 controls presenting with scoliosis, myeloma and trauma, were entered into the study. Clinical samples removed during surgery, including intervertebral disc and surrounding soft tissue were cultured for microorganisms by direct and enrichment methods. Serum levels of antilipid S IgG were determined by ELISA.
Results: Seventy-six out of 207 (37%) patients yielded positive cultures within 7 days of incubation ($P = 0.001$). *P. acnes* was recovered in pure culture from 49 (64%) of the positive clinical samples ($P = 0.03$) and in mixed culture with CNS in 8 (11%). Eleven (14%) of patients yielded a pure growth of CNS. Microorganisms isolated in pure culture from the remaining samples included *Propionibacterium granulosum* (1%), *Corynebacterium propinquum* (1%) and *Micrococcus* species (1%). Elevated levels of antilipid S IgG were detected in 26 (34%) patients with positive cultures. In comparison, *P. acnes* was recovered from only 2 out of 27 (7%) control patients, both of whom had scoliosis. Anti-lipid S IgG was undetectable in the serum samples of 21 (78%) control patients.
Conclusion: Low-virulent microorganisms, in particular *P. acnes* may be associated with chronic low-grade infection in the intervertebral discs of patients with severe sciatica.

P1592 Liver abscess due to *Bacillus cereus*: a case report

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It is well known that *Bacillus cereus* is widely recognized as a food-borne pathogen that causes a self-limiting gastroenteritis, which requires only symptomatic treatment. We describe a case report of a 72-year-old woman who was admitted to the hospital because of acute abdominal colic pain. On the first day of her admission whole blood and plasma laboratory investigations were almost normal, with the exception of the liver transaminases, which were only mildly elevated (AST: 48 U/L, ALT: 31 U/L). On the second day the patient became febrile (39 °C), jaundiced (billirubin: 21.6 mg/dL) and her liver function deteriorated rapidly, as shown by the laboratory examinations (AST: 1988 U/L ALT: 953 U/L). The clinical setting deteriorated as well, as the colic abdominal pain became worse with the appearance of acute abdomen. Emergency ultrasound and CT investigation of the abdomen was performed, which showed a big abscess (diameter ~3 cm) of the right lobe of the liver. The patient underwent emergency laparotomy, and the abscess was found ruptured to the free peritoneal cavity with subsequent generalized peritonitis. The final clinical diagnosis was acute peritonitis due to a ruptured liver abscess. Culture of the pus obtained perioperatively from the liver abscess and the peritoneal cavity isolated *Bacillus cereus*.
Conclusion: *Bacillus cereus* is a Gram-positive organism that usually causes a self-limiting gastroenteritis, rarely food poisoning and extremely rarely fulminant liver failure. However, no case of liver abscess has been reported up to now due to this organism.

P1593 Long-term followup after community-based oral azithromycin treatment of hyperendemic trachoma

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A major part of the SAFE strategy to reduce or eliminate blinding trachoma as a major public health problem is broad based treatment with oral azithromycin treatment. It is uncertain how long the effects of a single course of such therapy will be. This is obviously an important issue for the control program. In the ACT (Azithromycin in Control of Trachoma) project a single course of therapy (three doses given at 1 week intervals) resulted in 64–93% reduction in *Chlamydia trachomatis* infection rates in trachoma endemic areas. The best results were obtained in Egypt where the population in the villages was quite stable. Relatively good results (up to 77% reduction in prevalence of infection) were also seen in the control villages where a 6-week course of daily topical tetracycline ointment was administered. These treatments were begun in January of 1995. In early 2002 a follow-up study is planned to assess whether beneficial effects of the treatment regimens could still be detected 7 years after the treatment. Village wide surveys will be done assessing clinical status and using LCR positivity to diagnose chlamydial infection. Results will be compared to the baseline in these same villages prior to treatment in 1995, and to results obtained 1-year posttreatment. Results will also be compared to results seen in adjacent villages where no treatment had been done in an effort to assess whether some of the changes that were seen could actually be a result of a secular trends rather than the direct outcome of treatment. We plan to present the results of the 2002 follow-up survey of infection status in preliminary form because of the short period for analysis between generation of the data and time of the meeting.

P1594 Microbiological examinations of corneal discs removed with keratoplastical surgery

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Objectives: A total of 100 cornea removed by keroatoplastic surgery were invesitgated for the presence of bacteria, fungi or viruses. Indications of the operations were degenerations of cornea epithelium, dystrophy as well as ulcers of cornea. *Propionobacterium acnes* was isolated most frequently from damaging of the cornea. The possible role of the cytotoxic effect of *P. acnes* was investigated.
Methods: One piece of the cornea was cultivated in infusion-, Schaedler-, Sabouraud-broths, respectively, during 10 days. Another part of the cornea was tested for the presence of DNA of the herpes- and adenoviruses by PCR methods. Cytotoxic effect of the supernatants of *P. acnes* cultures on epithelial tissue cultures (HeLa) in a 96-well plate was examined. During the incubation time of 2, 6, 10, 24, 32 and 48 h, cells were subjected to a treatment with the dilution of 1 : 10 prepared from the supernatants of 5-day Schaedler broth cultures. Viability of cells was detected by means of the tetrazolium-based colorimetric assay (MTT, Sigma). Cytotoxic effect on epithelial cells was investigated by microscopic examinations.
Results: Out of 100 specimens 37 bacterial strains were isolated including 10 aerobic and 27 anaerobic ones. No fungus was isolated. Most frequent isolates were *P. acnes* (14), *Peptostreptococcus* sp. (7), *Stomatococcus* sp. (3), *Gemella* sp. (1), *Clostridium bifermentans* (1), *Staphylococcus* sp. (3), *Micrococcus* sp. (1), *Streptococcus oralis* (1), *Corynebacterium* sp. (5). DNA of herpes- and adenoviruses could not be detected by PCR method. Examining 10 *P. acnes* strains, in each case a significant, nonlytic, cytotoxic effect was detected in HeLa tissue after 10 h of incubation. After an initial perturbation effect a concentration dependent 30–50% decrease in the activity of mitochondrial dehydrogenase was obtained between 10 and 30 h of incubation in the epithelial cells. Microscopic examination revealed no morphological changes until 10 h of exposure, in contrast after 24 h, a slight cytopathic effect could be observed.
Conclusion: Only bacterial strains could be detected in corneal tissue, of which anaerobic species, first of all *P. acnes* was the most frequently isolated microorganism. *P. acnes* strains are able to survive under anaerobic conditions for 3 months or even longer and meanwhile they can produce toxic

metabolites. Our results suggest *P. acnes* to be harmful for human tissues of different sites of the body in case of a chronic persistence.

P1595 A case of endophthalmitis caused by *Bacillus cereus*

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Up to the last years *Bacillus cereus* has not generally been regarded as an important pathogen and is commonly dismissed as a laboratory contaminant but nowadays *B. cereus* infections have been reported with increasing frequency. In this report an invasive endophtalmitis caused by *B. cereus* is mentioned. A 10-month-old girl had a bilateral congenital cataract operation from one eye. After 48 h following the operation endophtalmitis was developed. The vitreous liquid was taken and cultured to Blood, Chocolate and EMB agar and incubated in 37 °C, 24 h in aerobic medium. Colonies, which are 3–8 mm in diameter, raised irregular with greyish to greenish frosted-glass appearance, surrounded by a large zone of β-hemolysis are isolated. In Gram staining: Gram-positive, subterminal spore-forming bacteria are taken for further identification. The bacteria which are lecithinase positive, penicillin resistance and that do not have parasporal toxin crystals are called *B. cereus*. In disc diffusion test, the microorganism is susceptible to gentamycin, vancomycin, and ciprofloxacin and is resistance to penicillin, oxacillin, erythromycin, clindamycin. As the patient couldn't get response to gentamycin, vitrectomy was done.
Conclusion: Endophthalmitis caused by *B. cereus* results as irreversible loss of vision by the effect of hemolysin B2. So it will be very useful to add effective antibiotics to the empirical treatment in the traumatic suspicious cases that may be caused by *B. cereus*.

Helicobacter pylori II

P1596 Antibiotic-resistance of *Helicobacter pylori* isolated from biopsy of symptomatic patients

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Objectives: The increasing resistance to therapy is an important factor for eradication failure of *Helicobacter pylori* infection. When metronidazole-based therapy is given to a patient infected by a resistant strain, the change of success decreases by 20% whereas as far as clarithromycin is concerned, it decreases by more than 50%. Generally, treatment is given on an empirical basic. The antimicrobial susceptibility testing is reserved for patient not eradicated in order to have indication on the choice of a quadruple therapy. The aim of this study was to evaluate the in vitro antimicrobial resistance of *H. pylori* isolated in symptomatic patients previously treated with triple therapy.
Methods: Symptomatic patients previously treated for *H. pylori* infection underwent endoscopy, were included in this study. Two gastric biopsy specimens, from both gastric antrum and corpus, were obtained for the culture and for the rapid urease test. A patients was considered *H. pylori*-positive if culture and/or rapid urease test was positive, and *H. pylori*-negative when both tests were negative. Antimicrobial susceptibility testing was performed by disk diffusion method (Kirby–Bauer modified). All isolates were tested for the following antimicrobials agents: metronidazole, tetracycline and clarithromycin.
Results: A total of 38 patients were investigated (21 females and 17 males). Seven resulted *H. pylori*-negative and were evaluated as eradicated. In 22 of 31 not eradicated patients, *H. pylori* grew in culture and antibiotics susceptibility tests was performed. Four strains were found to be susceptible to all antimicrobial agents tested. The resistance rates in *H. pylori* were as follows: metronidazole 60%, clarithromycin 60%, tetracycline 4.5%. The combined resistance in *H. pylori* was: metronidazole + clarithromycin 40%, metronidazole + tetracycline 5%, tetracycline + clarithromycin 5%, tetracycline + clarithromycin + metronidazole 5%.
Conclusions: The high rate of resistance to clarithromycin and metronidazole observed in this study can explain the eradication failure. The low percentage of the resistance of *H. pylori* to tetracycline indicates that this antibiotic can be used in quadruple therapy. This study support the utility to perform antibiotic susceptibility testing of *H. pylori* isolated in not eradicated patient in order to check the susceptibility and to address the physician to a correct treatment.

P1597 The importance of QuickStripe (QS) *H. pylori* (HP) in differentiating patients with gastrointestinal symptoms

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Objective: The paper aims at demonstrating the role of QS HP in our medical practice as a unique available noninvasive test method in detecting of antibodies to HP. It also demonstrates the test's correlation to gastrointestinal symptoms as a starting point in specific treatment, as well as differentiation and further diagnostic of diseases associated with HP infection, with more specific reference tests.
Material and methods: In the period 1999–2000, a group of 108 patients were observed and antibodies to HP were detected with QS in 57 of them. Gastrointestinal symptoms (dyspepsia, epigastralgia, diarrhea) were present in all of them. Besides anamnestic and epidemiological data, in making the diagnosis, standard biochemical laboratory analysis, bacteriological and parasitological investigations, stomach and duodenum Rtg and endoscopy were conveyed.
Results: The mentioned 57 patients were at the age of 18–73 years; 32 were male and 25 female patients. 44 of these patients were at the age of to 45. Various gastrointestinal symptoms, including symptoms of gastritis, dyspepsia and diarrhea (for which there had been previous negative bacteriological and parasitological findings) were present in them. After the obtained positive QS test of antibodies to HP, a specific treatment was conveyed (klaritromycin, metronidazole and omeprazole) leading to a clinical improvement and disappearance of subjective problems. Thirteen patients above 45 years of age with a positive QS HP test and with presence of the above mentioned symptoms, were recommended stomach and duodenum Rtg and endoscopical examinations for a definite diagnosis with HP infection (with rapid urease test) and possible association to duodenal and gastric ulcer or malignant disease.
Conclusion: QS HP as a rapid, immunochromatographical screening test in detecting antibodies to HP is the only noninvasive test method available in our medical practice. It directs as towards starting a specific treatment in the younger group of patients, which enables accomplishing an obvious clinical improvement and disappearance of gastrointestinal symptoms. At the same time, it is a significant direction for further diagnosing of ulcer and malignant diseases associated with infection to HP in the elder group of patients. We expect the application of more specific noninvasive reference tests (ELISA) to obtain a more exact diagnosis and follow the infection with HP.

P1598 Eradication of *Helicobacter pylori*: the effect on histological lesions of gastric mucosa in a follow-up study

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Objectives: *Helicobacter pylori* eradication heals gastritis and prevents the recurrence of peptic ulcers. We investigated the histological changes of biopsies obtained from five different regions of the stomach in a 12-month follow-up study, compared the results with serology and evaluated the *cagA* status and variability using RFLP-PCR.
Material and methods: A total of 18 patients (10 duodenal ulcer (DU), one gastric ulcer, seven gastritis) were given lansoprazole 30 mg, amoxicillin 1 g and metronidazole 500 mg bid for 2 weeks. Biopsies were collected before and at 1 and 12 months after treatment as follows: five biopsies for histology from lesser to greater curvatures of the antrum and body and incisura angularis and

another three for CLO, PCR and culture. A serum sample was also collected at similar dates. Statistical analysis was performed using SPSS 10.1.
Results: All patients were *H. pylori* positive by CLO, histology, PCR and ELISA before treatment. Histological scores of 0–3 (absence, mild, moderate, severe) were done according to the updated Sydney system. *H. pylori* was detected in all five regions of the stomach with variable densities being highest in the antrum biopsies. Eight patients with atrophy including four with intestinal metaplasia. *H. pylori* eradication was achieved in 39% of patients. The score corresponding to chronic gastritis (CG) declined progressively after *H. pylori* eradication, with average values of 1.52, 1.34, 1.02 ($P < 0.05$) at 0, 1, 12 months, respectively. The corresponding score for active CG also improved progressively after eradication: 1.05, 0.26, 0.47 ($P < 0.05$). Nevertheless, no significant changes were observed regarding atrophy or intestinal metaplasia conditions. A 50% decrease in IgG anti-*H. pylori* titer at 12 months was recorded in six patients. RFLP-PCR results showed five different *cagA* positive patterns. A significant correlation was found between the detection of *cagA* gene and DU.
Conclusions: Eradication of *H. pylori* resulted in a histological improvement of gastric mucosa that started early and continued for the 12-month period after therapy. The improvement in gastritis is observed immediately after eradication, however, it is not followed by a significant improvement in atrophy or intestinal metaplasia. The overall improvement in the inflammatory component is slow and progressive.

P1599 Effect of cytotoxic properties of *Helicobacter pylori* on different cell lines

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Introduction: A number of *vacA* genes differing in signal sequence and middle-region alleles have been detected in the strains of *H. pylori*. It has also been suggested that the *vacA* subtype is associated with different cytotoxic activities. Some investigators have postulated that the *vacA* m2 subtype is in vitro less active for the HeLa cell line that for the RK-13 cell line or primary culture of humans gastric cells. The aim of the study was to determine the relationship between *vacA* genotypes and the activity of vacuolating cytotoxin, which was tested on the following cell lines: Intestine 407 and RK-13.
Methods: The *vacA* genotypes were investigated in 26 *H. pylori* strains isolated from patients with duodenal ulcer and in 50 strains from patients with gastritis only. The presence of *vacA* signal- and mid-region sequences was determined by PCR. Vacuolating cytotoxin activity was examined on Intestine 407 cells (human embryonic intestine cells) and RK-13 (rabbit kidney cells). The results were regarded as positive when at least 50% of the cells were vacuolated after 24 h.
Results: Combinations of s1a/m1, s1a/m2 and s2/m2 were found in 32, 43 and 25% of the tested strains, respectively. Patients with duodenal ulcer showed similar proportions of s1a/m1 46% (12/26) and s1a/m2 46% (12/26) *vacA* genotypes. S2/m2 genotype was found only in 8% (2/26) of the duodenal ulcer patients and in 34% (17/50) of the gastritis patients. Using Intestine 407 cells, cytotoxin activity was found in 71% (17/24) of the *H. pylori* s1a/m1 genotype, but only in 24% (8/33) of the s1a/m2 genotype and in 32% (6/9) of the s2/m2 genotype. With RK-13 cells, cytotoxin activity was found in 67% (16/24) of the s1a/m1 genotype, 36% (12/33) of the s1a/m2 genotype and in 32% (6/19) of the s2/m2 genotype.
Conclusions: The frequency with which the vacuolating cytotoxin produced by the *H. pylori* m2 subtype was detected in Intestine 407 and RK-13 cell was similar. To test the expression of the m2 allele it is advisable to use such cell lines that are the most specific for subtype B of cytotoxin.

P1600 Investigation of porin genotypes as possible markers of in vitro antibiotic resistance in *Helicobacter pylori*

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Objectives: The porins are a family of outer membrane proteins involved in the transport of molecules into the bacterial cell and in some bacteria, can provide a mechanism for antibiotic resistance through changes in outer membrane permeability. Five porin proteins with unspecified functions have been identified in *H. pylori*; HopA, B, C, D and E. Our aim was to investigate their possible role in antibiotic resistance by examining associations between clarithromycin (CH) and metronidazole (MZ) susceptibilities in vitro and porin genotype.
Methods: A total of 142 isolates of *H. pylori* with different MZ and CH susceptibilities, determined by disc diffusion and E-tests, were examined; 49 fully sensitive, 67 MZ resistant only, 10 CH resistant only and 16 with dual resistance. Novel oligonucleotide primer pairs were designed for each *hop* gene, and PCR assays were performed to detect the five genes in all isolates. Strains were also genotyped for *cagA* (presence or absence) and *vacA* (s and m type).
Results: *hopB*, *C* and *E* were highly conserved in 99% of *H. pylori* irrespective of antibiotic resistance. The *hopA* primers amplified two products, the expected product of 1329 base pairs and a larger product of approximately 1950 bp. The two products were amplified independently and in combination from isolates and an amplicon was observed in 99% of isolates. 5/9 strains lacking the expected *hopA* product were resistant to MZ. Eight strains had the enlarged *hopA* product (1950 bp) of which four were MZ resistant. Both *hopA* products were detected in 48 strains, these comprised 15 (31%) fully sensitive, 24 (50%) MZ resistant, three (6%) CH resistant, and six (13%) dual resistant. For *hopD*, no expected product was obtained for 24 (17%) of strains of which, seven (29%) were fully resistant, and 11 (46%) were resistant to MZ alone. 107 strains were *cagA* positive and on the basis of combined genotypes (*hop* type, *cagA* and *vacA*), the most common strain type was *hopA*+ (1329 bp), *cagA*+ and *vacA* s1m1 (34/142).
Conclusions: Strains were genotypically diverse with respect to the *hop* and other markers investigated irrespective of antibiotic resistance with the 16 strains that were resistant to both MZ and CH represented by 11 combined genotypes. Our results suggest that resistance is not directly linked to the absence of particular porins but is more likely to be due to specific mutations causing porin protein alterations that affect outer membrane permeability.

P1601 Infection with *H. pylori* strains: testing of drug sensitivity and genotypes

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Objectives: The study aimed at genotype analysis and at evaluation of drug sensitivity of *H. pylori* strains, isolated from patients before therapy and following ineffective eradication attempts.
Methods: The studies were performed on 56 patients with established diagnosis of duodenal ulcer with presence of *H. pylori*, treated by the triple therapy (PPI + amoxicillin + clarithromycin). Control studies were performed 12 months later. Biopsies of gastric mucosa were plated on Columbia-agar enriched with blood and antibiotics. The cultures were kept in microaerophilic conditions for 4–10 days. Sensitivity of strains to metronidazole, clarithromycin, amoxicillin and tetracycline was determined using E-tests. Genotypic analysis of the strains employed PCR-based RAPD fingerprinting using a single T7RNAPOL1.
Results: Following 12 months, 10 patients (17.8%) remained *H. pylori* positive. Genotype analysis demonstrated that in seven of the patients the same strains were involved before and after the therapy while in three patients distinct genotypes of *H. pylori* strains were detected following the therapy. High frequency of primary resistance to metronidazole (44.6%) was noted. 8.9% strains were resistant to clarithromycin.
Conclusion: Relapsing character of *H. pylori* – induced pathology may reflect both ineffective eradication attempts and re-infection.

P1602 Susceptibility to amoxicillin, clarithromycin and metronidazole of *Helicobacter pylori* strains isolated from Turkey

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Objective: To evaluate the susceptibility to amoxicillin, clarithromycin and metronidazole of *Helicobacter pylori* strains isolated from Turkey.
Methods: In this study, a total of 70 strains isolated from patients with *H. pylori* infection were included. Culture was performed using Mueller–Hinton agar (selective supplement) with 10% sheep blood. Strains were tested for amoxicillin, clarithromycin and metronidazole resistance with Epsilometer test (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar with 10% sheep blood.

Result: Of 70 *H. pylori* strains, nine strains (12.85%) were resistant to metronidazole. Four strains (5.71%) were found resistant to two antibiotics (Metronidazole + Clarithromycin). In vitro susceptibility testing against amoxicillin yielded three (4.28%) resistant strains. These strains were also resistant to metronidazole and clarithromycin.
Conclusion: Although metronidazole resistance was 50–90% in the world, especially in developing countries, we found low rates of the resistance to metronidazole among *H. pylori* isolates from Kocaeli, Turkey. Amoxicillin resistance was not found in many countries. On the contrary, *H. pylori* strains isolated from Kocaeli, Turkey were resistant.

P1603 Prevalence of antimicrobial resistance in *Helicobacter pylori* clinical strains

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Introduction: The objective of this study was to determine the prevalence of metronidazole, furazolidone, nitrofurantoin, tetracycline, amoxicillin, and clarithromycin resistance in *H. pylori* clinical isolates.
Methods: A total of 200 clinical isolates, 78 from females and 122 from males, were studied. Strains were obtained from antrum biopsy samples following the standard methodology. The MIC was determined by an agar dilution method using Mueller–Hinton agar supplemented with 7% horse blood containing each antibiotic at two-fold dilutions (256–0.008 mg/L). Plates were inoculated with 106 cfu/drop and incubated at 37 °C during 2–5 days in a CO2 increased atmosphere. The MIC was determined as the lowest concentration of the drug inhibiting visible growth.
Results: Forty-eight *H. pylori* isolates were resistant to metronidazole (24%, 95%IC 0.67–0.96). The MIC_{50} and MIC_{90} values were 1, and 16 mg/L, respectively. The distribution of metronidazole resistance was higher in *H. pylori* isolates from females than from males (34% vs. 18%, $P=0.007$). Thirty-six *H. pylori* isolates were resistant to clarithromycin (18%, 95% IC 0.90–1.14). The MIC_{50} and MIC_{90} values were 0.008, and 0.064 mg/L, respectively. The distribution of clarithromycin resistance was similar when *H. pylori* isolates from females and males (14.1% vs. 15.5%, $P=0.7$) were compared. All the strains tested were susceptible to amoxicillin (range 0.008–0.5 mg/L), tetracycline (range 0.008–1 mg/L), nitrofurantoin (range 0.008–2 mg/L) and furazolidone (range 0.008–2 mg/L).
Conclusions: Metronidazole resistance was higher in women than men. Furazolidone and nitrofurantoin may be good alternatives to metronidazole for treating *H. pylori* infection.

P1604 Mutant frequency and mutation rate in *Helicobacter pylori* clinical isolates

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Madrid, E

Introduction: The objective of this study was to determine the in vitro frequency of spontaneous mutations in *H. pylori* that confer resistance to metronidazole, furazolidone, and nitrofurantoin.
Methods: Ten *H. pylori* clinical isolates, as well as the reference strain NCTC 11637 were used in this study. metronidazole, furazolidone, and nitrofurantoin MIC were determined by agar dilution method (NCCLS, 1999); all strains were susceptible to this antibiotics. *H. pylori* strains were grown on Brain Heart Infusion (BHI) with 10% fetal bovine serum (FBS) at 37 °C under microaerobic conditions to late log phase (3 days). This culture was diluted 10–4 in BHI broth with 5% SFB. 15 aliquots were prepared, which were grown for 3 days to obtain parallel and independent cultures. The number of resistant mutants that emerged in each culture was determined by plating the all culture on BHI agar with 7% horse blood supplemented with 2 mg/L of nitrofurantoin or furazolidone or 8 mg/L of metronidazole. The total number of cells was determined by plating an appropriate dilution (10–5, 10–6, and 10–7) of three cultures on non selective medium. Colonies on both selective and non selective plates were counted after incubation for 4 days. The frequency of resistant mutants was expressed as the mean number of resistant cells divided by the total number of viable cells per culture. The mutation rate (a) per cell division was calculated as $a = m/Nt$, where Nt is the total cell number per culture, and m is the number of mutations that have occurred in the culture.
Results: No colonies grew on plates supplement with furazolidone or nitrofurantoin. Four strains show spontaneous metronidazole resistance:
- HP-1, no. of cells per culture: 4.5×10^8; resistant bacteria: zero fraction: 6/12; mean: 6; frequency of mutants: 1.3×10^{-8}; and mutation rate: 1.5×10^{-9}.
- HP-2, no. of cells per culture: 4.0×10^8; resistant bacteria: zero fraction: 4/12; mean: 18; frequency of mutants: 4.5×10^{-8}; and mutation rate: 2.25×10^{-9}.
- HP-3, no. of cells per culture: 3.9×10^8; resistant bacteria: zero fraction: 8/12; mean: 6; frequency of mutants: 1.5×10^{-8}; and mutation rate: 1.02×10^{-9}.
- HP-4, no. of cells per culture: 5.0×10^8; resistant bacteria: zero fraction: 10/12; mean: 3; frequency of mutants: 6×10^{-8}; and mutation rate: 4.0×10^{-9}.

Conclusions: Furazolidone and nitrofurantoin resistance occurs spontaneously at a much lower frequency than metronidazole resistance.

P1605 Resistance of *Helicobacter pylori* isolates to metronidazole, clarithromycin, tetracycline, amoxicillin, and cefixime in Israel

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Objectives: Antibiotic resistance of *Helicobacter pylori* differs according to geographical area and may have an important impact on the efficacy of therapy. The aim of the study was to determine the resistance of *H. pylori* isolates to metronidazole (MET), clarithromycin (CLR), tetracycline (TC), amoxicillin (AM) and cefixime (CEF) in Israel.
Methods: One hundred and thirty-eight isolates of *H. pylori* were isolated from specimens of 138 dyspeptic adults, including 28 patients previously treated for *H. pylori* infection. Antibiotic susceptibility was tested by E-test method (AB Biodisc). Resistance was defined as follows: MIC ≥ 0.5 µg/mL for AM; ≥ 1 µg/mL for TC; ≥ 2 µg/mL for CLR and CEF; ≥ 8 µg/mL for MET. *H. pylori* ATCC 43526 strain was used for quality control.
Results: All isolates were sensitive to TC, MIC ≤ 0.38 µg/mL; resistance to AM was found in one isolate (MIC = 1.5 µg/mL) sensitive to all other antibiotics, recovered from untreated patient. Resistance to CEF was found in two isolates from each group ($P=0.18$). The prevalence of resistance to MET and CLR was much higher in the isolates from the treated than the untreated patients: 60.7 and 38.2% for MET ($P=0.03$); 46.4 and 8.2% for CLR ($P<0.001$). In the treated group all the MET or CLR resistant isolates were from patients previously treated with MET or CLR, respectively. Dual resistance to MET and CLR were found in 5.5% of isolates from untreated and 32.1% of treated patients ($P<0.001$). High MIC value of ≥ 256 µg/mL for MET and CLR were observed, respectively, in 30 and 5.4% of isolates from untreated patients and 42.6 and 25% of isolates from treated patients.
Conclusions: TC and CEF are potential alternative agents for the treatment of *H. pylori* infection. In vitro susceptibility testing may improve outcome especially after treatment failure.

P1606 Antibody-dependent cell-mediated activity against *Helicobacter pylori* in mice

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Objectives: Antibody-dependent cellular cytotoxicity against *Helicobacter pylori* (Hp) was assessed using Hp-specific immune serum and purified IgG in an in vitro assay in which peritoneal cells (PEC) were used as effector cells.
Methods: Lymphoid cell suspension was incubated for 2 h at 37 °C with bacteria coated with specific antibodies and at the end of the incubation period, diluted aliquots of the mixture were plated. Plates were incubated in microaerophylic conditions for 4–7 days and then the colony-forming units counted.
Results: PEC possessed some natural antibacterial activity, which was significantly augmented by Hp-specific antibodies in a dose-dependent manner. Specificity was shown by the fact that preabsorption of serum with an excess of whole Hp cells significantly decreased the antibody-mediated killing,

whereas serum preabsorbed to *E. coli* retained this effect. These results were also confirmed qualitatively with different Hp strains. The PEC population was analyzed at the fluorescence-activated cell sorter. Most of antibacterial activity was associated with cells that were non adherent, DX5–, CD19–, CD11c–, Thy 1.2–, CD11b+ and CD16/32+, suggesting that the main effector population belong to the macrophage lineage. Similar antibacterial killing was also observed with the macrophage cell line GG2EE.
Conclusion: These results suggest that antibody-dependent antibacterial activity may be important in the effector mechanisms against Hp.

P1607 Morphological changes in upper gastrointestinal tract mucosa and *Helicobacter pylori* infection in HIV-infected patients

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Objectives: The aim of the study was to evaluate morphological changes in upper GI tract mucosa and prevalence of *Helicobacter pylori* (Hp) infection in HIV infected patients with CD4 lymphocyte count $<200/mm^3$ in comparison to patients with CD4 $>200/mm^3$.
Methods: A total of 68 HIV-infected patients with dyspeptic symptoms (median age 34 years) were studied, among them 36 (53%) had CD4 lymphocyte $<200/mm^3$ (group 1), while 32 (47%) had CD4 lymphocyte $>200/mm^3$ (group 2). During endoscopy, evaluation of GI tract mucosa was done and biopsy samples were taken from corpus and antrum of stomach for histological analysis and rapid urease test.
Results: Basing on gastroscopy, following macroscopic lesions were found in group 1: mycotic esophagitis in 13 patients (pts) (36%), reflux esophagitis in eight pts (22%), chronic gastritis of corpus in 16 pts (44%), antrum in 35 pts (97%), gastric erosions in three pts (8%), duodenitis in 21 pts (58%), duodenal erosions and/or ulceration in 4 pts (11%); in group 2: mycotic esophagitis in two pts (6%), reflux esophagitis in 10 pts (31%), chronic gastritis of corpus in 19 pts (59%), antrum in 27 pts (84%), gastric erosions in one pt (3%), duodenitis in 19 pts (59%), duodenal erosions in five pts (15%). Histological analysis revealed in group 1: chronic active gastritis in corpus in nine pts (25%) while in antrum in 12 pts (33%), chronic nonactive gastritis in corpus in 18 pts (50%) while in antrum in 19 pts (53%). It was found in group 2 that: chronic active gastritis in corpus was present in nine pts (28%) while in antrum in 20 pts (62%), chronic nonactive gastritis in corpus in 18 pts (56%) while in antrum in 10 pts (31%). Hp infection was detected in 16 pts (44%) in group 1 and in 26 pts (81%) in group 2 ($P<0.05$).
Conclusion: In HIV infected patients with low CD4 count, mycotic esophagitis and erosive gastritis were more frequently found, whereas reflux esophagitis was less often diagnosed. Chronic active gastritis with Hp infection was significantly less frequent in the group of patients with low CD4 count.

Lyme borreliosis, anthrax and tetanus

P1608 *Borrelia*-induced hyporesponsiveness is cross-reactive to endotoxin and Toll-like receptor (TLR)-2-dependent

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Constance, D

Objective: If left untreated, infection with *Borrelia burgdorferi* usually leads to chronic Lyme disease. It is still unknown, how this pathogen manages to persist in the host in the presence of competent immune cells. We recently reported that *Borrelia* modulate the host's immune response, so perhaps preventing their elimination. Here, we further characterize *Borrelia*-induced immunomodulation by desensitization experiments.
Methods: Human PBMC were pretreated and subsequently restimulated with *Borrelia* lysate, heat-inactivated *Borrelia* or endotoxin (LPS) in all combinations. TNF-α and IL-10 release in the supernatant were measured by ELISA. Further, the role of TLR2 and TLR4 receptor was tested in analogous desensitization experiments, using bone marrow lavage cells from C3H/HeJ (TLR4-mutated) and TLR2-knock-out mice in comparison to the respective wild-type cells.
Results: Macrophages pretreated with *Borrelia* lysate or heat-inactivated *Borrelia* showed significantly reduced cytokine release on restimulation with either *Borrelia*-specific stimulus. In addition, hyporesponsiveness to a heterologous, TLR4-mediated stimulus (LPS) could be induced and vice versa LPS could induce desensitization to *Borrelia*-derived stimuli. Furthermore, in the absence of TLR2-receptor, no cytokine release could be induced by *Borrelia* and no state of hyporesponsiveness to restimulation with LPS was achieved. Respectively, no desensitization to *Borrelia* stimuli could be induced by LPS in the absence of a functional TLR4-receptor.
Conclusion: *Borrelia* induce a state of desensitization in human PBMC and murine macrophage populations, which is cross-reactive to endotoxin-stimulation and is TLR2-mediated. Thus, *Borrelia*-induced hyporesponsiveness represents a possible mechanism enabling the survival of this pathogen in the host despite the presence of immune cells.

P1609 Modulation of cytokine release by antibiotics used in therapy of borreliosis

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Objective: Ten percent of borreliosis patients are unresponsive to antibiotic therapy, and thus suffer from a persistent infection with *Borrelia burgdorferi*. We recently reported that *Borrelia* attenuate the host's immune response which might prevent elimination and suggested that antibiosis should be supported by the reconstitution of immune competence in chronic borreliosis. We tested whether the antibiotics generally used in borreliosis therapy have stimulatory effects on the immune system in addition to their bactericidal activity.
Methods: Whole blood and isolated leukocyte populations from healthy volunteers were stimulated with *Borrelia*-derived stimuli or with endotoxin (LPS) in the presence or absence of antibiotics. TNF-α, INF-γ and IL-10 release in the supernatant were measured by ELISA.
Results: Of all the antibiotics used for borreliosis therapy, none showed clear pro-inflammatory effects. However, doxycycline exerted a strong inhibitory effect on IFN-γ release, and simultaneously reduced the release of IL-10 in low micromolar concentrations by up to 50%. This inhibitory capacity was further characterized by determining the blood cells which were affected: lymphocytes were directly influenced by the antibiotic, resulting in reduced IFN-γ release. The mechanism of this effect is being investigated further.
Conclusion: Thus, up to now, we could not identify a candidate antibiotic with immunostimulatory properties. However, doxycycline, a commonly used antibiotic in borreliosis treatment was shown to have immunosuppressive activities which may be less favorable in late-stage borreliosis cases.

P1610 Lyme borreliosis and human granulocytic ehrlichiosis in Bulgaria

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In recent years, emerging tick-borne infectious diseases are the focus of increasing scientific and medical interest. Lyme borreliosis is the most commonly reported tick-borne infection in Europe and North America. Bulgaria is endemic for Lyme borreliosis. About 400 cases of Lyme borreliosis are reported annually (incidence 5/100 000). *Borrelia burgdorferi* sensu lato were isolated from ticks and patients. Identification of the isolates by serologic and genetic methods showed high cultivation rate of *B. garinii* (44% of the isolates), followed by *B. burgdorferi* sensu stricto (35%) and *B. afzelii* (21% of the isolates). *B. burgdorferi* s.l. were detected in 32% of adult *I. ricinus* ticks by PCR. *B. afzelii* was highly prevalent, found in 54% (21/39) of the positive samples. *B. burgdorferi* s.s. was detected in 8% (3/39) of the positive ticks, *B. garinii* in 5% (2/39), *B. valaisiana* in 3% (1/39). Double and triple infections were also found. Human granulocytic ehrlichiosis (HGE) is recently described zoonotic nonspecific febrile illness, which appears to be the second most common tick-borne disease in USA. Antibodies to the agent of HGE were detected in Bulgarian patients with tick bites in 9.7% of patients with early Lyme borreliosis, in 8% of patients with lymphadenopathy, in 20% of those with fever, chills, and headache, and in 4% of persons without clinical symptoms

after the tick bite. More recently, the first clinically and serologically confirmed acute case of HGE in Bulgaria was described. The agent of HGE was detected by PCR in 34% of adult field-collected ticks. Coinfection with *B. burgdorferi* and the agent of HGE was found in 12.9% 98/62) of Bulgarian field-collected *I. ricinus* female ticks, 14% (7/50) of the male ticks, and 2.2% (2/90) of the nymphs. These data show that Lyme borreliosis and HGE are widely distributed in Bulgaria and that coinfection with both agents in patients is probably not uncommon.

P1611 Case study: problems of diagnosing Lyme arthritis in a boy with knee-joint inflammation

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Background: Borreliosis as a multistage infection, especially in the chronic form, might be a serious diagnostic problem. One of the distant consequence of *Borrelia burgdorferi* infection is Lyme arthritis. In the presented case of 17-year-old boy, the clinical presentations – knee-joint inflammations and information of twice bite by tick (9 and 12 years earlier, without arise of erythema) were the basis of suspected borreliosis.
Methods: In the presented case of the boy, there was observed chronic (for about 3 years) knee-joint inflammation. During acute periods, there was observed fever (above 39 °C) with abundant, growing exudation of synovial fluid with numerous granulocytes. The microscopic examination of synovial fluid in dark field microscope, showed alived spirochetes, with morphologic features of *Borrelia*. Furthermore, staining by Giemsa method showed spirochetes. There were also performed serologic tests (ELISA) detecting antibodies in classes IgM and IgG. The results were negative. The confirmation of *B. burgdorferi* infection was performed by PCR method (in synovial fluid, blood and urine) using inner primers (N-PCR). Ceftriaxon was used at the first stage of treatment. After a few days of improvement, the intense clinical symptoms appeared. The microscopic examination of synovial fluid during antimicrobial therapy showed that *Borrelia* transformed to cytoidal form, spherical, morphologically noncharacteristic for spirochetes. Then, the arthotomy and drainage was performed and treatment with amicacin (7 days) and cefotaxime (21 days).
Conclusions: In the presented case, the crucial diagnostic element was detecting *Borrelia* in synovial fluid using dark-field microscope, simultaneously with negative serologic studies. PCR method confirmed the presence of *B. burgdorferi*. The fact that during diagnosis of Lyme disease, negative serologic results and cases of transforming from normal form of *Borrelia* to cytoidal form are observed during antibiotic treatment, which poses a crucial diagnostic difficulty.

P1612 Lyme's disease in Arkhangel'sk province of the Russian Federation

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Objectives: The Arkhangel'sk province is located in the northern part of the Russia. Ticks *Ixodes* – vectors of Lyme disease (L.D.) – can be found in the southern forest part of the province and are absent almost completely in the northern area (polar and tundra). The goal is to study the geographical distribution of L.D. in Arkhangel'sk province.
Methods: Almost 150 samples of *Ixodes persulcatus* were tested with PCR to detect vectors infection with *B. burgdorferi* s.l. and to identify the genospecies characteristics of the agent. The ELISA kit 'Lymetest-IgG' (Helix, Ltd) with mix of recombinant *Borrelia* proteins was used for detection of IgG antibodies to *Borrelia*. Almost 200 human sera were tested: 101 samples from population of Nenetsky Autonomous District located in the northern tick-free area of the province and 83 samples of persons bitten by ticks in the southern area of the province, including 13 patients with clinical diagnosis of L.D.
Results: During 3 years (1999–2001), 5259 persons bitten by ticks and 106 patients with L.D. were registered. Most cases were diagnosed clinically, taking into consideration the epidemiologic data. The morbidity rate was 2.6 cases per 100 000 persons that is two times lower in comparison with the average rate for the whole country. The disease developed in 2% of bitten persons. Most cases (93%) were registered in May–August. The age distribution of patients was as follows: children: up to 14 years of age (5.4%); adults: 20–60 years (45.9%); retired persons: males over 60 years and females over 55 years (47.3%). There was no correlation between profession of people and morbidity rates. Most patients (56.8%) developed disease after being bitten by ticks at their private vegetable gardens or in cottage yards. Forty-four percent of ticks *I. persulcatus* were infected with *B. burgdorferi* s.l. The distribution of *Borrelia* that was found in ticks by genospecies was as follows: *B. afzelii*: 73.8%; *B. garinii*: 15.4%; the mixture of *B. afzelii* and *B. garinii*: 10.8%. In sera from persons living in the northern area (Nenetsky Autonomous District), antibodies to *Borrelia* were not found. Antibodies were detected in 17 persons bitten by ticks in southern areas and antibody titers increased in nine patients.
Conclusions: L.D. is widely spread in southern regions of Arkhangel'sk province. Two genospecies of *Borrelia* are transmitted in the province – *B. afzelii* and *B. garinii*. Many cases of L.D. are not diagnosed. It was proved with results of IgG detection with ELISA.

P1613 Prospective dynamic study on PCR in urine in Lyme neuroborreliosis

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Objectives: Nested PCR technique has been developed for diagnostic of Lyme neuroborreliosis. Preliminary results of prospective dynamic study of urine PCR in neuroborreliosis are presented.
Methods: Two sets of primers were used in nested PCR – first one for plasmid gene encoding OspC protein and second one for chromosomal gene 16S rDNA. PCR was proved for urine and CSF (CSF data are not presented here). CSF/serum antiborrelial antibody index has been established according to Reiber et al.
Patients: Till now 41 patients with neurologic involvement in Lyme borreliosis have been enrolled in prospective designed study. All patients were examined clinically before and after treatment (3 weeks intravenously administered penicillin) and later after 3 and 6 months. Examinations of CSF and serum antibodies and urine PCR were performed in the same periods. Laboratory diagnosis was established on positive CSF/serum-specific antibody index (AI), in five cases only on CSF PCR positivity (examined elsewhere). Clinical manifestations comprised Bannwarth's syndrome (26), acute encephalitis (10), polyradiculoneuritis (4) and myelitis (1).
Results: So far, 41 patients have been included. Urine PCR and AI were positive in 23 (56%) patients in the same time, positive AI and negative PCR were in 13 (32%). Only in five cases was DNA positive and AI negative. Urine PCR was positive in 20 patients before treatment, in 18 cases after treatment and in four patients on 3 months' control. No patient was positive after 6 months.
Conclusion: In sum, urine PCR was positive in 28 (68%) patients. The frequency of positivity decreased from 20 (49%) patients at the beginning of therapy to 18 (44%) after treatment (3 weeks). Four patients (13%) on 3 months' control were positive and no positive was found out after 6 months. OspC primer was positive in eight and 16S rDNA in 22 samples, both of them 7 times.
Acknowledgment: This study is supported by grant of Health Ministry CR 6244/3 and study 111 30 0003.

P1614 Cutaneous anthrax as an occupational disease in Central Anatolia, Turkey

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Objective: Zoonotic disease, anthrax, has increasing medical attention worldwide. Incidence and medical interest of cutaneous anthrax had been markedly decreased in developed countries, but it remains a considerable public health concern in Turkey, as so in many developing countries. The aim of this retrospective study is to describe epidemiologic and clinical features of our 13 cases of human cutaneous anthrax.
Methods: All of the cutaneous anthrax cases admitted to the Infectious Diseases Clinic of Ankara Hospital between July 1999 and September 2001 were enrolled in the study. Epidemiologic data, source of the exposure, occupation, age, gender, clinical presentations with incubation period, previous antibiotic use, previous surgical procedures and other findings and laboratory diagnostic works were noted.

Results: All of our patients had occupational exposure; they were middle-aged people with low income. Female to male ratio was 3/10, and they were all living in the suburban regions of Central Anatolia. Only one patient had the typical scar on face (lower eyelid) and all others had their lesions on upper extremity. None of the cases had fatal outcome, but one patient had vision lost due to cicatricial ectropion of left eye. Patients treated 10–21 days according to their clinical responses. Total of eight patients had positive smear and two of them also had bacteriologic confirmation. Main drug used to treat was parenteral penicillin and ampicillin plus sulbactam combinations and ciprofloxacine were used occasionally.
Conclusion: Anthrax is an occupational disease in our country and occurs more commonly in summers. All of the cases in our centers had an occupational history. Majority of these patients have been treated with various antibiotics elsewhere before the admission to our center. Although, there has been no report of a major epidemic neither in human nor in veterinary medicine in Turkey, but our findings emphasize that anthrax still remains a public health concern in rural areas, and animal products provided from these areas may continue to pose a great risk for exposed people.

P1615 Killing of anthrax spores by aqueous iodine

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Objectives: Although, the degerming activity of preparations containing free molecular iodine against nonsporulating microorganisms is well investigated, only few reliable studies on its sporocidal activity are known.
Methods: We have examined the action of aqueous iodine (5, 10, and 15 ppm free iodine) and of the commercial preparation Betaisodona solution standardized against spores of three virulent and one nonvirulent strain of *Bacillus anthracis* compared to spores of *B. subtilis* and *Clostridium* spp.
Results: Free iodine in aqueous solution demonstrated a high activity against *B. anthracis*, which manifested in killing times of 1–5 min.
Conclusions: Because of these results, iodine solutions can be recommended as a disinfectant in case of skin contamination with *B. anthracis*.

P1616 Rapid identification and virulence characterization of *Bacillus anthracis* by pyrosequencing

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Objectives: Inhalation anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. Infection can be prevented with antibiotics if administered within the first 24–48 h after exposure to *B. anthracis*. Any delay in treatment reduces chances of survival if initial symptoms of inhalation anthrax are present. Therefore, the ability to distinguish *B. anthracis* from closely related *Bacillus* species, and to distinguish virulent from avirulent strains is of great importance. Virulent strains of *B. anthracis* harbor two plasmids, pXO1 and pXO2, carrying genes encoding the anthrax toxin, and products necessary for encapsulation, respectively. A rapid and reliable technique for identification and characterization of *B. anthracis* is urgently needed. Pyrosequencing is a novel sequencing-by-synthesis technique used for rapid and accurate analysis of short, specific DNA sequences. Up to 96 DNA samples can be analyzed within a single, 1-h assay. We investigated the use of pyrosequencing for species identification and virulence characterization of *B. anthracis*.
Methods: Plasmids and chromosomal DNA from 10 *B. anthracis* strains were extracted. *B. cereus* and *B. subtilis* were used as negative controls. PCR primers were designed to amplify a 129-bp fragment of the *B. anthracis* chromosomal marker Ba813, a 177-bp fragment of the *lef* gene encoded on pXO1, and a 127-bp DNA sequence of pXO2. The PCR fragments were analyzed by pyrosequencing.
Results: Nine of the 10 bacterial strains examined were clearly identified as *B. anthracis* by pyrosequencing analysis of 20 nucleotides of Ba813. *B. anthracis* identity was also confirmed by culture. One sample was excluded due to PCR failure. Sequence analysis determined the presence or absence of pXO1 and pXO2, and thereby the virulence status of the bacterial strains examined. Seven of the 10 *B. anthracis* strains were positive for both virulence plasmids, while three strains lacked both or either of them. Accuracy of the sequence analysis was between 99.6 and 100%.
Conclusions: We found that pyrosequencing provided a convenient way to rapidly gain information on species identity and virulence status of *B. anthracis*.

P1617 Anthrax in pregnancy: report of two cases

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Objective: The purpose of this report is to review the rarely seen anthrax cases in pregnancy, its maternal and perinatal complications, and management of anthrax in pregnancy.
Case reports:

- Case 1: A 33-year-old pregnant women with 32 weeks of pregnancy was admitted to hospital with a 5-day history of flaying a dead cow. The patient had a submandibular eschar with surrounding vesicles that oozed clear yellow fluid, extensive edema on the face, neck and upper thorax as to embarrass respiratory function and fever (38 °C). Obstetrical examination confirmed the 32 weeks of normal pregnancy; the patient had no signs and obstetrical findings (without any uterine contraction and cervical dilatation) of preterm delivery at the time of admission. The white cell count was 28 300/mm^3. Direct microscopic examination of vesicle fluid revealed large Gram-positive bacilli. Penicillin G and prednisolone therapy was administered immediately with the presumptive diagnosis of anthrax; the isolated organism was later identified as *Bacillus anthracis*. The signs and symptoms of anthrax were disappeared within 10 days of therapy, but patient delivered a preterm baby on the 13th day of her hospital admission.
- Case 2: A 29-year-old pregnant women was hospitalized with a 4-day history of swelling of the right arm and weeping lesion at the right elbow. The patient was febrile (38.5 °C) and had a 2-cm open sore with surrounding erythema and induration that oozed serous fluid. Obstetrical examination revealed 33 weeks of normal pregnancy without any findings of preterm delivery. The white cell count was 19 600/mm^3. Direct microscopic examination of the vesicle fluid revealed large Gram-positive bacilli. The patient was treated with procaine penicillin for 7 days. On the day of discharge from the hospital, preterm delivery began which was not prevented with tocolytic therapy and the patient delivered a 34 weeks of preterm baby.

Conclusion: Since anthrax in pregnancy is rarely seen, its management in pregnancy, maternal and perinatal complications are not entirely known. These two cases showed that anthrax in pregnancy can be successfully managed as in a nonpregnant woman. As in progress in our two cases preterm delivery may be one of the major complications for perinatal outcome. The obstetrician must be aware of the probability of this complication even at the end of the anthrax therapy and may consider the early tocolysis in cases of anthrax in pregnancy.

P1618 Antimicrobial susceptibility of *Bacillus anthracis* isolated from patients with cutaneous anthrax

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Objectives: To test the antimicrobial susceptibility of *Bacillus anthracis* isolates to various antimicrobial agents.
Methods: The 28 *B. anthracis* isolates collected between 1992 and 2001 from cultures of the patients with cutaneous anthrax at Cumhuriyet University Hospital were tested for susceptibility to various antimicrobial agents by using Sceptor™ (Becton Dickinson Diagnostic Instrument Systems, Towson, MD) automatic system. They were identified by conventional methods. Susceptibility breakpoints for interpreting minimum inhibitory concentration (MIC) results for *B. anthracis* have not been determined by the NCCLS; thus, breakpoints for staphylococci were used.
Results: All isolates were sensitive to penicillin (MIC < 0.03 μg/mL), and did not produce β-lactamase. Although, all isolates were sensitive to cephalothin (MIC < 8 μg/mL), ciprofloxacin (MIC < 1 μg/mL), clarithromycin (MIC < 2 μg/mL), clindamycin (MIC < 0.05 μg/mL), tetracycline (MIC < 4 μg/mL) and gentamicin (MIC < 4 μg/mL), one of the isolates was resistant to

ceftriaxone (MIC < 64 μg/mL). All isolates were resistant to cefepime (MIC > 16 μg/mL) and cotrimoxazole (MIC < 4 μg/mL). The carbapenem antibiotics such as meropenem and imipenem, and glycopeptide antibiotics such as teicoplanin and vancomycin showed good activity with MICs of <4 μg/mL, <4 μg/mL, <8 μg/mL, and <2 μg/mL, respectively. Susceptibility of the six isolates was considered intermediate to ceftriaxone (MIC = 32 μg/mL).
Conclusion: *B. anthracis* isolates were susceptible to antimicrobials suggested the treatment of anthrax such as penicillin, cephalothin, ciprofloxacin. and resistant to fourth generation of cephalosporin and cotrimoxazole in our region (Sivas city, which is located in the Middle Anatolian of Turkey).

P1619 The outcome of 43 adult tetanus cases in a tertiary hospital in Turkey

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Objective: To evaluate the outcome of 43 patients with their clinical and epidemiologic characteristics.
Methods: Ankara Numune Education and Research Hospital is a tertiary hospital with 1100 beds. This is a retrospective study which includes all the patients who were diagnosed with tetanus and hospitalized in Clinical Microbiology and Infectious Diseases Department between 1990 and 2000. All the patients were treated according to the guidelines, isolated in a quiet environment, surgical debridement was performed if necessary, and penicillin G i.v., human tetanus immunoglobulin, diazepam, prophylactic heparin and supportive therapy were given.
Results: The majority of the cases were admitted in the first half of the last decade (67%). All the patients were >15 years of age, 28 (65%) patients were male and 15 (35%) were female. The mean and median age was 44.6 and 51 years, respectively. Most of the patients were from rural Anatolia (77%), 48.8% of the patients were farmers, 34.8% housewife, 13.9% worker, and 2.3% unemployed. The most common clinical manifestations were trismus (100%), loss of consciousness (100%), abdominal rigidity (93%), dysphagia (81%), risus sardonicus (72%), generalized convulsion (65%), opustotonus (63%), nuchal rigidity (63%), fever (37%), tachycardia (33%), sweating (30%), respiratory distress (26%). The mortality rate (MR) was higher in patients who had tachycardia (79%). The patients with incubation period less than 10 days had significantly higher MR (81%) compared to patients with incubation period of more than 10 days (19%) ($P < 0.001$). The MR of the patients who had wounds on the head and neck or the body (100%) was higher than the patients who had wounds at the extremities (55%). The MR was >90% in first 10 days of hospitalization, but decreased to 18% after 10 days. The most common form of trauma was caused by a piece of metal (39.5%) or splinters of wood or thorn (25.5%). In the patient histories obtained from their relatives, the tetanus immunization was absent or inadequate or not known.
Conclusion: Even though the MR rate of tetanus cases admitted to our clinic was declined in the last half of the last decade, the overall MR for 10 years was found to be high. One of the major reasons for the high MR was delay in diagnosis and consequently lack of adequate medical aid prior to admission to our hospital. The incubation period was a significant predictor of outcome of the disease.

Rickettsiae

P1620 Q-fever, human and animal morbidity in some regions of Bosnia and Herzegovina, 2000

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Objectives: *C. burnetti* causes Q-fever, which may present as an acute febrile illness with pneumonia or as a chronic infection with endocarditis. *C. burnetti* is an obligate intracellular Gram-negative, small bacterium. *C. burnetti* is very resistant and in nature it is maintained and disseminated by horizontal and vertical transmission, by ticks, wild and domestic animals. Reservoir of infections may persist for years or even decades. After the war in Bosnia and Herzegovina (1992/1996), we had to import cattle, sheep and goats from all parts of Europe and it was result of these dramatic changes of different infectious diseases.
Methods: Diagnosis of Q-fever relies on serologic examination. All sera were tested by the chekin-Q-fever enzyme immunoassay (EIA) or indirect immunofluorescence assay (ELISA-MRL Diagnostics) for the detection of IgM and IgG antibodies to *C. burnetii* phase I and phase II antigens. From January to December 2000, blood samples were collected from persons with clinical signs of Q-fever who living in urban and rural parts of FBiH and from 47 621 ruminants (23 546 cattle, 22 962 sheep and 1103 goats) from 67 different areas of Bosnia and Herzegovina. Blood samples from patients or animals were eked and were separated and kept at −200 °C until required.
Results: The seroprevalence of the present antibodies in the cattle was 2.17% or 512, in the sheep 1.85% or 425, and goats 0.27% or three positive. The prevalence of disease was observed in 44 locations of BiH. Cases were distributed throughout all months with peaks during May and June. From total investigated human samples, presence of *C. burnetii* antibodies were observed in 153 patients from six different canton of BiH.
Conclusions: The results of this study indicate that prevalence of *C. burnetii* antibodies in serum samples of cattle, sheep and goats is widespread in BiH. Antibodies to *C. burnetii* antigen phase II were detected positive in serum from 153 persons in six locations of FBiH. High level of seroprevalence of coxillosis in domestic ruminants is the primary reservoir from which human contamination occurs, because human infections were related to contact with livestock.

P1621 *Coxiella burnetii* hepatitis

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Objectives: To investigate the hepatic involvement of acute Q-fever infection.
Methods: Patients were identified from a retrospective study of adults hospitalized for a febrile illness associated with serologic evidence of acute *Coxiella burnetii* infection. Over a period of 7 years (1989–1996), serum samples from 3300 patients suspected of being infected by *C. burnetii* were assayed for the presence of antibodies against antigen phase II of the microorganism, using the indirect immunofluorescence antibody technique (IFA). We reviewed the medical records of 131 patients that fulfilled the clinical and serologic criteria of acute Q-fever infection. Hepatitis was defined as elevated liver transaminase enzymes over two times the upper normal value for the testing laboratory.
Results: A total of 53/131 patients (40.4%) had hepatitis as defined above. A total of 45/53 were male (84.9%). The mean age of the patients was 37 + 11 years. Total 51/53 (96.2%) patients had fever at admission and 7/53 (13.2%) patients were presented as hepatitis. The rest of the 46/53 (86.8%) patients were admitted in the hospital with community-acquired pneumonia. Additional clinical features were hepatomegaly in eight (15%), rashes in three (5.6%), splenomegaly in three (5.6%) and meningitis in two (3.8%) patients. Ascites were noted in one patient presented with hepatosplenomegaly, hepatitis and pneumonia. At 7 ± 4 days from the onset of the symptoms, the mean serum levels of ALT were 108 ± 86.8 U/L (range 11–396 U/L; normal range: 5–40 U/L). Patients with normal ALT levels at admission showed a significant increase in ALT levels on day 12 ± 4.4 (range 5–26 days) from the onset of the symptoms. Overall on day 12, the mean serum level of ALT were 129.6 ± 71 U/L. The mean time to normalization of ALT level was 29 ± 5.7 days (range 25–41 days). One patient had a high serum bilirubine level, while prothrombine time was within the normal in all patients tested. In 10 (18.9%) patients were detected low serum albumin levels, mean 3.11 mg/dL (range 2.1–3.4 mg/dL). Despite only six (11.3%) patients being treated with doxycycline, the drug of choice for acute Q-fever infection, all patients spontaneously recovered. Fever subsided to normal levels in a mean time of 10.1 days (range 4–31 days) from symptoms onset.

Conclusion: Our data suggest that hepatitis is a common feature of acute *C. burnetii* infection. The mean time of complete resolution of the disease was estimated to be up to 30 days. The outcome was favorable for all patients.

P1622 Treatment of boutonneuse fever with doxicyclini, ciprofloxacini and corticosteroids

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Objectives: The efficacy determination of some therapeutical protocols composed by 'classic' antirickettsial therapy like chloramphenicol, tetracyclini, and the 'modern' ones like doxicycline and ciprofloxacini associated or not with corticosteroids.
Methods: The study includes 50 cases with boutonneuse fever of age 13–61-year-old observed by us during the period of 1993–2001. The etiologic treatment chloramphenicol 0.5 × 4/die (10 cases), tetracyclini 0.5 × 4/die (14 cases), doxicyclini 0.1 × 2/die (12 cases). The etiologic treatment lasted 7 days. The patients were treated even with antipiretics, like aspirini 0.5 × 4/die or paracetamol 0.5 × 4/die, or novalgini (1–2) × 1 ampoule/die until the normalization of the fever and headache. The patients were divided in two groups: the first group of 20 patients was treated with corticosteriodes [prednisolone (2–4) × 25 mg/die or dexamethasone (2–4) × 4 mg/die], and the second group of 30 patients was treated without corticosteroids. The comparison of the two groups was based upon the course evaluation of these indications: fever, toxicosis, exanthema and 'tache noire.' It also made up the comparison of those indicators for the subgroup, treated by different antirickettsials. The Z test was used for statistical elaboration.
Results: We did not see significant changes of the efficacy between different antirickettsials ($P < 0.1$); doxicyclini and ciprofloxacini were better tolerated. Significant variations were seen regarding fever normalization and toxicosis improvement in favor of the first group ($P < 0.001$) and concretely – the fever in that group was normalized on the first day in five cases, on the second day in 13 cases, on the third day in two cases. The fever for the group without cortisone was normalized – on the first day in zero cases, on the second day in 11 cases, on the third day in 15 cases, on the fourth day in four cases. No substantial changes of exanthema and 'tache noire' course were seen between the two groups.
Conclusions: The efficacy of doxicyclini and ciprofloxacini was equal with that of the 'classic' antirickettsials. The corticosteroids usage obviously shortened the fever and toxicosis but not the course of the skin lesions of bouttonneuse fever.

P1623 Mourine typhus in childhood: report of 20 cases in Chania, Crete, Greece

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Objectives: To study the clinical, laboratory manifestations and treatment features of endemic typhus in childhood in our region.
Patients and methods: Twenty children with clinical and serologic diagnosis of murine typhus were studied from June to November 2001 in the general hospital of Chania on the island of Crete.
Results: Twelve patients (60%) were boys and eight (40%) were girls. The average patient's age was 9.8 years (1.5–14). Five out of 20 (25%) patients were residents of urban and 15/20 (75%) of rural areas. All cases presented with fever (100%), whereas rashes were found in 18/20 pts (90%). Maculopapular rash appeared within 3.78 days (range: 2–5 days) after the onset of the fever and lasted for 2 days (range: 1–3 days). Headache was the presenting symptom in 12/14 pts (80%), arthralgias in 12/14 pts (85.7%), myalgias in 11/14 (78.5%), and photophobia in 2/14 (14.3%). Spleen enlargement was found in 16/20 pts (80%), and liver enlargement in 13/20 pts (65%). Five out of 20 pts (25%) presented pulmonary, and 3/20 pts (15%) gastrointestinal manifestations of the disease. The most prominent laboratory findings registered were: leukopenia in 12/20 pts (60%), anemia in 4 pts (21.1%), thrombocytopenia in 3 pts (15.7%), increase of serum transaminases in 5.5% pts, and blood traces in urine in 6 pts (30.3%). Nine out of 20 patients (45%) were treated with chloramfenicol, 6/20 pts (30%) with vibramycin, 3/20 pts (15%) with cotrimoxazole, 2/20 pts (10%) with ciprofloxacin. The mean time elapsed until defervescence was 8.73 days (4–15). The outcome was favorable for all the patients and no relapse was observed.
Conclusion: Endemic typhus presents in children of our region mainly with fever, headache, and rashes.

P1624 Liver biochemical abnormalities in murine typhus: a study of 101 cases

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The most frequent biochemical abnormality in murine typhus is a mild elevation of AST, but ALT and LDH are often elevated in parallel. A hundred and one patients with compatible clinical status of murine typhus and high serologic titers of antibodies against *Rickettsia typhi*, were studied by our team between January 1993 and December 1998. For the study of their hepatic function, three serum samples were obtained from each patient. The first sample was obtained on admission, approximately 9 days after the onset of the disease. The second sample approximately 2 weeks after the first. The third sample, taken from half of the patients, was obtained 1 month after the second. On admission (first sample), 87/101 patients (86.1%) presented an elevation of AST, 66/101 patients (65.3%) presented an elevation of ALT and 81/101 patients (80.2%) presented an increase of LDH. The mean values of AST, ALT and LDH were 70.3, 60.5, and 314.6 U/L, respectively. Two weeks later (second sample), 85/101 patients (84.1%) presented an increase of AST, 73/101 patients (72.2%) presented an increase of ALT and 76/101 patients (75.2%) presented an elevation of LDH. The mean values of AST, ALT and LDH were 71.2, 70.5, and 288.3 U/L, respectively. One month later (third sample), 18/48 patients (37.5%) presented an increase of AST, 13/48 patients (27.0%) presented an increase of ALT, and 8/48 patients (16.6%) presented an elevation of LDH. The mean values of AST, ALT and LDH were 35.5, 33.6, and 154.1 U/L, respectively. Our study showed that the time of normal restoration of liver function was about 2 months after the onset of the disease.

P1625 Demographic features of 101 cases of murine typhus in Crete, Greece

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A hundred and one patients with compatible clinical status of murine typhus and high serologic titers of antibodies against *Rickettsia typhi* were studied by our team between January 1993 and December 1998. Sixty patients were men (59.4%) and 41 were women (40.6%). The median age of the patients was 47 (SD ± 19.0) years, with a range of 14–80 years. A higher incidence of infection was noted in the age group 20–29 years with 21 patients (20.8%) and in the age group 60–69 years with 23 patients (22.7%). The monthly distribution of murine typhus cases revealed a higher incidence of infection from July till November (91 cases, 90.0%). Seventy-four patients (73.3%) had a history of contact with animals, mainly rats, dogs, cats, rabbits, sheep and goats. Fifty-two patients (51.5%) were registered as residents of rural areas, 21 patients (20.8%) were staying in semiurban areas and 28 (27.7%) in urban areas. In the Province of Kydonia were traced 73 cases (72.3%), in the Province of Apokoronas 15 cases (14.8%), in the Province of Kissamos 11 cases (10.8%) and in the Province of Selinos two cases (1.9%). No one case was traced in the Province of Sfakia. Twenty-nine (28.7%) of the 101 patients were farmers, 17 (16.8%) were clerks, 24 of the women (23.7%) were housewives, eight patients (7.9%) were workers. Furthermore, seven patients (6.9%) were merchants, five (4.9%) were students, four (3.9%) were pensioners and there was one nurse and one physician (0.9%) of our hospital. Our study showed that murine typhus regards all the Chania County and especially the Province of Kydonia. The rural activity and the contact of rodents represent risk factors for this infection.

P1626 The Albanian features of endemic exanthematic typhus

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Objectives: The evidence of the epidemiologic features and laboratory findings of murine typhus in Albania.
Methods: In 150 cases of murine typhus of age 14–72 years, observed during 1990–2001, confirmed through positivity of Weil–Felix reaction (1:320–640–1280) or indirect imunofluorescence (1:16–32–64), we determined residence, age group, gender and also laboratoric-clinic spectrum.
Results: The most vulnerable age group was 15–40 years (60, 66%), rarely 41–72 years (39, 34%). Year frequency was irregular: 6–24 cases. The domination of rickettiosis was found in the urban areas (110:40). The males are more frequently infected (102:38). The type and frequency of signs and their percentage was as follows: fever: 100 (from 38 to 41.6 °C); exanthema: 100 (confluent in 10.6% of the cases); headache: 100; weakness: 85.3; anorexia: 60.6; myalgia: 56.6; chills: 54; typhic tongue: 52; hepatomegaly: 50; splenomegaly: 42; conjuctivitis: 21.3; encephalopathy: 19.3; petechiae: 12; epistaxis: 10.6; hypotension: 6.7; meningismus: 6; vomiting: 6; pneumonia 3.3; icter: 3.3; diarrhea: 3.3; feet flebothrombosis: 2.6; lymphocitar meningitis: 1.8; pulmonary thromboembolia: 1.3; myocarditis shock: 0.6. We have seen: leukocitosis: 21.3%; leukopenia: 12%; hypertransaminasemia: 10.6%; hyperasotemia: 6%; thrombocitopenia: 4%; hyperbilirubinemia: 3% of the cases. According to the gravity, we have distinguished: grave forms: 23.3%; medium forms: 64.7%; mild forms: 12%. The resulting mortality rate was 0%.
Conclusions: Independently of missing mortality, murine typhus appears as a serious disease.

Sexually transmitted diseases

P1627 STDs in women addicted to drugs: a 15-year study in an STD unit

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Objective: To study the correlation between drug use and sexually transmitted diseases in women patients of a STD Unit in a 15-year time span.
Methods: Patients of the STD unit between January 1986 and January 2001 who had a background of high risk activities for STD infection were analyzed. High risk activities included: drug use (intravenous or otherwise), prostitution, trading sex for drugs, homosexual sex, or unprotected sexual contact with four or more partners in the last year. The study group was composed with the women who were addicted to drugs. A medical history was gathered for all of them, as well as a clinical exploration, and a STD screening.
Results: Out of 702 patients (83%, of which were HIV+), 343 (43%) were women addicted to drugs, 295 of these women (86%) were HIV+, 85% of them traded sex for drugs. In addition to HIV, 50% of the women had two other STDs, 23% of them had three or more. The most common infection agents were human papilloma virus (HPV) in 280 cases (81%). There were 190 cases (55%) of vaginitis, 78 of which were caused by *T. vaginalis*, 75 by *Candida* sp., and 37 by *Garnerella vaginalis*. Twenty-five (7.2%) of the total had genital herpes, 15 had syphilis (4.5%), 6 *Chlamydia trachomatis* (2%), 4 *Neisseria gonorrhoeae* (1%), 1 *H. ducreyii* (0.2%), and 1 *S. scabiei* (0.2%). Twenty-seven (8%) presented pelvic inflammatory disease.
Conclusions: HIV infection among women is correlated to drug use, 85% of the addicted women ended up prostituting themselves in order to obtain drugs. One out of four has three or more STDs associated to HIV. In 280 cases (81%) these infections are of viral origin, making them difficult to eradicate. This increases morbimortality not only in the female population, but also among their sexual partners, and the children born to these women. Drug addiction in women constitutes a high risk to public health.

P1628 Evaluation of an enzyme immunoassay (EIA) for syphilis screening

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Because of concern about the lack of sensitivity of the RPR assay (Becton–Dickenson; Cockeysville, MD) in late stage syphilis as well as automation potential, CLS switched to an EIA Assay (Trinity; Jamestown, NY) for IgG treponemal specific antibodies (TSA) for screening purposes 4 years ago [E.S. Pearlman et al. Clin Chem 1998; 44: 1790]. The RPR was used as a supplemental assay. Recently a Western Blot (WB) assay for TSA has become available (Phoenix Bio-Tech; Mississauga, ON). The manufacturer of the WB assay also produces an EIA screening assay. We wished to evaluate the WB assay in a syphilis-testing algorithm and in a first study we evaluated the EIA from Phoenix-Bio-Tech (EIA-new) as a replacement for our current EIA (EIA-old). Both EIA assays and an RPR assay were run on all specimens ($N = 519$ consecutive patient samples). Discrepant resolution utilized RPR data and results from FTA (Zeus; Raritan, NJ). All assays were performed according to manufacturers' directions. Both EIA assays produced results based on an optical density ratio (ODR) of patient specimen to a low positive cutoff. We compared results using an ODR of ≥ 0.9 as a positive result for EIA-old and ODR ≥ 1.0 as a positive result for EIA-new. EIA-new produced a higher ODR in 509/519 patient samples [$P < 0.0001$; Sign test] but overall agreement in interpretation (positive/negative) was noted in 502/519 specimens (96.7%). The 17 discrepant specimens were all EIA-new [+] and EIA-old [–]. Of these samples, 10 were RPR [–] and FTA [–]; 5 were RPR [–] but FTA [+] while 2 were RPR [+] but FTA [–]. In two specimens strongly positive by both EIA-old (initial ODRs = 2.378, 2.267) and EIA-new (initial ODRs = 4.943 and 6.33) serial dilution resulted in negative EIA-old results at 1:16 dilution (ODRs = 0.534, 0.632) while both specimens remained positive using EIA-new (ODRs = 1.000, 1.182). The results suggest that there are difficulties with any single test used to screen for syphilis and any combination of tests will yield some discrepant data. As a referral laboratory patient data is not readily available to us, but the presence of 5 EIA-new [+], FTA [+] and 2 EIA-new [+], RPR [+] samples that were all EIA-old [–], and also the ODR and serial dilution data suggest that EIA-new has greater sensitivity than EIA-old for detection of IgG TSA. Such increased sensitivity is desirable in a screening assay and EIA-new has been implemented at CLS.

P1629 A new approach to syphilis diagnosis

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CLS is a large freestanding clinical laboratory serving an exclusively ambulatory population. Because of lack of sensitivity of the RPR assay (Becton–Dickenson; Cockeysville, MD) in late stage syphilis as well as automation potential, CLS switched to an EIA assay for IgG treponemal specific antibodies (TSA) for screening purposes 4 years ago [E.S. Pearlman et al. Clin Chem 1998; 44: 1790]. The RPR was used as a supplemental assay. Recently a WB assay for TSA has become available (Phoenix Bio-Tech; Mississauga, ON). The same manufacturer also produced an EIA assay for screening, which CLS has evaluated and implemented [E.S. Pearlman et al. this meeting]. We were interested in evaluating the role of the WB assay in a syphilis-testing algorithm and for this purpose did an RPR assay on 519 consecutive patient samples irrespective of the result of the initial EIA screen. Discrepant specimens were further evaluated by WB and where specimen quantity was sufficient, by FTA (Zeus; Raritan, NJ). All assays were performed according to manufacturers' directions. There was result concordance between EIA and RPR in 490/519 samples (94.4%). Of the 29 discrepant specimens, 28 (96.6%) were EIA [+], RPR [–]. Of the latter 28 specimens, 18 were WB [+] and all 18 of these samples were FTA [+]. Of the 10 EIA [+], RPR [–] and WB [–] specimens, FTA could be performed on nine samples and eight of these specimens were FTA [–]. Finally, there was 1 discrepant sample which was EIA [–] and RPR [+]. This sample was WB [+] but FTA [–]. Overall, there were concordant results in 26 of 28 (92.9%) specimens on which both WB and FTA had been performed. As the EIA [–], FTA [–], RPR [+], WB [+] specimen suggests, it is difficult to get completely consistent results when multiple serologic tests for syphilis are used. Given the labor-intensive character of the FTA and the presence of false [+] FTA reactions in

1% of the general population (ASM Man. Clin. Lab. Immunol., 5th ed. p. 519), CLS has decided to incorporate the new WB assay into a syphilis-testing algorithm. EIA (+), RPR (–) samples are reflexed to WB. These specimens are signed out as TSA (+), TSA (–) or TSA-indeterminate (suggest repeat testing in 6 weeks) depending on the WB result.

P1630 The re-emergence of syphilis

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Introduction: Syphilis is often referred to as a disease which has been successfully controlled during the second half of the 20th Century. However, three recent outbreaks in the UK provide an unpleasant reminder that infective syphilis is making a comeback.
Objectives: The clinical features of the disease are discussed before describing the recent increases in cases in the UK.
Methods: Literature review of articles from medline describing evidence for the re-emergence of syphilis.
Results: Recently, three outbreaks of syphilis have occurred in the UK have been reported. The first was in Bristol between 1997 and 1998, where a heterosexual outbreak occurred largely due to unprotected sex and multiple partners. This was followed by an outbreak in Manchester, where 34 cases, were seen between 1999 and 2000, compared to a normal of 2 per year. The cases in Manchester were mostly homosexual (70%), and of these, nine cases were HIV-1 antibody positive, eight were HIV negative, and seven had not been tested. The most recent outbreak occurred in Brighton which involved homosexual cases.
Conclusions: Consideration is given to the possible causes of syphilis epidemics, by reflecting on cases in the USA and the former Soviet Union. When considering the drive to control syphilis, the problems of HIV coinfectivity and congenital syphilis are evaluated. Finally, suggestions are made to prevent further outbreaks of syphilis by improving management, particularly by increased awareness and adequate screening.

P1631 Fifteen years of syphilis in an STD unit: clinical, epidemiological and evolutional aspects

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Objective: To study the clinical, epidemiological and evolutive aspects of patients diagnosed with syphilis during 15 years at the STD center in a University hospital.
Methods: Data was gathered on all prospective patients of the STD unit between January 1986 and January 2001. A complete clinical history, a physical examination, as well as a complementary battery of laboratory tests were obtained for all patients. The syphilis diagnostic was established by *Treponema pallidum* detection in the serous transudate of ulcerated lesions using an optical microscope with dark field method. Non-treponemic antibodies were detected with a VDRL (Venereal Disease Research Laboratory) test. Treponemic antibodies were detected using a FTA (fluorescent treponemal antibody-absorption) test and hemagglutination of *T. pallidum*.
Results: In 15 years 205 cases of syphilis were diagnosed from a total of 3606 patients, 2630 of which had STDs (3% of the total), 8% of these were infected with syphilis, 72 of the infected patients (35%) were women, 133 were men (65%) 33% of the infected men were HIV+, of which 73% were homosexual. The most frequent presentation was latent syphilis (80%). Three of these cases had neurosyphilis, one case was congenital. In 66 cases (30%) syphilis coexisted with another STD. Clinical evolution after treatment was favorable in all cases.
Conclusions: Syphilis has been a relatively rare STD, representing 8% of the patients in a 15-year time span; 16% of the cases coexisted with an HIV infection, the most affected were patients with homosexual practices. One-third of the syphilis patients had at least two other STDs. Evolution after treatment was favorable in all cases.

P1632 Seroprevalence, antibodies to anti-*T. pallidum* in a cohort study of pregnant women in Buenos Aires, Argentina

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Background and objectives: Syphilis is a sexually transmitted disease (STD) caused by the Spirochete *T. pallidum*. A pregnancy untreated woman with syphilis transfer the infection to her fetus, who give birth to a child with serious mental and physical problems, neonatal death, and disorders such as deafness, neurology impairment, and bone deformities. The present study was carried out in our hospital to determine the presence of maternal syphilis during: the consult in the pregnant, just given birth, or immediately post-delivery. This Retrospective Cohort Study, was performed from October 1999 to October 2001, fixing the following bias: (A) the patients must authorize the study. (B) They must not have other results, in order to avoid an excessive health cost. (C) They have to compromise themselves to complete the treatment so as to study the dynamics of infection. Those who were positive had to concur for previously established controls to follow up their treatment.
Methods: We included in the present study 3346 pregnancy women who accepted our conditions. The average of ages was 26, with a range between 15 and 42 years. We tested anti-*T. Pallidum* antibodies titers, screening of infection it was made with two nontreponemal test: RPR (Nosticon Distrib. L.A. by Org. Tek., Co. Bio-Mérieux Group) or VDRL (GenCellDiag.). The (+) samples were confirmed/quantified with a treponemal test: (TPHA-Nosticon) or (FAT-ABS).
Results: From the 3346 pregnancy women studied, we confirmed infection in 64 (189%) IC 95% (143–235). All positive samples presented (+) treponemal test with titer between 1/80 and 1/40.960, although the nontreponemal had not given us a greater titer than two dilutions in many of cases.
Conclusion: The importance of this epidemiological study made and the prevalence of infection reported is that 19% of persons which share the same geographical zone and the average of age, ignore their (+) condition, transmitting silently the STD to the sexual partners and/or her newborn by transplacentarial way. We emphasize that all pregnant women were studied without discriminating possible risk groups confirming the absence of selection bias. The evidence is clear that all people around the world are exposed to this infection. That is the reason why everyone should control STD.

P1633 Increasing incidence of gonorrhoea in Israel associated with the countrywide dissemination of a ciprofloxacin-resistant strain

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Objectives: At the end of 1999, high-level ciprofloxacin resistant *N. gonorrhoeae* organisms (MIC_{90}: >32 μg/mL), also exhibiting decreased susceptibility to penicillin and tetracycline, were isolated in Israel for the first time. A study was conducted to investigate possible changes in the incidence of gonorrhea in the country related to the emergence of these organisms.
Methods: The incidence rates of male gonorrhea in southern Israel for the January 1991 to June 1999 and July 1999 to December 2000 period were calculated separately, and the number of cases of gonorrhea diagnosed in a large Jerusalem hospital during the same period was determined. Isolates of *N. gonorrhea* resistant to ciprofloxacin recovered in southern Israel and Jerusalem, as well as in the Northern and Estern areas of the country were typed by pulsed-field gel electrophoresis (PFGE) and compared.
Results: The incidence of male gonococcal urethritis in the south increased in 1.5-year period from 3/100 000 to 12/100 000 ($P < 0.05$) as the result of increased isolation of ciprofloxacin-resistant organisms. A marked increase in gonorrhea was also encountered in Jerusalem where ciprofloxacin resistance affected 54.5% of isolates in 2000. PFGE typing of gonococci from different areas of Israel indicated that all ciprofloxacin-resistant isolates belonged to identical or related strains.

Conclusions: Fluoroquinolone-resistant gonococci may emerge and disseminate extensively over a short period of time. Continuous surveillance of antibiotic susceptibility of gonococcal isolates should be performed to guide empiric therapy.

P1634 Evaluation of the VIDAS PROBE *Chlamydia trachomatis* test in males and females within a high risk population

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Objective: The aim of this study was to evaluate the preclinical performance of the VIDAS PROBE *Chlamydia trachomatis* (CT) test* in "at risk" female and male study subjects.
Methods: The VIDAS PROBE CT test is an automated assay for the amplification and qualitative detection of *C. trachomatis* 23S rRNA. A built-in internal control sequence coamplifies in the presence or absence of target sequence and monitors the success of amplification in each individual sample and control. The system employs the existing VIDAS immunoassay instrument for detection and subsequent decontamination of amplified product. First void urine (FVU) and female endocervical or male urethral swabs were collected from 804 patients (592 female/212 male) attending a sexually transmitted disease (STD) clinic or urgent care OB/GYN clinic. Study subjects ranging in age from 19 to 67 years (average age 28) were deemed at risk for chlamydial infection based on several factors including promiscuous behavior, recent exposure to STD infection and risk history (number of sexual partners, change in sexual partners, and previous positive test for *C. trachomatis*). Specimens were tested by the VIDAS PROBE CT test (TMA), polymerase chain reaction (PCR) and/or ligase chain reaction (LCR) and compared to patient infectivity status which was deemed positive if two or more of the comparative tests were positive.
Results: Of the 592 women and 212 men tested, 80 (14%) and 25 (12%), respectively, were shown to be infected with *C. trachomatis*. In women, the preclinical sensitivity/specificity of the VIDAS PROBE CT test for endocervical swabs and first void urine was 99/99% and 95/99%, respectively. Pre-clinical sensitivity/specificity in male urethral swabs and male urine was 96/100% and 100/99%. Inhibition, as monitored by internal control amplification, was observed in less than 1.0% (7/804) of all swab specimens and less than 3.0% (23/804) of all urine specimens tested.
Conclusions: Results of this study suggest that the VIDAS PROBE CT test is a highly sensitive and specific test for the detection of *C. trachomatis* infection in at risk male and female study subjects.

* This product has not been cleared by the United States FDA and is not yet available for commercial use.

P1635 Polycentric analysis of *C. trachomatis* infections: an epidemiological study

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CT one of the most wide spread sexually transmitted bacterial diseases. Published data have already shown that 30–50% of infections start in an asymptomatic manner so that it often escapes diagnosis and it is difficult to prescribe a correct therapy.
Objectives: In this study, we wanted to evaluate the prevalence of CT infection in patients that were treated at five different centers for sexually infectious disease (STD), in the north and center of Italy. Correlation between infection and social and behavior aspects have been studied to find possible risk factors.
Methods: From January 1, 2000 to the June 30, 2001, 14 619 patients (11 904 females and 2715 males), coming from sexually transmitted infection centers were evaluated. CT infection diagnosis was evaluated using molecular biology techniques in 10 432 samples, hybridization techniques in 1910, cell cultures in 1300 and by immunofluorescence technique in 973 patients. Statistical data were analyzed by Chi-square test and Fisher test with 95% CI.
Results: In the studied population, the infection prevalence is 1.95% (1.61% in females and 3.43% in males). CT was the only pathological agent we found in 54.39% of our patients. CT infection is particularly associated with a subjective symptomatology, more frequently in males (5.62%) than in females (1.90%) ($P < 0.001$). Prevalence in cases with symptomatic partners is also significantly higher ($P < 0.001$): (3.87% in females and 4.35% in males) versus asymptomatic controls (1.49%). In consideration of the age correlation, as published data have largely demonstrated, CT infection has shown a progressive decrease beginning with the younger patients (3.71% under 21 years of age and 2.38% between 21 and 30 with a $P < 0.001$ and <0.005, respectively) to the older patients (1.67% between 31 and 40 years of age and 1.63% over 40). Among other possible risk factors we inquired the number of sex partners in the last 6 months and the countries of origin with a high incidence of STD.
Conclusion: Infection resulting from CT is decidedly more prevalent in men than in women, it is accompanied by a more frequent subjective symptomatology and it is more frequent in younger subjects. Among the other risk factors that have emerged are the elevated number of sexual partners and subjects who come from countries with a high incidence of STD.

HBV–HCV

P1636 Influence of HBV–HCV replication on the clinical course of chronic dual (B, C) viral hepatitis

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Objective: The aim of this study was the investigation of dual chronic HBV and HCV infection clinical course.
Methods: A total of 42 HBsAg and anti-HCV-positive patients with chronic hepatitis were studied. Serological markers of disease were assayed using commercial enzyme immunoassays, HBV-DNA and HCV-RNA in serum were detected by means of a polymerase chain reaction (PCR) technique. Comparative analysis was done in next patients groups: (1) dual hepatitis with HBV-viremia (HBV-DNA+, HCV-RNA–), six patients; (2) dual hepatitis with HCV-viremia (HBV-DNA–, HCV-RNA–/+), 19 patients; (3) dual hepatitis with HCV-viremia and HBV-viremia (HBV-DNA+, HCV-RNA–/+), eight patients; (4) dual hepatitis with no signs of any virus replication (HBV-DNA, HCV-RNA) nine patients.
Results and conclusion: The clinical course of disease was characterized by cytolitic (65%), inflammatory syndrome (11%), rarely – changes in bilirubin metabolism with cholestasis (6%), the rest – subclinical cases (18%). Chronic hepatitis frequency with highest activity was the greatest in one (84%) and three (79%) groups. In these both groups, the disease activity was associated with replication of HBV, however, HCV had no significant influence on the disease course. Clinic and laboratory changes were less expressed in patients with integrative type of the HBV-infection (group 2): the frequency of chronic hepatitis with high activity was 31%. The cases with expressed clinical signs of hepatitis were observed also in the four groups of patients, in spite of absence of viremia, 7%. Probably, it is connected with hepatic injury by other factors, such as autoimmune antibodies, circulating immune complexes induced by viruses. Using of the complex analysis of clinical, serological dates and viral load was distinguished the following forms of chronic dual (B, C) hepatitis: (1) hepatitis with HBV–HCV-replication and high disease activity (HBsAg+ HBeAg+ HBV-DNA+ AntiHCV+ HCV-RNA+); (2) monoreplicate forms – clinical changes were less expressed; often: HCV-viremia and integrative type of HBV-infection (HBsAg+ HBeAg– HBV-DNA– AntiHCV+ HCV-RNA+); (3) HBV–HCV hepatitis with no signs of any virus replication (HBsAg+/– HBeAg– AntiHBc+ HBV-DNA– Anti-HCV+ HCV-RNA–); usually this form was subclinical, rarely observed, so-called postviral HBV–HCV induced autoimmune chronic hepatitis.

P1637 Serum iron, total iron binding capacity and ferritin levels in chronic hepatitis B and C patients

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Objective: This study has been planned to evaluate if there is any change in iron metabolism of chronic hepatitis B and C patients.
Methods: The level of serum iron, total iron binding capacity (TIBC) and ferritin levels together with liver enzymes in 260 chronic hepatitis B and 30 chronic hepatitis C patients. Control group consists of 80 people. Ferritin measurements were held with the Spectria Irma Test based on immunoradiometric assay principles. The levels of TIBC and serum iron were determined by DMA iron procedure at Olympus auto-analyzer.
Results: Patients with chronic hepatitis B have lower serum iron and ferritin levels and high iron binding capacity when compared to healthy control groups ($P < 0.05$). But chronic hepatitis C patients have higher serum ferritin and serum iron levels with normal iron binding capacity when compared to controls. No significant difference was observed between chronic hepatitis C patients and control groups.
Conclusion: In chronic hepatitis B and C patients, iron metabolism changes are reported in the literature. As in our cases serum iron and ferritin levels are reported to decrease. In chronic hepatitis C patients, serum iron and ferritin levels increase but there is no significant difference. Iron metabolism changes should be monitored and when necessary further investigations such as iron staining of liver biopsy specimens should be considered.

P1638 Extrahepatic disorders associated with chronic hepatitis B and C virus infections. A retrospective analysis of 435 patients

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Objectives: Chronic B and C hepatitis is a global public health problem in the world. The incidence of both infections seem to increase in both developed but mainly in developing countries. In Turkey, despite all recommendations and preventive controls hepatitis B and C virus infections still have a 5–7 and 3% incidence, respectively. Recent studies show a strong suggestion of hepatitis B and C can go together with various extra-hepatic diseases such as diabetes, autoimmune diseases, etc. We evaluated the incidence of both clinical and laboratory findings of extra-hepatic diseases associated with chronic hepatitis B and C virus infected patients.
Methods: In the study, 400 chronic hepatitis B and 35 chronic hepatitis C patients followed in outpatients clinic of Ankara University Ibn-i Sina Hospital Infectious Diseases Department between June 2000 and September 2001. Results were evaluated retrospectively. Patients were followed with 6 months' intervals for activation of the diseases. The prevalence of rheumatologic disorders (rheumatoid arthritis, systemic lupus erythematosus, Sjögren disease, etc.), hematologic disorders (iron deficiency anemia), endocrinologic disorders (diabetes mellitus, hypothyroidism or hyperthyroidism) were examined.
Results: The most common extrahepatic clinical manifestations observed frequently in both groups were diabetes mellitus, thyroid diseases, rheumatoid arthritis. Iron deficiency anemia and chronic disease anemia was observed only in chronic hepatitis B patients. There were no significant difference between chronic B and C hepatitis groups.
Conclusion: The results demonstrates the relationship between extrahepatic manifestations of chronic B and C hepatitis patients. These patients need further investigations for extrahepatic disorders and if necessary they also need therapies in order to prevent probable serious complications.

P1639 The presence of serum auto antibodies in patients with chronic hepatitis B and chronic hepatitis C

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Objective: Several auto antibodies can be seen in the sera of patients with chronic hepatitis B and C infection. These auto antibodies may be due to interferon therapy, accompanying autoimmune disorder and cross reactions but commonly due to primary disease itself. We aimed in our study to evaluate the presence and the titer of several autoantibodies including RF (Rheumatoid Factor), ANA (Antinuclear Antibody), Anti Sm (Smooth muscle), Anti LKM (Liver-Kidney-Muscle), Anti cardiolipin (ACA) IgM and IgG, anti TPO and Anti Tg in the serum of patients with Chronic hepatitis B and C patients.
Methods: Serum was obtained from 300 chronic hepatitis B (178 male, 122 female) and 30 chronic hepatitis C (19 male, 11 female) patients. The presence and titer of serum autoantibodies were detected by IFA and ELISA methods.
Result: No significant difference was detected between groups with autoantibody positivity. Extrahepatic manifestations and autoantibodies are frequently observed in chronic hepatitis B and C patients. The most frequent immunologic abnormalities include ACA IgG, ACA IgM, AntiTPO, RF, ANA postitivity in chronic hepatitis B group whereas ACA IgG and RF positivity in chronic hepatitis C group.
Conclusion: Autoantibody positive patients should be closely followed up for developing extra-hepatic manifestations such as autoimmune disorders when compared with autoantibody negative chronic hepatitis B and C patients.

P1640 Transfusion transmitted virus (TTV) infection in patients with hepatitis B or C

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Objectives: In 1997, the genome of a novel DNA virus, termed TTV, isolated from sera of patients with post-transfusion non A-G hepatitis, and was sequenced by representational difference analysis. TTV is an enveloped, circular, single-stranded DNA virus and comprises 3852 nucleodites. The TTV genome has three possible open reading frames, capable of encoding 770, 202, and 105 amino acids, respectively. TTV most closely resembles members of Circoviridae family. Many studies demonstrated that TTV viremia is frequent in general population and that is a lack of significant liver damage. On the other hand, TTV sequences were detected in sera and liver tissues from patients with liver disease, suggesting that TTV could be responsible for liver disease. We investigated the prevalence of TTV in patients with cirrhosis and/or chronic hepatitis B or C and also in cases of hepatitis of unknown etiology. Influence of TTV superinfection on hepatitis B or hepatitis C was also analyzed.
Methods: The sera from blood donors and from patients with different form of hepatitis were tested for the presence of TTV DNA, HBV DNA and HCV RNA by PCR systems. TTV DNA was detected by seminested PCR using primers from open reading frame 1. HCVRNA was evaluated by nested PCR with primers directed to the highly conserved 5′ noncoding region of the HCV genome.
Results: TTV DNA was detected in sera:
- from 5 out of 33 (15.2%) patients with chronic hepatitis B or C;
- from 3 out of 11 (27.2%) patients with cirrhosis B or C;
- from 1 out of 5 (20%) patients with cirrhosis C or B together with alcoholic cirrhosis;
- from 3 out of 12 (25%) patients with hepatitis of unknown etiology;
- the prevalence TTV was comparable with normal blood donors (13.3%).

In case of TTV, superinfection in hepatitis B or C cases, mean values of ALT were the same as in cases of non-superinfected patients (ALT in patients only with HBV infection was 66.6 IU/L; in patients with HBVand TTV infections were 90.3 IU/L; ALT in patients only with HCV infections was 72.8 IU/L; and in patients with HCV and TTV infections was 68 IU/L.
Conclusions:
- TTV does not seem to influence the clinical course HBV or HCV infected patients.
- TTV may play a role in the development of acute and chronic liver disease of unknown etiology.

P1641 Investigation of chronic viral hepatitis, of HBV and HCV and CA 19–9 serum level

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The frequent causes of chronic viral hepatitis are mainly HBV and HCV. Several tumor markers have been used for the diagnosis of malignant

diseases. False positive results are a common event in many non-neoplasmatic diseases.
Objective: The aim of our study was to investigate the chronic viral hepatitis for HCV and HBV and the serum level of tumor marker CA 19–9 in the patients with chronic hepatitis.
Materials and methods: A total of 51 patients of our hospital with chronic viral hepatitis were included in this study. We detected the antibody to hepatitis C virus with microparticle enzyme immunoassay (AXSYM, hepatitis C virus Encoded Antigen MEIA, ABBOTT) and the HbsAg, HbeAg, anti-HBc, anti-Hbe, anti-HBs, anti-HBc IgM with microparticle enzyme immunoassay (AXSYM, MEIA, ABBOTT). Additionally, the patients underwent liver's biopsy and we detected the serum levels of transaminases. CA19–9 was detected using microparticle enzyme immunoassay (AXSYM, CA 19–9 MEIA, ABBOTT).
Results: Fifteen (29.4%) of 51 patients had chronic hepatitis B and 36 (70.6%) chronic hepatitis C. Liver's biopsy proved the diagnosis and the levels of transaminases were higher than double of the upper normal limit. In 8 out of 51 patients (15%), the levels of CA 19–9 were raised 2–6 times the upper normal limit. Complete examination to detect neoplasmatic disease and attendance for a 3-year period, was negative.
Conclusion: (1) The causes of the chronic viral hepatitis are mainly hepatitis C and B viruses. (2) Chronic hepatitis B or C can cause false positive results for the tumor marker CA 19–9.

P1642 The significance of cryoglobulin levels in patients with chronic hepatitis B and C virus infection

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Objective: One of the extra-hepatic manifestation of chronic hepatitis B and C infection is mixed cryoglobulinemia. Typical presentation of cryoglobulinemia consists of purpura, neuropathy and arthralgia. Cryoglobulins can also be discovered in asymptomatic chronic hepatitis C and B patients with an incidence ranging between 11–54 and 15%, respectively. We tried to find the importance of cryoglobulin levels in patients with chronic hepatitis B and C infection in the study. Association between cryoglobulin levels and clinical symptoms is also discussed.
Methods: A total of 300 cases of chronic hepatitis B (178 male, 122 female) and 30 chronic hepatitis C patients (19 male, 11 female) were included in the study. Cryoglobulin levels were detected.
Results: The Study showed that cryoglobulin levels are low in both chronic hepatitis B and C patients in Turkey when compared with the results in the literature. Mixed cryoglobulinemia is more frequent in chronic hepatitis C patients than chronic hepatitis B group (16.6 and 4.6%, respectively). The most common symptoms with cryoglobulinemia were weakness and arthralgia in both groups.
Conclusion: The presence of positive results is not associated with extra-hepatic manifestations in our cases. The clinical significance of positive cryoglobulin level remains unclear.

P1643 Cytomegalovirus coinfection in patients with chronic hepatitis type B and C

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Objectives: The aim of the study was to evaluate the incidence of cytomegalovirus (CMV) infections in patients treated for chronic hepatitis type B and type C.
Methods: Fifty-two patients without any symptoms of CMV infection were randomly selected. IgM and IgG anti-CMV were examined in blood serum by ELISA tests. Anti-CMV IgM(+) was treated as confirmation of the active infection. There were no assessment of the primary infection or the reactivation of a latent one.
Results: A total of 23 patients were infected with HBV, 28 with HCV and one patient with both HBV and HCV. Twelve patients with chronic hepatitis type B were treated with interferon-α (IFN-α) and lamivudine (LAM). Eleven patients with chronic hepatitis type B and one with HBV and HBC infections did not receive any antiviral therapy. Seventeen patients with chronic hepatitis type C were treated with combination therapy of IFN-α and ribavirine; 11 patients were not treated. Anti-CMV IgM were detected in one treated and in two untreated patients with hepatitis type B, and in two treated and in three untreated patients with hepatitis type C. Anti-CMV IgG were detected in nine treated and 10 untreated patients with chronic hepatitis type B and in 15 treated and in nine untreated patients with hepatitis type C; it proves the 80% incidence of CMV infection in the population.
Conclusion: The results confirm the frequent infection of CMV (80%) in the patients' population. The incidence of active CMV infection was observed in three patients treated due to the chronic hepatitis type B and in five patients with chronic hepatitis type C without antiviral therapy. The results of this study with the CMV infections should be confirmed will the use of PCR techniques.