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# Scientific Abstracts – Invited Presentations

# Keynote lectures

## Recent advances in treatment of nontuberculous mycobacteria diseases

Hsueh PR[1]

[1]*Divisions of Clinical Microbiology and Infectious Diseases, Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan*

The isolation rate of nontuberculous mycobacteria (NTM) species and the prevalence of NTM-associated diseases are on the rise worldwide; however, the species distribution of NTM isolates and the types of diseases caused by NTM species vary from region to region. Treatment of a NTM disease is complicated and there is no comprehensive guideline regarding the in vitro susceptibility of each antimicrobial agent against NTM. Therefore, appropriate anti-NTM treatment can only be recommended based on individual NTM species and local surveillance studies of anti-NTM resistance. Previous studies on the in vitro susceptibility of Mycobacterium avium complex (MAC) to clarithromycin in some Asian countries have revealed a low rate of resistance to that antimicrobial agent. Thus, a clarithromycin-based anti-MAC regimen should be effective for MAC infections. However, clarithromycin resistance due to the mutation of the 23S rRNA gene in MAC strains has been detected in many countries. Therefore, physicians should avoid monotherapy with clarithromycin and consider the possibility of clarithromycin resistance in patients who do not respond to clarithromycin-based regimens. Rifampicin is the critical component of successful management of Mycobacterium kansasii diseases. Although most M. kansasii isolates are susceptible to rifampicin in western countries and in Japan, this agent may not work well in Taiwan. Rapidly growing mycobacteria (RGM) is a prevalent NTM group worldwide, particularly in Asia; however, each NTM species in this group may have its own distinct antibiotic susceptibility pattern, and close monitoring of the antibiotic-resistance patterns of RGM is necessary. Most important of all, the in vitro susceptibility may not represent the in vivo activity until the confirmation of the clinical study. Therefore, further investigation of the clinical effectiveness of the anti-NTM agents is warranted.

# Epidemiology of *Clostridium difficile* infection in Slovenia and other regions of SE Europe

Rupnik M

*National laboratory for health, environment and food (NLZOH), Maribor, Slovenia*
*University of Maribor, Faculty of Medicine, Maribor, Slovenia*
*Centre of Excellence Cipkebip, Ljubljana, Slovenia*

## 1. Introduction

*Clostridium difficile* is currently the most common reported pathogen in health care –associated infections in the USA (Magill, 2014). In European point prevalence survey of healthcare associated infections in acute care hospitals (ECDC, 2013) *C. difficile* was ranked as eighth most common pathogen, but high increase was noted since the last point prevalence survey.

Although CDI is often associated with age >65, hospitalization and prior antibiotic use, the recent new trends in epidemiology include increase in community CDI, emergence in younger populations without prior hospitalization and antibiotic use and emergence in populations with underlying diseases.

PCR ribotyping is the method of choice for further differentiation of the strains and at the time more than 350 PCR-ribotypes are recognized. Certain PCR-ribotypes (027, 078, 018, 053) are more often associated with severe disease and with outbreaks (Bauer et al., 2012). In particular PCR ribotype 027 (BI/NAP1/027) has spread worldwide since 2003 and has received much attention.

## 2. CDI in Slovenia

CDI situation used to be stable in Slovenia until the year 2008. At that time the number of reported CDI cases has started to increase from 39 (year 2008) to 266 cases in year 2012. Ribotyping is available in a single centre in Slovenia and comparison of typed strains with number of reported cases clearly indicates that the infection is still underreported.

In 2012 all laboratories performing *C. difficile* diagnostics in Slovenia have participated in large national study. In four months time interval (including two winter and two summer months) altogether 253 strains were collected and were distributed into 42 different ribotypes. However, the majority of strains belonged to only two ribotypes: 027 (43% of all strains) and 014/020 (17.7% of all strains). This national survey has shown for the first time the distribution of laboratory confirmed CDI cases and ribotypes in different Slovenian regions. Ribotype 027, which was first detected in Slovenia in year 2010 with only 16 strains, was in 2012 the most prevalent type on national level but was present only in single region.

In 2013 a point prevalence study on CDI was performed in five different hospitals. During a daily and a subsequent weekly sampling all diarrheic patients irrespective of the clinical diagnosis and independent of routinely ordered microbiological test were included in the study. Fecal samples were analyzed in a single laboratory and *C. difficile* was detected with culturing and molecular approaches. Hospitals/wards

included in the study had from 32 to 828 occupied beds per day. In daily sampling 44 diarrhoeic patients were found, six of them (13,6%) had toxigenic *C. difficile* and three had nontoxigenic *C. difficile*. Comparable results were obtained in weekly sampling. The percentage of diarrhoeic patients ranged from 0,6 % to 6,2% and the proportion of patients with toxigenic *C. difficile* was from 0,1% to 1%.

No data is available on community CDI in Slovenia. But in 2010 patients in two different wards in two different hospitals were screened at admission for colonization with *C. difficile*. In adult patients 11,5% (15 of 131) were colonized with *C. difficile* and strains were distributed into 11 ribotypes. In paediatric population 9,8% of patients (7 of 71) were colonized with *C. difficile* at admission and strains belonged to 4 ribotypes. This indicates high diversity of strains being introduced into the hospitals from the community.

## 3. CDI in other regions within SE Europe

Partial information for some countries within SE European region can be extracted from their participation in large European studies such as EUCLID (Davies K A et al., Second report from the EUropean, multi-centre, prospective bi-annual point prevalence study of CLostridium difficile Infection in hospitalised patients with Diarrhoea (EUCLID); Poster P0753, ECCMID 2014), ECDISNet (http://www.ecdisnet.eu/) and ECDC Point prevalence survey of health care associated infections and antimicrobial use (ECDC 2013). For some countries no published information is available at all. Publications for some others describe only the proportion of CDI within a single or a small number of hospitals (e.g. Turkey, Bosnia and Hercegovina). Only few countries have reported the *C. difficile* ribotypes (Croatia, Romania, Hungary, Bulgaria, Slovenia). Although publications describe the ribotype 027 only in Slovenia, Hungary, Austria and Romania (Jones et al., 2013; Rafila et al., 2014) our preliminary data indicate that this type is present also in some other countries. Therefore it seems that this particular ribotype has spread from Western Europe towards central and SE European regions.

## 4. Conclusions

The prevalence of CDI and distribution of *C. difficile* ribotypes is not well documented for SE European countries. However, the available data indicate that CDI is increasing and that ribotype 027 is present and/or associated with outbreaks also in this region.

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# Symposium lectures

Symposium 1: Hepatitis C: A challenging infectious disease

## HCV treatment in PWID: It works!

Matičič M[1]

*Clinic for Infectious Diseases and Febrile Illnesses, University Clinical Centre Ljubljana, Slovenia*

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Keywords: hepatitis C, people who inject drugs, treatment

### 1. Introduction

Hepatitis C virus (HCV) infection represents a major global health problem, which in developed countries mostly affects people who inject drugs (PWID). Injecting drug use is a huge public health problem around the world as almost 16 million people injected drugs in 2007. According to the European estimations from 2010 five million PWID are anti-HCV positive, with the yearly incidence of 5-45% (1). With 50-80% of PWID infected in the developed world, HCV is much more prevalent than either hepatitis B virus infection or HIV in this high-risk population. The existence of easily accessible testing and efficient and safe treatment for HCV has the potential to be successfully used in the majority of infected PWID.

### 2. Treatment of hepatitis C in people who inject drugs

Several studies indicate that HCV treatment outcomes for PWID are comparable to those for other patients in terms of adherence to treatment, completion of the full treatment course and attainment of a sustained viral response (SVR) (2). HCV treatment of PWID has proved to be safe and the risk of re-infection has been shown to be very low (1-5% per year). Moreover, modelling studies suggest that treatment of active PWID could control the burden of HCV in this population by decreasing the viral load among current drug injectors and reducing transmission rates. Besides, opioid substitution treatment (OST) has been shown to facilitate HCV treatment and reduce HCV acquisition among PWID. Early detection of HCV infection leading to early HCV treatment enables higher rate of sustained virological response therefore prevents HCV-related morbidity and mortality and has proved to be cost effective. Therefore, PWID should represent one of the most important target populations for treatment of HCV infection.

Unfortunately, HCV treatment in PWID has been controversial for decades and the treatment uptake in developed countries has been estimated only 3-4%. Historically, the international and national treatment guidelines have excluded PWID, particularly current injectors, from being treated for hepatitis C. Barriers to HCV treatment are present on patient (lack of knowledge, financial resources and fear of adverse effects and stigma), provider (concerns on serious side effects, adherence, high rates of concomitant alcohol abuse, mental health issues, and the risk of re-infection) and system level (no national strategies, action plans and

treatment guidelines). Another barrier is the setting for HCV treatment which needs to handle different needs in this vulnerable population group.

Since the cohorts of non-treated PWID with chronic hepatitis C are ageing they represent a significant proportion of patients with advanced liver disease and liver-related mortality. Twenty to 25% of deaths among PWID are related to the latter. Besides, daily use of cannabis may be associated with advanced liver fibrosis and heavy alcohol consumption which is commonly present in PWID is associated with a higher risk of cirrhosis. Therefore, the 2014 Clinical Practice Guidelines of the European Association for the Study of the Liver (EASL) consider substance users as a treatable patient group at risk provided they wish to receive treatment and are willing and able to maintain follow-up visits on an individual basis, preferably using a multidisciplinary approach (3).

## 3. Management of HCV infected PWID in European countries

The extremely varied political, economic and social circumstances of different European countries lead to very different health needs and health outcomes at the national level. HCV infection shows great diversity among European countries not only in its prevalence in the general population and among PWID, but also in the treatment of hepatitis C. Treatment funding, availability of anti-HCV drugs and the settings for HCV treatment in PWID vary immensely among the European countries. Policies, strategies and guidelines for HCV management therefore must be tailored to the specific national or sub-national context. In a recent study of 33 European countries data on available national strategy, action plan and guidelines for HCV treatment in general population and in PWID specifically were collected prospectively from mostly non-governmental organizations and public health institutions by means of a structured electronic questionnaire and analyzed accordingly (4). Fourteen (42.4%) countries reported on having national strategy and/or national action plan for HCV treatment, from which 10/14 also reported national strategy and/or national action plan for treatment of HCV infection in PWID. Nearly three-quarters (24/33 or 72.7%) reported to have national HCV treatment guidelines, in the majority (22/33 or 66.7%) PWID were included into the guidelines. Fourteen (42.4%) countries reported to have separate guidelines for treatment of HCV infection in PWID. Almost half of the countries involved in this study reported on having separate guidelines for HCV treatment in PWID on OST.

Settings for the management of drug use and for the treatment of HCV infection in PWID vary immensely among the European countries. Therefore, various models have been proposed for HCV treatment in PWID to be performed inter-disciplinarily in either community-based clinics, drug treatment settings, viral hepatitis clinics/hospitals or being integrated into the primary/secondary/tertiary medical setting. Countries should decide on the most appropriate model for their local facilities.

## 4. Treatment of HCV infected PWID in Slovenia

Slovenia provides an example of a multidisciplinary model of good practice that was introduced through a national strategy, action plan and clinical guidelines.

Within a two million population, approximately 10 000 PWID, with an estimated HCV seroprevalence of 23.4% (the second lowest in Europe); HIVco-infection also is extremely rare, the HIV seroprevalence among PWID consistently remaining under

1%. In 1995, 18 Centres for the Prevention and Treatment of Drug Addiction (CPTDA) were founded, managing approximately 4,500 PWID yearly, three quarters of whom receive OST. The CPTDA provide HCV testing to PWID entering the programme, and an offer of 6 to 12 months of testing for HCV-negatives. In the first national study evaluating the HCV treatment rate among PWID managed by 18 CPTDA in 2006, the prevalence of HCV RNA among 1,450 PWID was 15.6%, but only 3% of infected have received HCV treatment by the time of the study. The extremely low treatment rate among HCV-positive PWID urgently called for action.

HCV treatment in Slovenia is generally delivered by infectious disease specialists at five hospital-based clinics. This is fully funded by the health insurance system with no limitations except that treatment must be prescribed by approved infectious disease specialists (and hepatologists in case of advanced liver disease) according to the guidelines established by the National Viral Hepatitis Expert Group in 1997, not excluding PWID from HCV treatment.

In 2007, a national multidisciplinary healthcare network for the treatment of HCV infection in PWID was established, regionally integrating the existing medical settings of 18 CPTDA and five specialised clinics for treatment of viral hepatitis. The multidisciplinary network team includes clinical care providers (addiction therapists and infectious disease specialists), psychiatrists and councillors (nurses, social workers) who have undergone additional medical education and training, and peers (former HCV-positive PWID) and other supportive systems (family, friends, co-workers, etc.).

Accordingly, national consensus guidelines for the management of HCV infection in drug users were developed in 2007, providing procedures for the complex management of HCV-infected PWID, including improvements in screening for HCV treatment-eligible PWID, highly qualified education, discussion, and motivation-enhancing techniques individually tailored by addiction therapists, and referral to infectious disease specialists for the treatment of HCV according to the best standard of care (detailed pre-treatment optimisation, individualised HCV treatment plan, on-treatment optimisation with aggressive management of treatment side effects) with at least monthly interventions performed individually in close cooperation with an addiction therapist throughout the treatment period (5).

Analysing the database of all Slovenian patients that have ever been treated for hepatitis C since 1997, in the first few years of follow up the proportion of those who reported intravenous drug use as a risk factor for infection was 5% (1997-1999), 16% (1999-2001) and 36% (2002-2004), respectively. After the introduction of the national multidisciplinary healthcare network in 2007, among all the patients in Slovenia, treated for HCV during the following period 2008-2010, the proportion of those who reported intravenous drug use as a risk factor has significantly increased to 78%, compared to previous period (6). The HCV treatment rate of infected PWID in CPTDA has increased from 3% in 2006 to 13% in 2010. A 95.7% HCV treatment adherence rate and an all over sustained virological response of 82% followed by significant improvement in certain lifestyle variables and a major decrease of drug use and OST compared to pre-treatment condition justify the use of the multidisciplinary network model in Slovenia.

## 5. Conclusion

Given the huge burden of HCV-related morbidity and mortality in PWID management of HCV infection should become one of the healthcare priorities in all European countries starting with setting up or precise using already existing national strategies, action plans and guidelines for this vulnerable population. With several new direct acting antivirals on the horizon, there is hope that HCV infection can be cured in the majority of patients in the nearest future. However, the most highly potent regimens may remain under-utilised until the group at greatest risk for HCV infection is recognized as urgently needing access to this treatment.

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Symposium 2: Highlights of recent advances in clinical microbiology

# Tuberculosis and molecular epidemiology – old and new challenges

Žolnir-Dovč M, Svetina P, Bidovec Stojković U

*University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia*

## 1. Introduction

Although tuberculosis (TB) is a curable disease, it still causes 1.4 million deaths every year. TB is usually transmitted from person to person by airborne droplet nuclei generated by coughing of the persons with active disease. Therefore, rapid and reliable identification of infectious individuals, early beginning of treatment with appropriate drugs, accurate and reliable contact tracing are very important for TB surveillance and essential components of any tuberculosis program. For this purpose, molecular epidemiology and various molecular genotyping methods have been established, which enabled significant progress in understanding of *Mycobacterium tuberculosis* transmission in the last 25 years.

## 2. Typing methods and molecular epidemiology

There is a wide range of methods available for genotyping of *Mycobacterium tuberculosis* (MT). First typing methods were phenotypic. In 1963, Schuitemaker developed a way to differentiate between MT isolates by exposure to a predefined selection of bacteria-lysing viruses **(bacteriophages)**. This method had a very low discriminatory power. Another phenotypic method, **anti-TB drugs susceptibility profiling** in combination with other bio-chemical features, allowed a very limited subdivision of MT isolates. This technique was never widely used for typing purposes, because its discriminatory power is limited to drug resistant strains only and many resistant strains have the same resistance profile.

In the 1980s, under the influence of advances in molecular biology, DNA-based typing methods were developed, whose discriminatory power was improved compared to phenotypic methods. RFLP IS*6110*, spoligotyping and MIRU VNTR are the most commonly used techniques for genotyping tubercle bacilli. Different techniques have different roles in phylogenetic analyses and epidemiological person-to-person transmission studies. Each method has advantages and disadvantages; none of them is ideal. The choice of the optimal typing depends on the sample under investigation, the setting in which typing is performed, and the expected outcome.

*Restriction fragment length polymorphism* - **RFLP IS*6110*** was first widely used DNA-based (also called DNA fingerprinting) technique for discrimination of tubercle bacilli at the end of the 20$^{th}$ century. This technique is based on discrimination of number and position of IS*6110*, a repetitive mobile insertion sequence (IS) element in the genome of MT. The method has a very high discriminatory power, but a special software is required to compare the results. Important disadvantages of the method include problems with reproducibility and time needed to perform the test. Therefore, in 2006, **24-locus MIRU-VNTR typing** was proposed as a new gold

standard for typing of MT isolates. This is a multiplex PCR-based technique performed on an automated, fluorescence-based sequencer. The final result is a 24-digit code (the so-called MIRU-VNTR code), easy to compare and with almost similar discriminatory power as that of RFLP IS*6110*.

The third frequently used genotyping technique is **spoligotyping** which serves for both strain typing and phylogenetic analyses. Spoligotyping is PCR-based, fast, relatively simple, cost-effective, reproducible method, giving a binary (presence/absence) result, but with the lowest discriminatory power among all three genotyping techniques.

Current DNA fingerprinting methods are not without limitations. Although they target especially polymorphic genetic regions, they interrogate less than 1% of the genome. Consequently, they are often incapable of distinguishing between genetically closely related isolates of tubercle bacilli, for instance between the isolates from an outbreak. Therefore, in the last years, the application of **whole-genome sequencing (WGS)** is sometimes used to clarify recent outbreaks. First studies on outbreak clones showed that WGS provides higher resolution than classical genotyping methods and correlates better with contact tracing information. Due to high price, this technique is currently useful for clarifying large longitudinal MT outbreaks only. With the decreasing sequencing costs and elimination of standardization problems in the next years, WGS has the potential to become the ultimate tool for TB genotyping.

## 3. Usefulness of molecular genotyping methods

In the epidemiology of TB as a typical infectious disease that is transmitted through the air, the key issue is to define the source of infection and to disclose the routes of transmission. Such an inquiry needs a good tool. DNA-based techniques stand out as an excellent tool which has revolutionized the epidemiology of TB, as they allow querying the genome which is relatively stable for each isolate.

Molecular genotyping methods have been successfully applied in many phylogenetic and epidemiological investigations of tubercle bacilli. With their assistance, answers to a wide variety of epidemiological research questions were found:

- metropolitan or country-wide population-based analysis,
- investigation of TB outbreaks,
- international transmission of drug resistant TB,
- discrimination between endogenous reactivation and exogenous re-infection,
- high risk population groups (alcoholics, homeless population),
- the evidence of multiple MT infections,
- survey of laboratory cross-contaminations.

## 4. Genotyping and molecular epidemiology in Slovenia

In a number of developed countries, including Slovenia, molecular epidemiology has become an important part of TB surveillance and important tool of national TB programs. In our country, RFLP IS*6110* was used nationwide from 1999 until the end of 2009. In 2007, standard 24-locus MIRU VNTR typing was introduced and

replaced RFLP IS*6110* in 2009. The use of this faster, PCR-based technique greatly reduces the time required to identify clusters and 24-digit code simplified reporting to the Slovenian Registry for Tuberculosis. Molecular typing methods in connection with data of classical epidemiology (molecular epidemiology) have increased our knowledge and understanding of TB transmission. Before the era of molecular epidemiology, it was not readily recognized that in our country: (i) we have a high degree of recently transmitted TB, (ii) alcohol abuse is one of the most important risk factors for clustering, (iii) pubs are the most important places for transmission of tubercle bacilli, (iv) a high number of unsuspected microepidemics have been revealed, (v) a non-cooperative patient or a patient with the delay in the diagnosis can cause an outbreak of large extent, (vi) the transmission of tubercle bacilli in Slovenian hospitals by airborne droplet nuclei and by endoscopes is possible, (vii) laboratory cross-contamination happens also in our laboratories. All these new findings and knowledge were immediately incorporated into our everyday practice and into national TB program. Consequently, we have:

- introduced separate TB wards with negative pressure rooms for treatment and diagnosis of TB,
- used more strict DOTS in hospitals and in extension phase of treatment at home,
- accelerated and improved TB diagnosis,
- diagnosis by bronchoscopy was largely replaced by induced sputum,
- supplemented contact investigations,
- introduced checking of all positive cultures on possibility of laboratory cross-contamination.

Renovation of National TB Program in 1996 and implementation of molecular epidemiology in the national program coincided with a substantial decrease of TB incidence in Slovenia from 22.1/100.000 in 1999 to 6.7/100.000 inhabitants in 2012.

## 5. Conclusion

For thousands of years tuberculosis has remained, and even now in the 21$^{st}$ century it still remains one of the most challenging and interesting infectious diseases which still causes more adult deaths than any other infectious disease. To have a tool for discrimination between MT isolates was the great desire of many TB researchers in the past, which was realized with the development of molecular biology at the end of the last century. Many DNA fingerprinting methods have been developed for phylogenetic and epidemiological purpose and many molecular studies have been published in the last 25 years. Molecular methods have brought significant insight into the epidemiology of TB and radically changed the understanding of TB transmission. In many regions of the world, molecular epidemiology has become a part of TB surveillance and is an important tool of national TB programs.

Molecular-guided TB control was adopted years ago also in Slovenia, when nation-wide molecular epidemiological studies identified the most important risk factors and new routes of TB transmission. Implementation of national TB program with molecular epidemiology coincided with a substantial decrease of TB incidence in our country in the last years.

RFLP IS*6110*, spoligotyping and 24-locus MIRU VNTR are today the most commonly used techniques for genotyping tubercle bacilli which interrogate only about 1% of MT genome. Therefore, they have restricted discriminatory power. In the last four years, WGS was tested in some studies which revealed that WGS has the potential for more accurate molecular genotyping and consequently improved molecular epidemiology and surveillance of TB in the near future when some of its shortcomings will be eliminated.

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# Epidemiology of candidemia in central region of Slovenia from 2001 - 2012

Matos T, Pirš M

*Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia*

*Candida spp.* are the most important causative agent of human invasive fungal disease. The aim of our study was to analyse epidemiology of *Candida* bloodstream infection (BSI) and to determine local antimicrobial susceptibility of BSI isolates of *Candida spp.* in the two tertiary centres in Central Slovenian Region in 12-year period. The study was approved by the Republic of Slovenia National Medical Ethics Committee.

## 1. Methods and materials

Patients with at least one *Candida* BSI who were treated at University medical centre Ljubljana or Institute of Oncology between 1.1.2001 and 31.12.2012 were included in the study. The data on underlying host factors and risk factors for *Candida* BSI, antifungal treatment and the outcome (30-day mortality) were collected retrospectively from patient records using a structured questionnaire. The total number of Candida BSI isolates was retrieved from laboratory information system at Institute of microbiology and immunology, Faculty of Medicine Ljubljana. Successive *Candida spp.* blood cultures from a patient were considered to be distinct episodes if they occurred 21 days or more apart or were caused by different species. While full clinical information was not available for all patients, the data were obtained for 344 of the 467 episodes, microbiological data were available and analysed for all episodes. During the study period there were no changes in microbiological laboratory techniques. Most departments used the BacT/Alert blood culture system (bioMerieux, Marcy l'Etoile, France), and one used the Bactec blood culture system (Becton Dickinson, Franklin Lakes, NJ). The blood culture bottles were cultivated in total for 10 days. Antimicrobial susceptibility testing was performed using gradient-diffusion test using E-test according to manufacturer's instructions (BioMerieux, Marcy l'Etoile, France). The results were interpreted according to valid CLSI (Clinical Standards Institute) breakpoints with the exception of amphotericin B. Statistical analysis was performed using statistical software IBM SPSS Statistics version 20.0 (IBM corp., NY, USA). We used Mann–Whitney U-test to compare continuous variables and Fisher's exact test or chi-squared test for categorical variables.

## 2. Results

During the 12-year period 467 episodes of *Candida* BSI were identified in 422 patients with average incidence of 0.568/10.000 patient-days. The incidence of candida BSI was increasing from 0.230/10.000 patient-days in 2001 to 0.844/10.000 patient-days in 2009. In the last three years of the study (2010-2012) the incidence of *Candida* BSI was slightly lower with 0.746/10.000 patient-days. Majority of patients had a single episode of candida BSI, in 18 (4.3 %) multiple episodes of *Candida* BSI occurred. In 25 (5.6 %) multiple *Candida spp.* were isolated during an episode; most commonly *C. albicans* and *C. glabrata* were isolated simultaneously

(17 patients, 68.0 %). The most commonly isolated species were *C. albicans* (60.0 %) and *C. glabrata* (22.0 %), followed by *C. parapsilosis* 8 %, *C. tropicalis* 4 % and *C. krusei* 2 %; the remainder of isolates (4 % in total) were *C. kefyr*, *C.utilis*, *C. dubliniensis*, *C. lusitaniae* and *C. pelliculosa*. We did not note any changes in proportion of *C. albicans* and *C. glabrata* over the 12-year period.

Among the 422 patients 250 (59.2 %) were male and 172 (40.8 %) were female. Sixteen (3.8 %) were younger than 1 year, 20 (4.7 %) were aged between 1 and 20 years, 35 (8.3 %) were aged between 21-40 years, 101 (23.9 %) were aged between 41 and 60 years, 214 (50.7 %) were aged between 61-80 years and 37 (8.8 %) were older than 80 years.

Antimicrobial susceptibility to 4 systemically active antifungal agents was determined. The rate of susceptibility to fluconazole was 99.6 % (MIC90 0.5 mg/l) for *C. albicans*, 97.3 % (MIC90 4 mg/l) for *C. parapsilosis* and 100 % (MIC90 1 mg/l) for *C. tropicalis*. Decreased susceptibility to fluconazole was mostly seen as expected with *C. glabrata* (14.3% in susceptible range and 37.8% in a dose-dependent range, SDD; MIC90 256 mg/l). The rate of susceptibility to voriconazole was 100% for *C. albicans*, *C. tropicalis* and *C. parapsilosis*; C. *glabrata* was susceptible in 49.5 % of the cases with MIC90 4 mg/l. The rate of susceptibility to caspofungin was 99.4 % (MIC90 0.25 mg/l) for *C. albicans*, 97.1 % (MIK90 0.38 mg/l) for *C. glabrata*, 100% (MIC90 0.25 mg/l) for *C. tropicalis* and 100% for *C. parapsilosis* which demonstrated higher MIC than the rest of the isolates (MIC90 1.5 mg/l). MIC90 for amphotericin B in *C. albicans* was 0.5 mg/l and 0.75 mg/l for both *C. parapsilosis* and *C. tropicalis*. MIC90 for *C. glabrata* was 1.5 mg/l. The data for newer echinocandins, anidulafungin and mycafungin, are not shown as a relatively small number of isolates were tested.

Clinical information was obtained for 344 episodes, some of the clinical data was missing for some patients which was taken into account when proportions were calculated. Among underlying host factors the most frequent was malignant disease in 129 (37.7 %), among those patients 90 (69.8 %) had solid cancer and 39 (30.2 %) had hematologic disease. Second most common underlying host factor was diabetes mellitus (58 patients, 17.0 %), followed by chronic pulmonary disease (25 patients, 7.3 %), alcohol abuse (21 patients, 6.1 %) and liver cirrhosis (17 patients, 5.0 %). Twelve patients (3.5 %) had rheumatologic or connective tissue disease and nine (2.6 %) had an organ transplant. Among children below 1 year of age, 6 (37.5 %) were neonates or preterm neonates.

Majority of the patients (332, 97.4 %) had indwelling vascular catheters at the time of Candida BSI and were hospitalized in the ICU prior or during Candida BSI (253 patients, 74.2 %); 216 (63.9 %) received antibiotic treatment in 14 days prior to Candida BSI and 178 (52.4 %) were on parenteral nutrition. Almost a third of patients (124, 36.1 %) has surgery prior to Candida BSI, most frequently abdominal (60 patients, 48.4 %) and cardiovascular (25 patients, 20.2 %) surgery. Other notable risk factors were corticosteroid therapy (96 patients, 28.2 %) and chemotherapy (44 patients, 12.9 %); gastrointestinal disease (bowel necrosis or perforation, short bowel syndrome or other disease affecting oesophagus or bowel) was present in 72 patients (21.1 %); 37 patients (10.9 %) were neutropenic and 33 (9.7 %) experienced traumatic injury prior to Candida BSI (14, 42.4 were polytraumatic patients). Pancreatic and gallbladder disease was present in 14 (4.7

%) and 9 (3.5 %) respectively. Six patients (1.8 %) were intravenous drug users and six patients (1.8 %) experienced CMV infection or reactivation.

Concurrent bacteraemia was present in 75 (21.9%) of the 342 episodes, enterococci and coagulase-negative staphylococci were the most frequent isolates (both 19 patients, 5.6 %), followed by enterobacteria (15 patients, 4.39 %) and Staphylococcus aureus (5 patients, 1.75 %). Prior fungal isolates of the same species as BSI isolate were identified from other body sites in the 14 days prior to BSI in 157 patients (45.9 %), most frequently from lower respiratory tract (112 patients, 71.3 %), followed by urine (54 patients, 34.4 %).

In 246 Candida BSI episodes the antifungal treatment was initiated within 7 days prior to first positive blood culture. The treatment was adequate in 222 episodes (90.2 %). Fluconazole was prescribed most often (148 patients, 60.2 %), followed by echinocandins (74 patients, 30.1 %); the remainder of the patients received amphotericin B (15 patients, 6.1 %), voriconazole (8 patients, 3.3 %) and itraconazole (1 patient, 0.4 %).

Overall 30-day mortality data was available for 317 patients, crude 30-day mortality was 56.6 % (180 patients have died in the first 30 days following BSI). In 79 patients (43.9 %) Candida BSI directly contributed to death.

## 3. Conclusion

*Candida* BSI are a significant source of morbidity and mortality in patient populations at higher risk of infection. The incidence of *Candida* BSI in the two institutions in Central Slovenian Region has remain stable in the last three years (mean incidence 0.746/10.000 patient-days). The predominate causative agent is *C. albicans* (60.0 %), followed by *C. glabrata* with relatively large proportion of 20.0 %. Susceptibility of all species of *Candida* BSI isolates to caspofungin which is treatment of choice according to current European guideliness is excellent. As expected we have found significant proportion of *C. glabrata* to be non-susceptible to azole compounds (47.9 % of isolates non-susceptible to fluconazole and 50.5 % non-susceptible to voriconazole). Our results have demonstrated a high burden in the two hospitals in Central Slovenian Region with a high, 56.6 % crude 30-day mortality.

# MALDI-TOF MS in identification of anaerobes

Nagy E[1]

[1]*Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary*

Anaerobic bacteria predominate in the normal flora of humans and are important, often life-threatening pathogens in mixed infections originating from the indigenous microbiota. Clinically relevant anaerobic bacteria need special culture conditions and even in proper anaerobic environment they multiply more slowly than usual aerobic pathogens. Because of this relatively long time is needed for their isolation in pure culture and their identification is hindered also by the inactivity of many species in biochemical tests. There are difficulties to reach enough inokulum for their identification by the conventionally available phenotypic identification kits as well. Even DNA-based molecular methods are time-consuming and laborious for routine laboratories to identify these bacteria at a species level. Following the successful adaptation of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the routine laboratory identification of bacteria, the extensive development of the anaerobe database has been initiated to use this method for the identification of clinically relevant anaerobic bacteria. Several studies proved its better performance in routine identification compared with automated identification kits or classical methods. The possibility of this technique to be used successfully is highly influenced by the development of the database of the systems available. The aim of the ENRIA project supported by the ESCMID Study Group for Anaerobic Infections (ESGAI) and ESCMID Study Group for Epidemiological Markers (ESGEM) is to optimize the Bruker Biotyper database for anaerobic species identification. The participating laboratories from 6 European countries provide a great variety of clinically important anaerobic isolates for this purpose. Not only frequently isolated anaerobic species, but also newly recognized and taxonomically rearranged genera and species can be identified by using direct smear samples or whole cell protein extraction. Typing of anaerobic bacteria such as *Bacteroides fragilis, Propionibacterium acnes, Clostridium difficile* on sub-species level was carried out successfully. The clinical relevance of these typing results needs further evaluation. Determination of antibiotic resistance and direct identification of blood culture isolates will revolutionize anaerobe bacteriology in the near future. Several examples will be discussed during the lecture how MALDI-TOF MS can be used in routine anaerobic diagnostic laboratories.

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Symposium 3: European clinical trials' and laboratory network for bringing new anti-infectives into medical practice

# Healthcare associated *Staphylococcus aureus* infections: Epidemiology and prevention

Bode LG

*University Medical Center Utrecht, the Netherlands*

## 1. Healthcare associated infections

The aim of hospital admissions generally is to cure, or at least to support the patient, without doing any harm. Nevertheless, hospital admissions pose patients to the risk of complications of medical interventions. One of these complications is the development of healthcare associated infections. After urinary tract infections and lower respiratory tract infections, surgical site infections are the third most frequently reported healthcare associated infections. *Staphylococcus aureus (S. aureus)* is the leading cause of surgical site infections, accounting for approximately 20% of these infections. Bloodstream infections are also frequently caused by *S. aureus.*

Healthcare associated infections lead to increased morbidity, mortality, length of stay, and hospital costs. Societal costs rise as well, and consequences of infections for patients can be devastating, for example in the case of infection of prosthetic joint infections, due to permanent functional impairment.

## 2. Carriage of *S. aureus*

The human nose harbours many bacteria, predominantly gram-positive cocci.[2] Carriage of these bacteria is usually not of clinical importance. *S. aureus* however, is a more virulent organism than the other gram-positives. It colonizes the skin and mucosae of approximately 20-30% of the general population. The nose is the primary niche, but other body sites are frequently colonized as well.

In 1959, Weinstein reported that *S. aureus* nasal carriers are at increased risk of developing “infectious complications”, mainly attributable to *S. aureus*. A review of the literature in 1995 showed that the incidence of *S. aureus* wound infections in nasal carriers of *S. aureus* ranged from 5 to 19%, while in non-carriers, the reported incidence was 2-10%. Approximately 80% of healthcare associated infections (range 30-100%) are of endogenous origin, i.e. caused by the patient’s own *S. aureus* strain.

## 3. Prevention of healthcare associated *S. aureus* infections

Elimination of *S. aureus* nasal carriage can be achieved by a course of mupirocin, a topical antibiotic that has to be administered to the nose. This strategy also eradicates *S. aureus* from extranasal body sites, although less effective.

We aimed to prevent endogenous infections by decolonization of *S. aureus* carriers. In a randomized, placebo-controlled multicenter trial, patients were screened for *S.*

*aureus* carriage with rapid diagnostics upon admission. Carriers were treated with a five-day course of mupirocin nasal ointment and chlorhexidine gluconate medicated soap, or placebo ointment and placebo soap, starting within 24 hours after admission to hospital. The primary outcome was the incidence of healthcare associated *S. aureus* infections: these were prevented by almost 60% in the mupirocin/chlorhexidine group compared with the placebo group. Length of stay, one of the secondary outcomes, was reduced by 1-2 days.

Furthermore, we conducted an observational follow-up study, to compare long-term mortality between patients who received mupirocin/chlorhexidine and placebo. We compared mortality rates one and three years after admission in all surgical patients allocated to the intervention, and in different subgroups. Mortality was not reduced when all patients were taken together, but a significant reduction in one-year mortality was shown in patients who had undergone a clean procedure and had received mupirocin/chlorhexidine, as compared to these patients who had received placebo.

Finally, we compared hospital costs of cardiothoracic and orthopedic patients who were allocated to mupirocin and chlorhexidine in the RCT, to the costs of those who received placebo treatment. Total costs of care for patients who had received placebo were on average higher than the costs for patients who had received mupirocin and chlorhexidine.

## 4. Future research

Future research will focus on the identification of risk factors associated with the development of healthcare associated *S. aureus* infections, other than *S. aureus* carriage. In 2015, an observational study will start to describe the epidemiology of healthcare associated infections among intensive care unit patients in the European Union. Sites interested to participate are invited to contact the investigators (see WP6A at www.combacte.com).

Symposium 4: Infectious diseases in areas affected by war conflicts

## Infectious diseases among Iraqui and Syrian refugees in Lebanon

Suvada J

*St. Elizabeth University of Public Health and Social Work, St. Charles Foucauld Health-care Refugee Centre in Lebanon and Health Initiatives Association*

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Around 230 million people has crossed international borders for various reason. By the end of May 2014 official bodies estimate that through an exodus of Syrians to Lebanon 8% of estimated population size of Lebanon makes Syrian refugees and around 4% Iraqi refugees. These numbers reflect only registered refugees but situation is more complicated in the field. Both populations have different immunity and microbial background due to different antibiotic policies too. The refugees are the weakest and most vulnerable category in a conflict setting also from epidemic´s and source of infections´ point of view for rest of the community.

We have provided systematically clinical, social and microbiological examinations among patients with acute infectious diseases, those who came first time to the camp (refugees, visitors) and general screening of the general population of refugee camps.

Prevalence of communicable infectious diseases in two UNHCR camps in Beirut was assessed separately among children and adults population. Among adults were observed mainly upper respiratory tract infections, skin infections, soft tissue infections, urinary tract infections, lower respiratory tract and eye infections. Among children population were predominantly observed gastrointestinal (enteritis, gastroenteritis), upper and lower respiratory tract infections, soft tissue and skin infections, then otitis and parasitic infections as special group. There were observed measles, hepatitis as imported cases. Tuberculosis of the bones, kidney and one LTB were also very probably imported to camp also as a little unvaccinated boy with susp. poliomyelitis. Between spectre of infections with special interest were: an 23-years old adolescent with rabies, a newborn baby with tetanus. Then there were infections classified as imported group of special interest: one Zika virus and two cases with MERS-coronavirus visiting family in camp. An increasing prevalence of antimicrobial-resistant bacteria has become observed more often among refugees coming from Syria and among group of those who were coming as new refugees from larger Syrian cities and who had stop longer than 10 days in mountains near borders. The most prevalent resistance was observed in MRSA, ampicillin-resistant E. faecium. Gram-negative bacilli were mostly resistant to ampicillin and ampicillin-sulbactam, trimethoprim-sulphamethoxazol. There were observed also ESBL Enterobacteriacaeae and in some hospitalized patients also carbapenem-resistance, which was not detected among general population living in the camps.

Knowledge of local epidemiological situation of newly coming refugees will be beneficial but is unrealistic. The general screening of new refugees and antimicrobial resistance patterns of camps´ inhabitants is important for proper management and development of antibiotic policies. Travel history of refugees and

visitors is essential to protect population from new emerging and old infections with outbreak spread potential.

# Epidemiological situation and spectrum of diseases in migrants and internally displaced people during conflicts in South Sudan

Mamova A

*St. Elisabeth Univeristy - Tropical institute*

## 1. Background

Currently, there are several ongoing long lasting armed conflicts on the world and South Sudan is one of them. At the end of 2013, there were 132 000 internally displaced persons recorded by UNHCR, with the total concerned population of more than half a million people (UNHCR Global Appeal, 2014). These situations entail the influx of migrants from unstable countries to countries, where security situation is stable. Poor living conditions, malnutrition and stress contribute to the development of infectious diseases, which have a chance to spread in the susceptible population (Brzoska, 2014). Doctors of the University of St. Elizabeth operate in areas of conflicts with refugees and internally displaced populations. Clinicians encountering these patients have an essential role in recognizing, and communicating associated public health risks (Albuja, 2014).

## 2. Methods

To investigate the morbidity of war or conflict associated diseases in migrants and internally displaced people, we analysed diagnoses of 9414 ill patients who presented in 2013 to our clinics and hospitals in South Sudan.

## 3. Results

Malaria accounted for 27,1% of illnesses, followed by other respiratory diseases (21,4%), STIs (14,9%), diarrhea (6,8%) and intestinal parasites (5,5%).

Gastrointestinal conditions were divided to acute watery diarrhea (6,9%, n=1429) with presumed bacterial cause and intestinal parasites (5,5%, n=1157) where most common were giardiasis and intestinal amoebiasis. Among febrile systemic illnesses with identified pathogens, malaria was leading diagnose (27,1%, n=5733) divided to complicated, uncomplicated and not-confirmed. Due to lack of other tests for febrile diseases, such as Dengue, Chikungunya and others in many rural areas, we stated them under the others. Dermatological conditions were not very common (3,2%, n=672), dominated by bacterial infections, arthropod bites, animal bites, cutaneous anthrax and even 4 cases of leprosy. Most common respiratory illness included influenza like illnesses, bronchitis and URTI. Pneumonia accounted for 4% of total diseases, with most of the patients under 5 years. 7 cases of TB were diagnosed, none of them was resistant for TB drugs. Sexually transmitted infections (STI) accounted for 15% of total diagnoses, included HIV infection and syphilis.

## 4. Conclusions

In 2013, a broad spectrum of diseases were diagnosed at our clinics and hospitals in or near conflict zones. Diagnoses varied according to regions and climate. The spectrum of armed conflict associated morbidity also shows that there is a need to pay more attention to communicable diseases, especially in children under five years old. Alarming rate of sexually transmitted diseases should also be targeted. Management of health care therefore must reflect the fact that 15% of the population in conflict-affected area has any kind of STI and it is necessary to begin the process of health education in population of internally displaced people in South Sudan.

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## Communicable diseases among migrants to EU from Ukraine and CIS

Krcmery V

*St. Elisabeth University, Bratislava, Slovakia*
*Comenius University, School of Medicine*

Within the last 6 months, the Schengen border represents a place where illegal travellers (migrants) from Crimea and east Ukraine transit Slovakia on their way seeking better economic and political future in Europe.

In UNHCR Emergency Transit Centre in Humenné, Slovakia, during the last 18 months, around 1440 migrant transited, most of the from CIS countries, central and south Asia as well as from Syria, Iraq and Lebanon. More than 60% of children and 49% of adults have geohelmints in their stool; however most of them are clinically asymptomatic. Commonest infectious diseases (ID) are respiratory tract infections (RTI; such as pneumonia, tonsillitis, acute otitis), gastrointestinal infections and skin and soft tissue infections (SSTI) exacerbating due to prolonged immune suppression during long-term travelling. Out of the 1440 cases, only 2 cases of tuberculosis (TB) were diagnosed and only 3 patients were HIV-positive (0,5%). Thirty-six patients were colonised with methicillin-resistant *Staphylococcus aureus* (MRSA) and 21 with penicillin-resistant pneumococci.

Regarding our previous research in asylum seekers during 2009-2013, the most frequently acute ID found were upper respiratory infections (URTI)- 32% (pneumonia 12%), gastroenteritis - 7%, followed by SSTI - 26% (impetigo, scabies, other ectoparasites). Intestinal parasites were found among 15% cases. Interestingly, TB was detected only in 118 patients (less than 1%), however 51% of TB cases were due to multiresistant (MDR) strains. Sporadic cases of morbilli, diphtheria and pertussis were noticed (less than 1%) during the last 4 years.

# Infections and Outbreaks among Refugees from DRC to Rwanda and from Sudan to Kenya

Kafkova J

*St. Raphael Clinic, Tropical Programme in Nairobi, Field office of St. Elizabeth University College of Social Work and Public Health, Bratislava, Slovakia*

New conflicts, violence and human right abuse continue to create new displacement emergencies in the region of sub-Saharan Africa, thus large populations are forced to move into temporary settlements or camps. This has had a significant impact on human health. Overcrowded and rudimentary shelters, and poor access to safe water and sanitation, chronic undernutrition and micronutrient deficiencies can increase the risk of proliferation of infectious diseases in such environments. Pre-existing immunity of the populations is according to the country of origin usually low and in addition, information about previous vaccinations is often not available. Most frequent communicable infectious diseases in refugee populations in Kenya and Rwanda include respiratory tract infections, diarrhoeal diseases, parasitic infections, TB, malaria, hepatitis B and C, STDs, and measles. High prevalence of malnutrition significantly contributes to the higher morbidity and mortality in such populations. Outbreaks of infectious diseases in refugee camps are not uncommon and have strong coincidence with large influx of refugees within a short period of time and concomitant outbreaks in the countries of origin. The most effective measures to prevent mortality, morbidity and outbreaks of infectious diseases in refugee camps include access to clean water and sanitation, access to health care services, preventive services and vaccination coverage, mother and child health, and food security.

Majority of refugees in Rwanda originate from DRC. In January 2014 the number of refugees residing in Rwanda reached 73,349 located in four refugee camps and one transit centre (1). Majority of refugees residing in Kenya originate from Somalia, Ethiopia and South Sudan with total number of refugees from the respective countries reaching 534,938 (January 2014) located in six refugee camps (2).

One of the refugee camps in Rwanda, Kiziba, between January and August 2014 reported a total population of 16,414, with 7,471 men and 8,943 women. Number of children under 5 years of age was 2,317. Crude mortality rate (CMR) was 0.2. In this period it was reported a total of 15,196 cases of URTI (crude morbidity rate [CMR] 46%), 2,451 cases of watery diarrhoea (CMR 7%); 1,752 cases of intestinal worms (CMR 5%); 518 cases of lower respiratory infections (CMR 2%); 88 cases of bloody diarrhoea; 369 cases of STIs (non-HIV/AIDS; CMR 1%); 32 cases of confirmed malaria and 3 cases of tuberculosis. In total, 3 cases of acute severe malnutrition were reported. No cases of measles were reported. Coverage of vaccination against measles was 95.4%, against polio 100.6%, and against DTP 104.1%. Cumulative number of patients on ART was 63. (1)

Outbreaks of infectious diseases in refugee camps have strong coincidence with large influx of refugees within a short period of time and concomitant outbreaks in the countries of origin. Also, pre-existing population immunity to preventable infectious diseases such as measles, rubella, and polio is very low (in 2010 and 2011

estimated MCV 1 coverage was only 46%(1)). Since the establishment of the refugee camps Kakuma and Dadaab, numerous outbreaks have been reported namely outbreaks of mumps, measles, polio, diphtheria, cholera, and multi-drug resistant TB. With globally increasing vaccination outbreaks have become less frequent and there is a shift of age distribution towards older age groups. Kenya UNHCR Annual Report, 2011, reported several outbreaks of measles, cholera, and shigellosis in several camps in Dadaab area (5). Also, Kakuma refugee camp reported an outbreak of malaria in 2011 (4). From January 2013 till June 2014 no outbreak was reported in Kiziba refugee camp, Rwanda (6).

In Kiziba camp, Rwanda, the highest proportion of infections is upper respiratory tract infections followed by watery diarrhoea and intestinal parasites. Lower respiratory tract infections were ten times less prevalent than the upper respiratory tract infections. Cases of malaria were rare due to the high altitude (estimated altitude – 1,958 metres above the sea level) and effective malaria control measures (distribution of ITMNs and indoor residual spraying).

The number of refugees in both the countries reached in January 2014 almost 606,000. Given the fact that the capacity of most of the refugee camps is exceeded and that the influx of refugees is practically continual, measures to decrease the proliferation of infectious diseases. In order to cease the transmission of measles more than 90% of the population must be effectively vaccinated. However, despite the vaccination initiatives the collective immunity still remains low in the refugee populations. The most effective measures to prevent mortality, morbidity and outbreaks of infectious diseases in refugee camps include access to clean water and sanitation, access to health care services, preventive services and vaccination coverage, mother and child health, and food security.

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Symposium 5: Multidrug resistance in community and hospital

## Rationalization of antibiotic therapy as part of HIV management in developing countries

Suvada J

*St. Elizabeth University of Public Health and Social Work, Health Initiatives Association and St. Charles Foucauld Health-care Refugee Centre in Lebanon*

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The improvement in use of antiretroviral therapy (ART) has entirely changed the incidence and management of human immunodeficiency virus (HIV) infection worldwide. It has been observed that due to this policy it was dramatically reduced the rates of opportunistic infections. However, non-opportunistic infections continue to cause significant morbidity and mortality in developing countries where presentation with advanced infectious complications is common among both children and adults groups. HIV-associated T and B cell dysfunction increases the incidence of respiratory infections at all CD4 counts which may be caused by a number of different opportunistic and non-opportunistic microbial causes. An infectious diseases of the central nervous system are a common occurrence in HIV and may present differently also geographically. The gastrointestinal tract may be affected by a wide range of pathogens, from the types of bacterial and viral infections that are common in non HIV population to opportunistic fungi, viruses, and parasites too. Currently due to ART access it is observed that the incidence of many of known opportunistic infections has diminished enormously but in late presenters and those who use to fail to ART, it has been still observed to cause chronic infections. Among HIV-positive children living in some developing countries due to the low immunity it has been observed detection of bacterial blood-stream infections. The access to care, propped EBM-evaluation, diagnosis with antimicrobial resistance evaluation and appropriate therapy is usually limited. From conducted studies the overall in-vitro antimicrobial susceptibility pattern showed that high bacterial resistance was detected in different areas where no rationalized antibiotic guidelines and policies has been made.

The findings of multi-centric studies revealed that infectious diseases common amongst HIV- infected population have unique distribution, incidence and antimicrobial resistance related to many local, host and (social) health-care factors which have direct and indirect impact on mortality and infectious morbidity among these patients. Local intervention, screening programs development, a conducting of epidemiological research, open policy-makers and community discussion, preventive programs improvement and EBM-approach implementation through local rationalization of pharma sale market and prescription according local antibiotic guidelines seems to be essential for further improvement of quality care for HIV infected population living in resource limiting settings.

# Occurrence of extended spectrum β-lactamase (ESBL)-producing isolates from Enterobacteriaceae family and their antimicrobial resistance development

Koreň J, Záborská M, Slobodníková L

*Institute of Microbiology, Faculty of Medicine, Comenius University and University Hospital Bratislava* (FMCU and UHB)*, Slovak Republic*

Keywords: ESBL, *Enterobacteriaceae*, antimicrobial resistance, nosocomial infections

## 1. Introduction

Currently is not so easy to ensure efficient initial empiric therapy, that should not be compromised. Increased evidence and prevalence mainly in healthcare facilities, as well as growing antibiotic resistance of ESBL-producing *Enterobacteriaceae* poses a worldwide concern. These extended spectrum enzymes belong to the most frequent resistance mechanisms, from that a 30 years ago, since they were discowered in Europe (1). Concerning of selection pressure of antibiotics, failure of hygienic regime and insufficient monitoring of nosocomial infections, ESBL-producing isolates have still emerging and spreading character. Coicidence of the same plasmid transfer results in co-expression of resistance to β-lactam antibiotics, fluoroquinolones, aminoglycozides, tetracyclins and cotrimoxazole in these multidrug resistant (MDR) pathogens (2). Nowadays ESBLs show geographical variation or diversification and they are represented by 587 distinct enzymes – TEM (*Temoniera*, name of first patient) 219, SHV (*Sulfhydryl variable*) 189, CTX-M (activity to cefotaxime, first isolated in Munich, cefotaximasis) 160, and OXA (hydrolysis of oxacillin) 19 variants (www.lahey.org/studies, 3). Additionally, ESBLs can be combined with each other, with other molecular classes and types. This can be done occasionally with class C, AmpC β-lactamases (first was encoded by *ampC* genes) or could be done exclusively with class A or class B carbapenemases. Moreover, ESBL hyperproduction combined with other resistance mechanisms (porin defect and bacterial efflux) may lead to carbapenem resistance (4).

## 2. Objectives

We focused on assessment of ESBL-producing isolates occurence in the University Hospital in Bratislava - Old Town during years 2007 to 2013. Likewise, we also monitored the development of antimicrobial resistance in the most frequent ESBL-producing bacterial species.

## 3. Methods

All types of the clinical specimens were included in the examination and the samples were consecutively recovered from the hospitalized patients during the last 7 years. All samples were simultaneously submitted to cultivation on Brilliance ESBL chromogenic screening-medium as well (5). Following the standard biochemical identification, isolates were examined by double disc synergy test (DDST), confirmatory disc diffusion ESBL and confirmatory dilution ESBL tests to prove their ESBL activity (1, 4). Susceptibility testing was carried out by a

quantitative colorimetric micro-method against 10 selected antiinfectious agents: ampicillin, aminopenicillin/β-lactamase inhibitor (amoxicillin/clavulanic acid or ampicillin/sulbactam), cefuroxime, ceftazidime, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin and cotrimoxazole.

## 4. Results

The numbers of ESBL-producing isolates from *Enterobacteriaceae* family during the particular years were the following: 131 (6%), 169 (7%), 383 (13%), 314 (12%), 269 (9%), 293 (8%) and 511 (15%). Out of 19 725 overall investigated isolates, 2070 (10.5%) were ESBL-producers. The incidence of ESBL-producing isolates per 1 000 admissions was during the individual years of the monitored period equal to 13.8 in the year 2007, 17.8 in 2008, 40.3 in 2009, 33.1 in 2010, 28.3 in 2011, 30.8 in 2012 and 53.8 in 2013. During the 7-year observation period, the distribution of ESBL-positive isolates from *Enterobacteriaceae* family was the following: 40% (835 isolates) came from the 1st internal medicine clinic, 30% (626 isolates) from the 2nd internal medicine clinic, 15% (298 isolates) from the Surgical clinic, 6% (129 isolates) from the Neurological clinic, 4% (83 isolates) from the Dermatovenerological clinic and 5% (99 isolates) from the Psychiatric clinic. The total number of ESBL-producing *Klebsiella spp*. isolates during the 7-year study period was 832 (40%, 12.5 isolates/1000 admissions), *Escherichia coli* 743 (36%, 11.2 isolates/1000 admissions), *Proteus spp.* 329 (16%, 4.9 isolates/1000 admissions), *Enterobacter spp.* 114 (5%, 1.7 isolates/1000 admissions) and others (*Morganella spp., Serratia spp*. and *Citrobacter spp*.) 54 (3%, 0.8 isolates/1000 admissions). The proportion of ESBL-producing isolates of *Klebsiella spp*. decreased from the initial 53% (70 isolates of ESBL-producing *Klebsiella spp.* from tho total number of 131 ESBL-producing isolates) in the year 2007 to 32% (165 isolated ESBL-producing *Klebsiella spp*. isolates out of the total number of 511 ESBL-producing isolates) in the year 2013. In the case of *E.coli*, it was a slight increase from 27% to 38%, in *Proteus spp*. from 12% to 20%, in *Enterobacter spp*. and others from 4% to 5%. When analyzing the trend of resistance of all tested *Klebsiella pneumoniae* isolates (ESBL positive and negative), the resistance against protected aminopenicillins (amoxicillin/clavulanic acid, or ampicillin/sulbactam) was 67% in 2007, and at the end of the reporting period (2013) it was 47%; against cefuroxime 64% and 37%, against ceftazidime 48% and 37%, gentamicin 52% and 33%, tobramycin 62% and 49%, amikacin 20% and 19%, ciprofloxacin 69% versus 40%, cotrimoxazole 54% vs 41%. Only the resistance to meropenem slightly increased from 1% to 2%. The resistance of all investigated *E. coli* isolates (ESBL-producing and non-producing) observed at the beginning of the period (2007) was 51% against ampicillin, and at the end (2013) it was 59%, against protected aminopenicillines 24% and 37%, against cefuroxime 26% and 28% , ceftazidime 11% and 25%, gentamicin 13% and 18%, tobramycin 19% and 28%, amikacin 2%, and 6%, ciprofloxacin 35% and 34%, resistance to cotrimoxazole was 21% versus 38%. Resistance to meropenem increased only minimally, from 0% to 2%. The resistance in all surveyed strains of *Proteus mirabilis* to selected antibiotics was as follows: the resistance to ampicillin retained 76% with the small fluctuation during the observation time, to the protected aminopenicillines had a value of 44% in 2007 and 39%in the year 2013, to cefuroxime 39% vs 46%, ceftazidime 12% vs 27%, gentamicin 49% vs 51%, tobramycin 38% vs 46%, 1% amikacin vs 5%, ciprofloxacin 60% vs 57%, and for the cotrimoxazole rose from 67% to 72%. Meropenem resistance remained unchanged, and both at the beginning and at the end of the investigated period was 0%.

## 5. Conclusion

It is essential to point out that the ESBL positive *Klebsiella spp.* dominated worldwide at the end of 20th and early 21st century. Considering our results, the occurrence of ESBL- producing *Klebsiella spp.* in our hospital has a slight downward trend since 2009, however, these strains are still important agents of nosocomial infections. In 2013 alarmingly and sharply started to increase in our hospital the occurence of ESBL-producing *E. coli* and *Proteus spp*. isolates, and increase their antimicrobial resistance to non-betalactam antibiotics as well. These MDR strains constitute a real threat to our healthcare facility for their expansion and the possibility of treatment failure. Resistance in these isolates increased mainly against 3$^{rd}$ generation cephalosporins, aminoglycosides and cotrimoxazole. Meropenem and amikacin antimicrobial activity was maintained against the majority of ESBL-producing strains at unchanged levels. Fears of ESBL isolates are, that they may acquire carbapenem resistance.

Therefore, the prevention of emergence and spreading of ESBL-producing isolates of *Enterobacteriaceae*, as well as the reduction of the selection pressure of the broad-spectrum antibiotics on microbial flora, have irreplaceable importance, not only with respect to the cost of the treatment, but first of all to the treatment results of our patients.

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## Elevated MIC in Gram-negative bacteria

Kulková N

*St. Elisabeth University, St. John Neumann Institute, Příbram, Czech Republic*

Multidrug resistant (MDR) Gram-negative bacteria (GNB) have only limited options for effective treatment. Antimicrobial susceptibility testing belongs to one of the most important techniques in laboratory of clinical microbiology and microorganism susceptibility or resistance according to MIC is the key factor of therapeutic practice guidance. However, MIC value (*in vitro*) alone does not always reflect the activity *in vivo* and may not be predictive for clinical outcome. Especially, in bacteria with decreased susceptibility, the success of therapy may be negatively influenced. More treatment failures could be expected with decreasing susceptibility of GNB. It is now clear that categorization into groups of "sensitive", "intermediate susceptible" and "resistant" according to MIC levels cannot fully predict activity of particular antibiotic in the context of real clinical outcome. Negative impact of higher MIC values in GNB was proved in some studies [1,2] leading to reasonable concerns and starting discussion on MIC levels revision. It was shown for *Acinetobacter baumannii* infections, where carbapenem MIC increase of one dilution led to increased risk of death by two fold [1]. Similar was observed in studies with S. enterica, which showed more treatment failures in cases with fluoroquinolone resistant infection [2]. Infection with GNB with high fluoroquinolone MIC was also shown to lead to longer hospital stay and inferior clinical outcome [3]. The lecture will provide review of currently known data on elevated MIC and its impact on mortality and other outcomes.

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Symposium 6: HCV disease burden in Egypt

## Epidemiology of hepatitis C virus infection in Egypt; overt and occult

Amer FA, Gohar M

*Microbiology and Immunology Department, Zagazig Faculty of Medicine, Zagazig, Egypt*

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Hepatitis C virus is an epidemic in Egypt which contains the highest prevalence in the world; 14.7%. Explanations for this unique epidemic may be dated back to iatrogenic role of parenteral antischistosomal therapy campaigns to control endemic schistosomiasis. Other routes of infection are contributing to the ongoing HCV transmission. The prevalent genotype in Egypt is type 4 (73%), the origin, evolution, and dynamics of which are difficult to determine. Risk factors for acquiring HCV infection include: history of antischistosomal injection treatment before 1986, old age, male gender, and residence in rural areas. Other risk factors include; injection therapy, blood transfusion prior to 1994, exposure to various facility-based medical procedures, and occupational transmission among health care workers. In community settings, a set of risk factors, mostly related to prevailing social and cultural conditions, are responsible for maintaining the high rates of HCV transmission. Sexual transmission is a matter of controversy. Chronic HCV is the main cause of liver cirrhosis and liver cancer in Egypt and, indeed, one of the top five leading causes of death. It kills an estimated 40,000 Egyptians a year.

When talking about children, current HCV seroprevalence is high, approximately 5-8%. It is to be emphasized that HCV infection is not always benign in the childhood period in Egypt. It has been shown that blood transfusion, surgical procedures, dental treatment, male circumcision and age above 10 years are significant risk factors associated with anti-HCV antibody prevalence. In addition, household transmission, vertical transmissions are routes that are under investigation. Finally, approximately 70% of acquired infections in children are due to unidentified risk factors.

Occult hepatitis C virus (HCV) infection is defined as elevated liver function tests and negative HCV antibodies in serum, while HCV RNA is detectable in liver tissue and PBMCs. Interest in occult HCV has been emerged recently in Egypt. Studies at a national level are being carried out, but no results have yet been released. Many small scale studies have been performed among particular patient groups, which have highlighted the importance of this disease entity.

# Egyptian experience for management of HCV genotype 4 infection

Monkez M Yousif

*Faculty of Medicine, Zagazig University, Egypt*

Keywords: Hepatitis C Virus, Epidemiology, Prevalence, Incidence, Egypt, Interferon

## 1. Introduction

Egypt has the highest prevalence and incidence of HCV infection worldwide. Genotype 4 is encountered in more than 90% of HCV infection in Egypt [(1)]. Parenteral anti-Schistosomal Treatment (PAT) campaign prior to 1985 was accused to be responsible for the high prevalence of HCV infection in Egypt [(2)]. Currently the formal and informal health care settings are responsible for the persistently high prevalence and incidence of HCV infection in Egypt. Chronic hepatitis C (CHC) is responsible for most of the liver diseases, cirrhosis, hepatocellular carcinoma (HCC) and mortality among Egyptians. The Egyptian Ministry of Health and Population (MOHP) implemented a program for HCV infection control and treatment. Sustained virologic response (SVR) to standard of care therapy (pegylated interferon and ribavirin) is about 50%. The new national guidelines using the first direct acting antiviral drug (DAA) sofosbuvir has been published in July, 2014 and patients will receive the new drug in the mid of October, 2014 with the hope of HCV elimination by year 2030.

## 2. Epidemiology of HCV in Egypt

The most recent estimates for HCV prevalence in Egypt were obtained from Egypt demographic and health survey in 2008. HCV antibody prevalence (nationwide) was 14.7% while HCV RNA was detected in 9.8% [(3)]. There are a few prospective studies estimating the incidence of HCV infection in Egypt. Interpolating the data of these studies together, an average incidence of HCV infection was 2 per 1000 py which translates into around 150,000 new infections annually [(4)].HCV genotype 4 is the commonest in Egypt representing more than 90% of nationwide cases. HCV subtype 4a is the most commonly prevalent in Egypt [(1)].

The initiator of the epidemic is believed to be the extensive campaigns of PAT led by Egyptian Governments between 1950s and 1980, while nosocomial infection represents a major source for the ongoing intense spread of HCV infection in Egypt, because of improper infection control practices [(1)] .

## 3. Natural history of HCV infection in Egypt

HCV patients may present as acute or chronic hepatitis. Most acute hepatitis cases are asymptomatic with the majority of cases passing into chronicity. Chronic infection is characterized by various degrees of inflammation and fibrosis with about 10-40% of patients developing liver cirrhosis over decades of infection. Cirrhotic patients are at risk of mortality and risk of HCC development. Studies on the natural history of HCV infection in Egypt are lacking in literature. Patients from Egypt are unique in having HCV genotype 4 , mixed infection with schistosomiasis,

absence of alcohol, absence of coinfection with HIV in most cases, as well as having different health care system and different population genetic background.

## 4. Control of HCV infection in Egypt

In response to the high burden of HCV infection (morbidity and mortality, mostly from chronic liver disease), the Egyptian government and MOHP implemented a program in 2001 to reduce health care associated HCV spread, created the National Committee for Control of Viral Hepatitis (NCCVH) in 2006, and launched the Egyptian National Control Strategy for Viral Hepatitis in 2008. Prior to formation of NCCVH, several measures were taken in the last 20 years to control spread of HBV & HCV. Since 1993, the national blood supply has been screened for HCV [(2)].

## 5. National infection control program

Egypt's infection control program (ICP) was designed in collaboration with the WHO in 2001 [(2)]. The national infection control guidelines developed in 2003 and infection control programs were established in 450 MOHP facilities. Among 60 facilities with dialysis units, the annual incidence of HCV infection decreased from 28 % (before program implementation) to 6 % (3 years after implementation). In 2011, the program underwent an international health regulation assessment, with the conclusion of its success in substantially reducing iatrogenic transmission of HCV [(5)].

## 6. National Control Strategy for Viral Hepatitis (2008-2012)[(2)]

This is the first comprehensive approach tailored in Egypt to combat the burden of HCV & HBV disease in Egypt. The main goals of the strategy were the following: accurate tracking of prevalence and incidence of HBV & HCV in Egypt, reducing the prevalence of chronic HBV and HCV infection in the 15-30 age groups by 20% of 2008 levels by 2012, treating 20% of persons needing treatment by 2012 under subsidized schemes and conducting high-quality scientific research on viral hepatitis. To achieve these goals, the NCCVH established "Viral Hepatitis Treatment Centers" in different regions of the country. These centers are prepared to treat and care for patients with HCV according to the national guidelines.

The Egypt national guidelines for treatment of HCV infection were published in 2007 [(2)] and revised in 2010 to treat patients with chronic hepatitis C with ages 18-60 ys, compensated liver disease and favorable hematologic profile with body mass index up to 30. The treatment consists of 48 weeks of combined pegylated interferon and ribavirin.

## 7. Evaluation of the national strategies to control HCV in Egypt

In the last 10 years, there is an increasing trend of the Egyptian health authorities towards containment of HCV epidemic. Following implementation of the infection control program in MOHP facilities, there is success to some extent in reducing HCV infection and transmission. As well, the NCCVH provided care and treatment for almost 350,000 patients with CHC in the period from 2008 to 2014 with average SVR about 50%. About 40% of treated patients are covered by government (about$ 80 million annually), while the remaining 60% is covered by insurance companies and patients. The Egyptian government succeeded in bringing down the price of medications from approximately $12,000 to <$2000 for the course of 48 weeks

duration. In spite of the apparent governmental efforts to disseminate and intensify the infection control program in MOHP facilities, only $ 800,000 was dedicated to the program in 2011, which represents only 1% of government expenditures for viral hepatitis care and treatment [(5)]. The hope we have is the recognition of the epidemic magnitude by health authorities and participation of universities and research institutes in intensive campaigns and initiatives aiming at adopting comprehensive and sustained educational and preventive programs to combat the epidemic. In addition, the availability of highly effective and safe medications that attack HCV directly will help much in overcoming this epidemic.

Currently, the Egyptian guidelines for management of HCV infection is changing with the introduction of sofosbuvir (HCV polymerase inhibitor), into the Egyptian market. The MOHP succeeded in making a deal with the producer company to sell it to Egypt at 1% of its price in USA. Adding sofosbuvir to HCV-4 treatment regimen in clinical trials gave SVR results more than 90%.

To eliminate HCV infection in Egypt we need to use a treatment with more than 90% response efficacy plus increasing the number of patients diagnosed and treated annually up to more than 300,000 in addition to reducing the incidence of new infection by 10% annually. Implementing these measures which are affordable after introduction of the new DAA into the Egyptian market will bring down HCV prevalence to below 2% by year 2030.

## 8. Conclusion

In conclusion: hepatitis C represents a major health problem in Egypt, which has the highest prevalence and incidence worldwide. More than 90% of cases are due to genotype 4 which is difficult to treat. Unsafe injections and invasive procedures are the major risk factors of HCV spread. HCV is the major cause of chronic liver disease, hepatocellular carcinoma and related morbidity and mortality. Most of the governmental expenditure and efforts are paid to disease treatment and lesser and inefficient measures are given to prevention and infection control which is responsible for the intense ongoing epidemic. Establishment of a clear and sustainable surveillance system and tailoring a more comprehensive infection control programs as well as using the recent directly acting antiviral drugs suitable for HCV-4 is mandatory for overcoming the epidemic.

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Symposium 7: Epidemiology, diagnosis and treatment of *Clostridium difficile* diarrhoea

# Clostridium difficile infection, epidemiology, pathogenesis and diagnosis

Petrikkos G

*4th Dept. of Internal Medicine, Attikon University Hospital, National and Kapodistrian Universityof Athens*

## 1. Introduction

*Clostridium difficile* infection (CDI) is considered to be the main cause of bacterial infectious diarrhea in hospitalized patients. Since the beginning of the new century a continuous rise in the incidence of CDI, severity and mortality, has been observed worldwide. Even though some CDI cases are not associated with previous antibiotic exposure, this remains as the principal risk factor for the development of CDI.

## 2. Epidemiology

During the last decade the incidence of severe CDI is continuously increased worldwide as has been observed in Canada, in the USA and in Europe. In North America, there was a fivefold increase in the incidence in the whole population and an eightfold increase in the elderly. In Europe, this continuous increase has been associated with outbreaks, first in the UK from 2003 to 2004, then in the Netherlands and Belgium from 2005, followed by France and other European countries. Barbut *et al.* reported a mean incidence of nosocomial CDI in 23 European hospitals of 245 per 10,000 patient-days.

During the last 10 years, epidemiological changes are observed including an increased rate of severe manifestations, such as septic shock, toxic megacolon and intestinal perforation. Hypervirulent strains that causes severe disease as well as community acquired infections not always associated with antibiotic, have appeared. Furthermore, by the emergence of these superbugs strains, both an increase in treatment failure with metronidazole and several relapses were occured.

In 2005, an increase in CDI rate was observed in North American hospitals, which after molecular analysis was identified as a new hyper-toxigenic strain of *C. difficile,* causing a large number of severe cases, capable of producing *in vitro* 10 times more of toxins A (TcdA) and 23 times more Toxin B (TcdB); this isolate is also referred as ribotype 027 by PCR ribotyping. Another postulated reason for the hypervirulence of *C. difficile* BI/NAP1/027 is the production of a binary toxin, called CDT which is found in approximately 6% of *C. difficile* isolates and was discovered by Popoff *et al.*

A further characteristic of *C. difficile* BI/NAP1/027 is an increased sporulation rate *in vitro* in the absence or presence of non-chloride cleaning agents. This may lead to a better survival and spread of the strain in the environment.

Clostridium difficile was previously consider to be a hospitalized acquired infection (HAI), but nowadays cases of community acquired CDI (CA-CDI) are also appeared, but in a considerably lower rate than the hospitalized cases. Groups of healthy individuals previously considered at low risk, without prior exposure to hospitals or prior use of antibiotics such as children or pregnant women but also peripartum women, patients with inflammatory bowel disease or cirrhosis, organ transplant patients and immunocompromised patients consists the vulnerable groups exposed to the disease. Possible community sources of CDI include soil, water, pets, animals used for food, meats and vegetables. *C. difficile* can also be a cause of infection in animals, with similar mechanisms of pathogenicity.

In contrast with historic strains of 027 *C. difficile*, the new hypervirulent strains are resistant to fluoroquinolones and erythromycin. High levels of resistance to clindamycin have recently been described in Europe regarding *C. difficile* BI/NAP1/027; this strain is usually susceptible to standard therapy (metronidazole and vancomycin). Isolates with reduced susceptibility to metronidazole have been found to be transmitted between patients, but their clinical significance in terms of response to antibiotic treatment remains unclear. There is evidence that sub-inhibitory concentrations of metronidazole, vancomycin and linezolid induce TcdA and TcdB gene trascription and toxin production. Multiple antibiotics have been shown to promote *C. difficile* spore germination, vegetative cell growth and toxin production, including both older and newer fluoroquinolones and cephalosporins.

Wilcox *et al.* have recently published an accurate analysis on the current epidemiology in the UK. An increase in the number of outbreaks of severe CDIs was observed in Europe, with more than 55,000 CDI cases only in England from 2007 to 2008.

From 2007, due to this increase in incidence and mortality, all NHS hospitals in England were asked to report all cases of CDI and a *C. difficile* Ribotyping Network (CDRN) was created. In the 3 years of the analysis, ribotype 027 was the most frequently detected ribotype and more than 85% of the samples belonged to patients older than 65 years. These two characteristics, plus a severe disease (PMC, toxic megacolon and the need of abdominal surgery for CDI) and the exposure to at least two antibiotics, significantly correlated with an increased mortality.

Interventions aimed at the reduction in the use of fluoroquinolones and cephalosporins, clindamycin and broad-spectrum penicillins through a strict antibiotic stewardship clearly demonstrated a reduction in the incidence of CDI cases, also when associated with measures aimed at limiting the spread of the spores within the hospital environment, such as isolating or cohorting positive cases.

## 3. Risk factors

Various risk factors for CDI are important to consider; the antibiotic therapy during the previous 3 months was the most known factor since the early emergence of the disease. The antibacterials with the highest risk of CD colonization includes clindamycin, second and third generation cephalosporins and fluoroquinolones; macrolides, ampicillin, amoxicillin/clavulanic acid presents a moderate risk of infection, either aminoglycosides, vancomycin, trimethoprim, tetracyclines, benzylpenicillin piperacillin/tazobactam carries a lower risk of infection. Other risk factors consists of the older age (>65 years), severe underline disease,

immunocompromised patients, nasogastric tubes, gastrointestinal medication including gastric acid reduction therapy, recent endoscopy, prolonged hospitalization stay including ICU stay.

## 4. Pathogenesis

*C. difficile* spores are highly resistant to desiccation, chemicals and high temperature. Spores frequently contaminate the environment around patients with CDI, potentially persisting for months or even for years.

In optimal conditions the *C. difficile* spores germinate and vegetative cells multiply. The bacteria adhere to the cells through their adhesins and colonize the gut. Thereafter they penetrate the mucus layer that covers the enterocytes, via flagella and proteolytic enzyme activity. The second phase of the pathogenesis of CDI is the production of the two main virulence factors, toxins A (TcdA) and B (TcdB). These are potent cytotoxic enzymes that can damage the colonic mucosa by causing cell cytoskeleton disorganization. As mentioned above, some strains including *C. difficile* BI/NAP1/027, produce a third toxin called binary toxin CDT, and it might increase the toxicity of TcdA and TcdB, leading to a more severe disease.

## 5. Recurrences

Recurrence may constitute one the most challenging aspect on the management of CDI. After a successful first-line treatment with standard therapies with metronidazole or vancomycin, 20–30% of patients may present a second event within 60 days from discontinuation, but usually this occurs within the first 2 weeks. A second cycle of treatment with metronidazole or vancomycin can be efficacious, but 40–60% of patients have one or more relapses.

Lack of restoration of enteric microflora, persistence of *C. difficile* spores within the gut with the chance of recurrence. Some patients may even experience multiple recurrences, with the consequence of many courses of antibiotic therapies, which may be potentially toxic and very expensive. Hospitalized patients who are colonized by the bacteria or that experience acute or recurrent infection may represent a reservoir of infection for other patients who share the same environment which leads to the need for isolation of the patient and infection control measurements.

## 6. Diagnosis and prevention

Since the diagnosis of CDI is usually based on clinical history and diarrhea, in combination with laboratory tests, rapid and accurate microbiological diagnosis is important. Preemptive antibiotic therapy is often started by clinicians based on CDI clinical evidence, prior to the availability of diagnostic test results. However, the value of a CDI rapid diagnostic algorithm is enforced by the possibility of reducing unnecessary antibiotic treatment and implementation of infection control precautions.

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# Laboratory diagnosis of *Clostridium difficile* infection (CDI)

Alexandrou M

*Microbiology Dept. of Larnaca General Hospital, Larnaca, Cyprus*

## 1. Introduction

*Clostridium difficile* is a gram positive anaerobic sporulating bacillus originally described in 1978. In the way became known as a complication of antibiotic therapy, causing diarrhea from mild to severe, in the form of pseudomembranous colitis or even of aggressive life threatening colitis. Due to the severity of the infection and the increasing incidence and mortality rate, especially after the emergence of the ribotype 027 strain in the recent years, a rapid and accurate diagnosis of CDI is important, not only for the patient's management but also for the infection control and prevention.

The diagnosis of CDI is usually based on the clinical history in combination with laboratory tests bearing in mind that only toxigenic strains cause disease due to the production of toxins A and/or B and the colonization rate of non toxigenic strains is 1-2% between the out patients, while this rate increases to 20-30% among the hospitalized patients.

## 2. Laboratory Methods

Various laboratory tests are currently available for the detection of *C. difficile* or its toxins. The diagnostic tests for *C. difficile* can be divided into culture and non-culture techniques: (i) Culture methods for the detection of toxin-producing *C. difficile* strains (ii) *C. difficile* products (glutamate dehydrogenase – GDH, toxins A and/or B), and (iii) tests for *C. difficile* genes (PCRs for 16S RNA, toxin genes, genes for GDH).

Routine stool cultures for CDIF isolation performed on commercially selective media CCFA (cycloserine, cefoxitine, fructose agar) after 48h of anaerobic incubation at 37$^{o}$C, without testing the toxin production lacks specificity and appear to be useless since non toxigenic strains colonize the intestine taking part of the normal flora.

Cell culture cytotoxicity assay (CCA), first describe in 1978, detects the toxin B and together with the toxigenic culture (TC), which means culture followed by *in vitro* toxin detection of the isolated strains, are regarded as reference standard methods for the diagnosis of CDI. Since these standard methods are time consuming taking 2-5 days and specific laboratory facilities are required to be performed, over the time they have been replaced by enzyme immunoassays (EIA). These are rapid and easy to perform assays designed to detect *C. difficile* toxins or the enzyme glutamate dehydrogenase (GDH), which is a metabolic enzyme specific to *C. difficile* but produced by both toxigenic and non-toxigenic *C. difficile* strains. Molecular methods based on PCR for the amplification of the toxin A genes are used recently, but seems to have a few advantages comparing to EIA. Direct detection of the

toxin genes of *C. difficile* in fecal specimen by RT-PCR (Real-time polymerase chain reaction) has been tested, but its sensitivity/specificity does not exceed that of EIA.

For CCA, stool filtrates are inoculated onto a monolayer of a cell culture which is then observed for a toxin B induced cytotoxic effect after 24 and 48h of incubation. For CCA, different cell lines can be used including HeLA cells, fibroblasts and Hep-2 cells. The cytotoxic effect is achieved by neutralization, using an antiserum (*C. sordelli* antitoxin or *C. difficile* antitoxin).

Toxigenic culture (TC) consists of culture performed on selective media CCFA (cycloserine, cefoxitine, fructose agar) after 48h of anaerobic incubation at 37°C, followed by *in vitro* toxin detection (by EIA, CCA or PCR) to determine the toxigenicity of the isolated strain. Pretreatment of the stools with ethanol-shock (mixing equal volumes of ethanol and feces staying for 1h before inoculation) inhibits overgrowth of the normal feces flora increasing the sensitivity of the culture.

Enzyme immunoasssays (EIAs) available for detection of *C. difficile's* toxins or its products include EIAs detecting toxin A only or both toxins A and B and EIAs detecting the enzyme GDH. A combination panel that includes both an EIA detecting toxin A and GDH is also available, although the sensitivity remains at the same level, however the combination test allows an excellent negative predictive value of the CDI. The detection of GDH alone is not recommended as it lacks of specificity since it can be detected in healthy carriers also of non toxigenic CDIF strains.

## 3. ESCMID recommendations for diagnosing CDI

After a systematic review for the evaluation of the various laboratory methods for CDI diagnosis the ESCMID Study Group for Clostridium difficile – ESGCD, on purpose to optimize CDI testing in patients with suspected of CDI, has published the recommendations for diagnosing the CDI. Recommendations are applied to both the sample selection and laboratory testing, as follows:

Sample selection

- CDI testing should only be performed on unformed stools.
- CDI testing should be performed on unformed stool samples of all patients with potential infective diarrhoea and negative tests for common enteropathogens, irrespective of age, prior antibiotic use, co-morbidity, co-medication, and onset of diarrhoea (community-acquired or nosocomial).
- All patients with diarrhoea who have been hospitalized more than 72h should be tested for CDI, irrespective of the physicians' request. This does not mean that CDI testing should not also be performed on samples submitted within the first 72h of hospitalization
- Patients with diarrhoea who have been admitted in a health-care facility within a period of 3 months prior to the development of diarrhoea should also be tested for CDI.

Laboratory testing

- The diagnosis of *C. difficile* infection should be based on clinical signs and symptoms in combination with laboratory tests.

- It is recommend testing patients with a two-step algorithms. In the first step, faeces samples could be investigated with an EIA detecting GDH, an EIA detecting toxins A and B, or molecular test detecting TcdB. Samples with a negative test result can be reported as negative. Faeces samples with a positive first test result should be re-tested with a method to detect free faeces toxins, or with a method to detect GDH or toxin genes, dependent on the assay applied as first screening test.
- If free faeces toxins are absent but *C. difficle*, TcdB or GDH is present, CDI cannot be differentiated from asymptomatic colonization.
- Repeated sample submission during the same episode is not recommended in an endemic situation, but may be useful in an epidemic situation.

## 4. Conclusions

According to various studies published, the mean sensitivity of the methods varies from 75-80% while the mean specificity is 97-98%. The available tests have low PPV (positive predictive value) at low prevalence rate of the population (30-50%), but they have high NPV (negative predictive value) a t low prevalences which reaches the 99-100%.

In conclusion, none of the currently available tests is suitable as a stand-alone test in endemic populations to diagnose CDI because of their low PPVs at low prevalences, while the NPVs of the tests are very acceptable at low prevalences and the tests can be used alone as screening tests in an endemic situation. When a negative test result is obtained, CDI can be very reliably excluded. However, when a positive test result is obtained, a confirmatory test must be performed to recognize a truly positive sample.

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# Current treatment options for *Clostridium difficile* infection

Potamitis GS

*Dept of Gastroenterology, Apollonion Private Hospital, Nicosia, Cyprus*

Some general principles are important managing CDI.

Stop offending antibiotics where possible may be sufficient in 10% of cases. Avoid opiates and antiperistaltic agents. Initial empirical treatment as soon as diagnosis is suspected for severe CDI.

In use for more than 30 years oral metronidazole and oral vancomycin are the first line treatment. Oral metronidazole is recommended for patients non severe CDI, 500mg Tds or 250mg QID for 10 days. For more severe cases Vancomycin 125mg QID orally for 10 days.

For patients who have second and subsequent reccurency treatment with vancomycin for a severe first recurrence either usual dose, tapered or pulsed dosing regimens.

Reccurency of CDI is a big proplem.

Up to 25% of patients recurrent within one month following treatment either with metronidazole or vancomycin.

For those patients whom are high risk for recurrence Fidaxomicin is a promising option of treatment.

For refractory CDI Tigecycline in combination with rifaximin have good results. IV immunoglobuding might help in those cases at a standard single dose 150-400mg/kg IV.

For refractory CDI fecal microbiota transplant is suggested with good results.

Early surgical consultation is assential for severe and complicated CDI. Medical treatment longer than 6 days before surgery was associated with high mortality in severe CDI.

New guideline should be suggested by ESCMID considering the approval of new antibiotics and other options of treatment mainly for those subgroups of high risk for recurrence.

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Symposium 8: Reversal of resistance by help of non-antibiotics

## Bypassing the efflux-pumps with new types of antibiotics

Christensen JB

*Department of Chemistry, University of Copenhagen, Denmark*

Bacterial efflux-pump mediated resistance to antibiotics is a serious problem that enables bacteria to develop simultaneous resistance to a broad variety of antibiotics with different mechanisms of action [1]. The best known example is MRSA, which is a problem both in humans and in livestock such as pigs in pig-farms and there is a need for solutions [2]. In my presentation, I will describe different approaches to solving this problem going from chemical compounds that inhibit the efflux-pumps to new ways of recycling classical, well-known antibiotics by modifying them, so they can bypass the efflux-pumps.

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# The potential of Thioridazine as a helper compound in the treatment of infections with *Staphylococcus aureus*

Poulsen MØ

*Institute of Clinical research, Research Unit of Clinical Microbiology, Southern Danish University, Odense, DK*

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The shortage of drugs active against methicillin resistant *Staphylococcus aureus* (MRSA) is a growing clinical problem. The neuroleptic antipsychotic derivative thioridazine has been shown to increase the susceptibility of methicillin resistant *Staphylococcus aureus* (MRSA) isolates towards dicloxacillin *in vitro* [1,2]. The combinatorial synergistic killing effect of thioridazine and dicloxacillin was also observed in methicillin sensitive *Staphylococcus aureus* (MSSA) [1]. The *in vitro* studies furthermore indicated, that the killing effect of the combinatorial treatment is independent of Penicillin Binding Protein 2a (PBP2a)-mediated resistance mechanism.

Known side effects of thioridazine such as QTc prolongation, cardiac arrhythmias and sudden death of patients with schizophrenia has been described in the literature [3,4]. Thioridazine is administered as a 50-50 racemic mixture of the enantiomers thioridazine (+) and thioridazine (-). Thioridazine (-) (**JEK47**) has been reported to display less challenging CNS pharmaco-dynamic activity, than thioridazine (+). Furthermore, thioridazine (-) is characterized by an anti-microbial activity against Staphylococcus aureus, Mycobacterium tuberculosis and a wide array of other microorganisms. The antimicrobial activity is well described in numerous both *in vitro* and *in vivo* studies [3,4].

*Caenorhabditis elegans* (*C. elegans*) infected with MRSA was introduced as an *in vivo* model to test the effect of thioridazine as a helper drug in combination with dicloxacillin. Because thioridazine is an anthelmintic, initial experiments were carried out to determine the thresholds of toxicity, determined by larval development and induction of stress-response markers. No measurable effect was observed at concentrations less than 64 mg/L thioridazine. *C. elegans* were exposed to the most virulent MRSA strain tested for 3 days and subsequently treated with 8 mg/L dicloxacillin and 8 mg/L thioridazine alone or in combination for 2 days. As control infected *C. elegans* were treated with water. Combinatorial treatment resulted in a 14-fold reduction in the intestinal MRSA load as compared with untreated controls. Each drug alone resulted in a two to threefold reduction in MRSA load.

Viability assays with thioridazine racemic, thioridazine (-) (JEK47) and dicloxacillin was performed simultaneously on a few selected MRSA and MSSA isolates as described in [1] and compared to each other. Among the selected isolates was also MRSA of the clone type CC398, which is supposed to originate from pigs. The results showed no significant difference between the two drugs with the aspect of killing effect. An investigation of the dose-response of the two thioridazine drugs performed on a MSSA strain indicated that above concentrations of 20 mg/L JEK47 seemed to be slightly better than the racemic thioridazine.

In conclusion our observations indicate that thioridazine is a quite potent helper compound in the treatment of *Staphylococcus aureus* infections and *in vitro* results can be reproduced *in vivo*. The results also suggest the superior potential of JEK47 in the context of antimicrobial treatment due to potentially fewer side-effects than thioridazine (+) and racemic.

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Cowriters

Kirstine Jacobsen[1], Mette Thorsing[1], Nadia R.D. Kristensen[1], Julie Clasen[1], Eva M.S. Lillebæk[1], Lone Schøler[3], Anette Nielsen[1], Jørn B. Christensen[4], Oliver Hendricks[5], Anders Olsen[3], Marianne N. Skov[2], Jette E. Kristiansen[6], Birgitte H. Kallipolitis[1], Hans Jørn Kolmos[2], Janne K. Klitgaard[1,2]

*[1]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*
*[2]Institute of Clinical Research, Research Unit of Clinical Microbiology, University of Southern Denmark, J.B. Winsløw Vej 21,2, DK-5000 Odense C, Denmark*
*[3]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*
*[4]Department of Chemistry, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*
*[5]King Christian X Hospital for Rheumatic Diseases, SDU, Gråsten, Denmark*
*[6]Member of Center for Biomembrane Physics, MEMPHYS, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

Symposium 10: Antibiotic stewardship in theory and practice

## Can antibiotic stewardship turn the tide of antibiotic resistance

Livermore DM[1]

[1]*University of East Anglia, Norwich, Norfolk*

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Profligate antibiotic use selects resistance for little or no health benefit; stewardship seeks to ensure that antibiotic usage is controlled and proportionate. The approach establishes multidisciplinary teams to agree antibiotic treatment guidelines for common infection types and audits prescribers' usage against these guidelines, with feedback on compliance. Multiple studies confirm that the approach leads to improved antibiotic use, ensuring that more patients receive appropriate therapy whilst reducing overall use and selection pressure for resistance. In a few cases reductions in resistance are achieved, though this is not universal, and it is easier to displace resistant bacterial clones by a combination of stewardship and improved infection control, than to reduce plasmid-borne resistance that has disseminated among bacterial strains. Beyond this, there remains the question of 'What is optimal stewardship'? Many programmes favour a single antibiotic for a particular infectious setting. This is easy to police but concentrates selection pressure on the favoured drug, potentially leading to sequential treatment destruction, as with gonorrhoea, where low-dose penicillin, high-dose penicillin, ciprofloxacin and cefixime were lost in turn. It may be better to seek to cap total use but to encourage diversity in which antibiotics are used. Various cycling and mixing models could be used to achieve this. In the longer term a different strategy is becoming feasible: to identify the genetic signatures of pathogens and their resistances in clinical specimens within hours, rather than the 2-3 days needed for conventional culture and susceptibility testing, and to individualise treatments. This will allow much more sophisticated stewardship than at present.

## Education of healthcare professionals on prudent antibiotic prescribing

Pulcini C

*Infectious Disease Department, Nancy University Hospital, France*

Education is a core component of antimicrobial stewardship programmes. All healthcare professionals (medical doctors, dentists, pharmacists, nurses, veterinarians…) should be trained, in all settings. Education should target both undergraduate students, as well as senior staff, and be continuous throughout professional lives (1,2).

Up until now, most efforts have focused on senior doctors in hospitals and general practitioners in the outpatient setting. Less has been done for undergraduates, and for non-medical senior healthcare professionals (1,2,3). National curricula integrating prudent antibiotic prescribing learning outcomes are still rare (4). There is considerable room for improvement, since several studies have consistently shown gaps in knowledge and a desire for further education on prudent antibiotic prescribing among healthcare professionals (1,2,5). Regulatory measures are needed to improve this situation.

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## Antibiotic stewardship in the region and beyond: sharing good practices

Beović B

*Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia*

The pandemics of antimicrobial resistance and the lack of effective antibiotics for the treatment of infections caused by multi-resistant bacteria increase the importance of antibiotic stewardship.

Antibiotic stewardship includes various types of interventions, from structural changes, education and other types of persuasive interventions, restrictions, and surveillance to the support of modern technology. The interventions have been proven successful in many studies. It has been shown that the interventions may decrease the antibiotic consumption and lower the resistance at no cost in the treatment efficacy. Recently, it has been shown that restrictive interventions work better on short term, but later on, the effect of persuasive and restrictive interventions is equal. Several medical specialities are involved in the antibiotic stewardship interventions. Most studies have shown the crucial role of infectious diseases specialists. Clinical microbiologists and pharmacists are inevitable part of the antibiotic stewardship team, and play the leading role in some settings.

In spite of well-known effect of the interventions, the map of antibiotic consumption and antimicrobial resistance in common bacterial pathogens jn Europe remains very colourful. The differences are huge. There are much less data on antimicrobial consumption and resistance in European countries outside European Union. The countries on the Balkan peninsula seem to be the most problematic part of Europe.

Recently, a report on antimicrobial consumption in some South-east European countries and the neighbouring countries in Asia has been published. Although the published data offer the first information on the antimicrobial consumption in the countries in the study, wide over-the-counter purchase of antibiotics lowers the reliability of the report. The data on antimicrobial resistance in the same European countries are scarce, but it seems that the resistance rates are very high. There are several reports on extremely drug resistant bacteria such as carbapenemase-producing enterobacteriae and pan-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Some resistant bacteria were isolated in patients who were transferred from the hospitals in the region to hospitals in other parts of Europe.

Activities in Slovenia include national antibiotic team since 2006 and mandatory antimicrobial consumption surveillance in hospitals. In addition, prudent use of antimicrobials is regulated by a ministerial rule. Each hospital is responsible for the antimicrobial stewardship program. The consumption of antimicrobials in the hospitals remains stable. In outpatients, a substantial decrease in consumption has been observed since the year 2000. In University Medical Centre Ljubljana, antibiotic consumption is the lowest in the units with regular infectious diseases physician visits. In UMC Ljubljana, antibiotic committee has been in place since

1976, and restricted list of antimicrobials has been used since 1999. Various interventions are introduced according to current problems.

The reports on antibiotic stewardship interventions in the region are not very common. Most reports come from Croatia, which have long tradition in antimicrobial resistance surveillance, activities in hospitals and in out-patients. More than ten years ago, Bulgaria reported to have a national antibiotic team, which was not in place in Romania, Former Yugoslav Republic of Macedonia and Serbia and Montenegro. All countries with the exception of FYROM reported hospital antibiotic teams. Four years later, in 2007, Serbia reported several antimicrobial stewardship structures and interventions in five hospitals included in the survey. A multi-centre survey in intensive care units, which included hospitals in Slovenia, Hungary, Bosnia-Herzegovina, Kosovo, Serbia, Romania, Macedonia, Albania, and Bulgaria showed that written antibiotic guidelines are only used in one quarter of hospitals, included in the study. Several reports on interventions come from Greece, the country with one of the highest antimicrobial consumption and resistance rates in Europe.

In spite of the activities in many countries, the antimicrobial consumption and resistance in the region remain high. The problems seem to be multi-factorial, the lack of well-trained experts is accompanied by the financial problems and recent radical changes in the health systems. International co-operation in the region with the exchange of good practices offers an affordable solution.

Symposium 11: Inflammation and cancer – Bulgarian experience

# Advances in immunotherapy in solid tumors

Konsoulova A, Donev I

*University Hospital "Sveta Marina", Varna, Bulgaria*

Immune system protects the organism from foreign antigens by producing antibodies, circulating until they find and attach to the targeted antigen, thus triggering immune response and destruction of the antigen-containing cells. Another important function of the immune system is to recognize normal cells and prevent self-destruction. To do this, it uses endogenous "immune checkpoints" that normally terminate immune responses after antigens activation of T-cells.

Human cancers arise from normal cells and harbour genetic and epigenetic alterations, generating potentially recognizable by the immune system neoantigens.[1] The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades.[2] Although an endogenous immune response to cancer is observed in preclinical models and patients, it is ineffective as tumors develop new resistance mechanisms, including local immune suppression, induction of tolerance or dysfunction in T-cell signalling, down-regulation of tumor-specific antigen expression and presentation, secretion of anti-inflammatory cytokines, and upregulation of inhibitory ligands.[3-5] Moreover, tumors may exploit several distinct pathways to modulate or evade immune recognition and destruction, including endogenous immune checkpoints inhibitors.

These observations have resulted in intensive efforts to develop immunotherapeutic approaches as cancer treatment options. The immunotherapeutic approach as potential cancer treatment has been evaluated over the past several decades. Initial attempts included non-specific immunotherapies that don't target cancer cells specifically (cytokines, interleukins, interferons, etc). Subsequent efforts tried to identify antigens of cancer cell and design monoclonal antibodies (MAB), targeting those antigens, including immune-checkpoint-pathway inhibitors such as anti–cytotoxic T-lymphocyte antigen-4 (anti-CTLA-4) antibody (ipilimumab), anti- programmed death 1 (anti-PD-1) inhibitor (pembrolizumab, lambrolizumab, nivolumab, pidilizumab), anti-PD-L1 inhibitor (MPDL3280A, BMS-936559, MEDI4736), etc. (Table 1)

Table 1 Immune-checkpoint-pathway inhibitors and their targets in currently running clinical trials

| Target | Drug name | Biological description | Pharma company | Tumor site |
|---|---|---|---|---|
| CTLA-4 | *Ipilimumab (BMS-734016)* | MAB | Bristol-Myers Squibb | MEL, CRPC, Liver cancer, NSCLC and other advanced solid malignancies |
| PD-1 | *Nivolumab (BMS-936558)* | Fully human IgG4 MAB | Bristol-Myers Squibb | MEL, CRC, CRPC, NSCLC, RCC and hematologic malignancies |
| | *Pembrolizumab (MK-3475)* | Humanized IgG4 MAB | Merck Sharp & Dohme Corp. | MEL, NSCLC |
| | *Lambrolizumab* | Humanized IgG4 MAB | Merck | MEL, NSCLC |
| | *Pidilizumab (CT-011)* | Humanized IgG1 MAB | CureTech | Hematologic malignancies |
| | *AMP-224* | IgG1 fusion protein | GlaxoSmithKline/Amplimmune | Solid malignancies |
| PD-L1 | *BMS-936559* | Fully human IgG4 MAB | Bristol-Myers Squibb | NSCLC, MEL, CRC, RCC, ovarian, pancreatic, breast cancer |
| | *MPDL3280A* | MAB | Roche/Genentech | MEL, RCC, NSCLC, bladder cancer |
| | *MEDI4736* | MAB | MedImmune | MEL, NSCLC, SCCHN |

Abbreviations: MAB – monoclonal antibody; Melanoma (MEL); castrate-resistant prostate cancer (CRPC); renal cell cancer (RCC); Non-Small-Cell Lung Cancer (NSCLC); Colorectal cancer (CRC); Squamous Cell Carcinoma of the Head and Neck (SCCHN)

So far, immunotherapy has had limited success in the treatment of solid tumors except in the treatment of melanoma and renal cell cancer.[6-9] Tumors overwhelm the immune system via multiple strategies, including alterations in antigen expression, interference with T-cell priming, and effects known as “immune editing,” whereby tumors manipulate their microenvironment during development in order to escape immune detection and eradication.[3] Blocking antitumor T-cell responses and checkpoint pathways benefits the tumor, preventing tumor destruction and leading to equilibrium between the tumor and immune system.

Newer drugs that target these checkpoints hold a lot of promise as cancer treatments. T-cell checkpoint regulators such as CTLA-4 and PD-1 are cell surface molecules that, when engaged by their cognate ligands (PD-L1 and PD-L2), induce signaling cascades down-regulating T cell activation and proliferation.

## 1. Role of the PD-1 Pathway in the Immune Response

Accumulating evidence shows correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in different malignant tumors.[10-23] The PD-1/PD-L1 interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1 under healthy conditions is to downmodulate unwanted or excessive immune response, including autoimmune reactions. PD-1 is expressed by activated T cells, mediating immunosuppression. PD-1 functions in peripheral tissues, where T cells encounter immunosuppressive PD-1 ligands PD-L1 and PD-L2, expressed by tumor cells, stromal cells, or both.[11,24-26] Inhibition of the interaction between PD-1 and PD-L1 enhances T-cell responses in vitro and mediates preclinical antitumor activity.[11,16] PD-L1 leads to inhibition of the T-lymphocyte proliferation, survival and effector functions (cytotoxicity, cytokine release), inducing apoptosis of tumor-specific T-cells and promoting the differentiation of $CD4^+$ T cells into regulatory T cells. The blockade of PD-1 / PD-L1 results in a potent and durable tumor regression and prolonged stabilization in patients with advanced malignancies.[27] Therefore, inhibition of PD-L1 binding to PD-1 represents an attractive strategy to restore tumor-specific T-cell immunity.

The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling protein. PD-1 was shown to be expressed on T-cells, B-cells, monocytes, and natural killer T cells, following their activation.[28,29] PD-L1 and PD-L2 are expressed in a variety of cell types, including non-hematopoietic tissues as well as in various malignancies. PD-L1 is expressed at low levels on non-hematopoietic tissues, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells in lymphoid tissue or chronic inflammatory environments. PD-L2 controls immune T-cell activation in lymphoid organs, whereas PD-L1 serves to protect healthy tissues from unwarranted T-cell immune-mediated damage.

Although healthy organs express little (if any) PD-L1, many cancers express abundant levels of this T-cell inhibitor. High expression of PD-L1 on tumor cells (and to a lesser extent PD-L2) has been found to correlate with poor prognosis and survival in various cancers, including RCC[30], pancreatic carcinoma[31], hepatocellular carcinoma[32], and ovarian carcinoma.[33] Furthermore, PD-1 has been suggested to regulate tumor-specific T-cell expansion in melanoma patients.[34]

The observed correlation of clinical prognosis with PD-L expression in multiple cancers suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

## 2. Adverse effects of immunotherapy

Adverse events (AE) are graded using NCI Common Terminology Criteria for Adverse Events Version 4.0. Their management is important as the population of treated patients frequently consists of patients with disseminated disease or patients who have been previously treated with multiple lines of treatments. Most frequent drug-related AE with potential immune-related causes are hepatitis, pneumonitis, infusion reactions, colitis, arthralgia and rash, necessitating sometimes the use of corticosteroids.[35] Fatigue, decreased appetite, nausea, dyspnea, constipation, vomiting, pyrexia, and headache are also described as drug-related AE.

## 3. Tumor response evaluation of immunotherapy

An issue that has been recently recognized is the measurement of antitumor effect of immunotherapy. The cytotoxicity of chemotherapeutic agents often produces measurable change in the size of target lesions within weeks of the initial administration. Response for solid tumors is assessed using WHO or RECIST criteria.[36,37] For cytotoxic agents, these guidelines assumed that an early increase in tumor growth and/or appearance of new lesions signaled progressive disease (PD) and the term "progression" became synonymous with drug failure. Cessation of the current chemotherapy is thus recommended once PD is detected.

On the other hand, immunotherapeutic agents enhance antitumor immune responses[38], stable disease (SD) may also be viewed as an indicator of meaningful therapeutic effect. Beyond that, additional novel response patterns observed with these agents raise concerns about the interpretation and characterization of WHO or RECIST criteria. In studies with cytokines, cancer vaccines, and monoclonal antibodies, CR, PR, or SD has been shown to occur after an increase in tumor burden characterized as PD by WHO or RECIST criteria. [39-42] Therefore, conventional response criteria may not adequately assess the activity of immunotherapeutic agents because PD (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Thus in order to systematically characterize additional patterns of response in patients, treated with immunotherapy underlying WHO criteria were evolved into immune-related response criteria (irRC).[43] The core novelty of the irRCis the incorporation of measurable new lesions into "total tumor burden" and comparison of this variable to baseline measurements (before and after WHO PD, but not after confirmed irPD). Clinical activity often appears to be delayed following immunotherapeutic treatment and a period of apparent progression (as defined by the existing response criteria) may occur, followed by response. Four types of distinct response patterns have been described: (two conventional and two unique to immunotherapy): 1. immediate response; 2. durable stable disease; 3. response after tumor burden increase and 4. response in the presence of new lesions. The apparent increase in tumor burden that sometimes precedes response in patients receiving immune therapy may reflect either continued tumor growth until a sufficient immune response develops or transient immune-cell infiltration into the tumor with or without edema.[43]

The use of irRC for response evaluation with immunotherapeutic treatment is considered clinically meaningful as they appear to be related to favorable survival. However, they are still in early development and prospective trials need to evaluate their role and potential association with survival.

## 4. Predictive and prognostic biomarkers for immunotherapy

Research is ongoing in order to identify potential biomarkers for cancer immunotherapy. PD-L1-positive cancers are associated with poorer prognoses than PD-1 negative. A correlation of PD-L1 expression and response rate was demonstrated in patients with the highest levels of PD-L1 expression and PD-L1-positive TILs.[44] The potential role of PD-L1 as well as TILs as a biomarkers remain to be elucidated.

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# Angiogenesis in cancer and inflammation - role, evaluation and clinical significance

Konsoulova A, Donev I, Dimitrova E

*Medical Oncology Clinic, University Hospital "Sveta Marina", Varna, Bulgaria*

The role of inflammation in carcinogenesis has first been proposed by Rudolf Virchow in 1863, when he described presence of leukocytes in neoplastic tissue samples.[1] Since thisVirchow's early observation that linked inflammation and cancer, accumulating data have supported that tumors can originate at sites of infection or chronic inflammation.[2] About 25% of all cancers are somehow associated with chronic infection and inflammation.[3] The possible mechanisms by which inflammation can contribute to carcinogenesis include induction of genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumor neoangiogenesis with subsequent invasion and metastasis, etc. While inflammation promotes development of cancer, components of the tumor microenvironment, such as tumor cells, stromal cells in surrounding tissue and infiltrating inflammatory/immune cells generate an intratumoral inflammatory state by expression or activation of proinflammatory molecules. They turn on the angiogenic switches mainly controlled by the vascular endothelial growth factor (VEGF), thereby inducing inflammatory angiogenesis and tumor cell-stroma communication. This ends up with tumor angiogenesis, metastasis and invasion.[4]

There are four cardinal signs of inflammation – rubor, tumor, calor and dolor. The first 3 of them in fact reflect the changes that appear in the vasculature in state of inflammation. Blood vessels are normally a well-organized structure of hierarchy - arteries, arterioles, capillaries, venules and veins. Each type of vessels has a distinct pattern of structural and functional characteristics. Vessels proliferate by sprouting from the pre-existing ones – a process known as angiogenesis.[5] Blood vessels change in phenotype in inflammatory diseases, cancer and other chronic inflammatory conditions – a process referred to as vascular remodeling. Those changes in the newly formed vessels reflect the adaptive processes, occurring as consequence of inflammation or cancer. In inflamed tissues the neovascularization and the remodeling processes lead to increased blood flow, plasma leakage and inflammatory cell infiltration. Blood vessels in tumors differ from those in inflammation - the sprouting of the endothelial cells, their proliferation is a disorganized process, leading to formation of new vessels that differ from normal ones with structural defects presenting by leakiness, high luminal resistance, poor blood flow, elevated interstitial pressure and altered immune cell traffic. The growing vasculature becomes dependent on the vascular endothelial growth factor (VEGF) and other growth factors for survival.

The process when the tumor transforms into a vascularized structure is referred to as angiogenic transformation and is characterized by several steps that are initiated by specific endothelial growth factors, produced by the tumor or the surrounding connective tissue.[6] Via migration and proliferation of the activated endothelial cells new capillaries are being formed; their basal membranes and

extracellular matrix is dissolved by enzymes such as the metalloproteinases. Adhesion molecules then enhance the connection between the endothelial cells of the new and the preexisting vessels; the smooth muscle cells and the pericytes stabilize the already mature vascular system.[7] Though abundant, the blood flow and the correspondent oxygen supply remain inadequate, due to those structural defects.

The angiogenesis is promoted by the so called proangiogenic stroma where imbalance with over production of angiogenesis stimulators and/or decreased levels of angiogenesis inhibitors exists.[6] Amongst the proangiogenic growth factors, produced by the human cancers, most significant are the basal fibroblast growth factor (bFGF), epidermal growth factor (EGF) and VEGF.[8] The expression of these proteins could be induced by oncogenes and could facilitate the transfer to angiogenic phenotype.[6,8] In healthy conditions the organism is protected by endogenous angiogenesis inhibitors in the tissues and/or the circulation.[8]

## 1. VEGF and VEGFR family

Angiogenesis contributes to the progression of cancer from a dormant *in situ* lesion to a life-threatening metastatic disease. The key role of vascular endothelial growth factor A (VEGFA) and its receptor VEGF receptor 2 (VEGFR2) in tumour angiogenesis is firmly established. Not surprisingly, the vast majority of anti-angiogenic strategies focus on inhibiting VEGFA and VEGFR2 signalling.[9] Although this has proved successful for some patients, anti-angiogenic therapy remains challenging.[10] First, a substantial fraction of patients with cancer is resistant to VEGF-based therapies.[11] Second, despite disease stabilization and an increase in the proportion of progression-free patients or the overall survival of patients with cancer treated with anti-VEGF therapies, tumours eventually escape and relapse, such that anti-VEGF therapies currently produce an absolute gain in duration of survival in terms of months rather than years.[12,13]

## 2. VEGF

VEGF is one of the most important pro-angiogenic factors in state of physiology and pathology.[14] VEGF was first identified in the late 80s by Ferrara and nowadays it is a well-established fact that the family of VEGF and their corresponding tyrosine-kinase receptors are key factors in angiogenesis both for lymph and blood vessels.[15,16] VEGF is a growth factor that produces its pro-angiogenic effects via its receptor - VEGFR.[17,18] It was first described as a factor, inducing extravasation of macromolecules through the endothelial-cell barrier and vasodilation by inducing the NO-synthase.[19,20] VEGF induces proliferation, migration and increases the endothelial survival.[14] The family of VEGF consists of several structurally related forms - VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF.[17,21]

The principal mediator of the tumor angiogenesis is VEGF-A or only VEGF. VEGF transmits its signal predominantly by VEGFR-2 that is mostly expressed in endothelial cells, involved in the angiogenesis.[18] VEGFR-2 binds to VEGF-A and thus playing role of a primary receptor, promoting angiogenesis in physiological and pathological conditions as inflammation and cancer.

The precise role of VEGF-B *in vivo* is unknown; there are data suggesting its role in the heart development.[22] VEGF-C and VEGF-D stimulate the lymph vessel

development.[23,24] PLGF seems to participate in the arteriogenesis and the formation of collateral arteries.[25]

The role of VEGF-R 1 is still unclear – the aphynity of VEGF for VEGFR1 is much stronger compared to that for VEGFR2, but the signal, transmitted via VEGFR1 is extremely weak.[16 26] Thus the signal is transmitted predominantly via VEGFR-2.[16] The weak tyrosine-kinase activity of VEGFR1 and the high aphynity for VEGFA was proved by a model where VEGFR1 serves as a receptor that attracts and modulates the angiogenesis by sequestering VEGFA, thus reducing the signal transmitted via VEGFR2,[27] avoiding the hyperactivation of signal pathways, stimulated by VEGFR2.[28]

Most of the human cancers highly express VEGF which might be induced by different genetic and epigenetic mechanisms. The over expression of VEGF is frequently related to bad prognosis. Hypoxia is very typical for solid tumors and is an important inducer of VEGF secretion, mediated by hypoxia induced factor 1 (HIF-1).[29] HIF binds to the promotor or the regulatory areas of the genes.[30,31] In ischemic tumors the VEGF levels are increased in proximity of necrotic areas.[32]

Other regulators of the *VEGF* expression are cytokines and growth factors such as epidermal growth factor (EGF), transformed growth factor (TGF), interleukin-1 (IL-1) and insulin-like growth factor-1 (IGF-1).[33-36] Stimulators of *VEGF* expression are the proinflammatory cytokines (e.g. IL-6), low pH, fibroblast growth factor, etc. The activation of different oncogenes or the deactivation or suppression of tumor-suppressor genes (e.g. *p53,*[16]*, RAS*, *Raf и HER2*[37-39]) also leads to induction of VEGF secretion.[40] VEGF secretion is also regulated at posttranslational level.

## 3. Signal transduction via VEGF

It has been well established in the last years that the development of the neural and the vascular systems follows same patterns. Nerves and blood vessels frequently run in parallel and both system present as a network of branches. The structuring of both networks is regulated by attractive and repulsive signals that appear to be common.[41-43]

Four ligands and their receptors have been described as regulators of the neuronal growth and the angiogenesis - Semaphorins, Ephrins, Slits and Netrins, as well as their corresponding receptors – neuropilin (NRP), Eph, roundabouts (Robo) and uncoordinated-5 (UNC5). All those receptors are expressed both in neurons and endothelial cells. They produce attractive or repulsive signals to their ligands. As an example for this regulation by attractive or repulsive signals is the binding of VEGF to NRP and its isoform NRP1, which leads to an increase in the endothelial cell migration [44], while the binding of semaphorin to NRP1 leads to inhibition of the endothelial cell migration.[45]

The biological and molecular similarities between both neural and vascular systems have always been interesting subject of scientific study especially after the discovery that the signals, acting as repulsive to the neuronal system are identical to those inhibiting the process of angiogenesis.

## 4. Conclusion

The discovery of the molecular mechanisms involved and guiding the tumor angiogenesis proved its significance for the tumor survival, progression and

metastasis development. That led to synthesis, introduction and incorporation of many angiogenic drugs into the cancer treatment which predominantly block the VEGF/VEGFR signaling pathway. The discovery of the family of VEGF and VEGF - receptors, as well as their mechanism of action revealed their role for the angiogenesis which is a crucial process in inflammation as well as a surrogate for survival and progression of tumors.

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# (18F)-FDG PET/CT neuroimaging: a diagnostic challenge to the brain neoplasms

Kaprelyan A[1], Bochev P[2], Tzoukeva Al[1], Georgiev R, Grudkova M[1]

*[1]Department of Neurology, Medical University "Prof. Dr. P. Stoyanov", Varna, Bulgaria*
*[2]Department of nuclear medicine and radiotherapy, Medical University "Prof. Dr. P. Stoyanov", Varna, Bulgaria*

Keywords: brain neoplasm, (18)F-FDG-PET/CT, tumor recurrence, posradiation necrosis

Although the recent advances in structural neuroimaging, the postoperative delineation of recurrent brain tumors and their differentiation from certain morphological changes in treated tumor site still present a great diagnostic challenge (7). Accordingly, the additional use of functional non-invasive diagnostic techniques, such as SPECT and PET improves the detection of cerebral neoplasms, based on the differences in cerebral blood flow and metabolic activity of brain and tumor tissue (2, 4, 14, 20). Data exist that brain consumes more than 60% of assimilated glucose, therefore (18F)-FDG is the most suitable radiotracer for assessment of normal cerebral function and dysfunction associated with tumor growth (15, 16). It is well known that (18F)-FDG PET most commonly demonstrates zones of increased glucose metabolism in relation to brain tumor type, location, distribution, and grade of malignancy (5, 9, 10, 11, 13). This technique also enables the postoperative follow-up of brain neoplasms and their response to different therapeutic strategies (1, 4). In addition, based on the evaluation of radiotracer accumulation, PET scanning is able to distinguish recurrent gliomas from postradiation necrosis and to improve the differential diagnosis between primary cerebral lymphomas and AIDS-associated neuroinfections (6, 12, 19). This technique provides important information about the disease severity and prognosis (1, 5, 14). For instance, hypermetabolism in gliomas correlates to higher histological grades (III and IV) and shorter survival rates. (18F)-FDG PET plays a crucial role in detection of malignant transformation of low-grade gliomas into high-grade variants, has an influence on the therapeutic choice and assessment of its response (8, 18). Accordingly, in cases of radio- and chemotherapy, the partial and total response is related respectively to significant reduction or normalization of metabolic activity in the tumor site. It is also useful in early diagnosis of paraneoplastic syndromes in cancer patients with unknown primary tumor site (17).

The aim of our study was to evaluate the usefulness of brain FDG-PET/CT to detect tumor recurrence and distinguish it from postradiation necrosis in treated patients with clinical deterioration.

## 5. Materials and methods

### 5.1. Study population

A total population of 26 patients (16 males and 10 females, aged between 37 and 69 years) with brain neoplasms (16 gliomas and 10 meningiomas) were included in the study. The final diagnosis was based on neuroimaging (MRI/CT) findings and tumor grading according to the World Health Organization classification.

### 5.2. Magnetic Resonance Imaging

MR images were acquired on a 1.5T MR-imaging scanner (Signa Excite HDxt; GE Healthcare) with the following technical characteristics: software version - 15.0IM4AI0947.a; ASSET, version 005.001 SH; Head coil, 8 channel and MR injection system Spectris Solaris EP Medrad. Standard study protocol included SAG T1, AX T2 FLAIR, COR T2 FLAIR, COR T2 FSEIR, 3D IR T1 techniques.

### 5.3. (18)F-FDG PET/CT scanning

All the patients performed (18)F-FDG PET/CT using Philips Gemini TF PET/CT (2009) with 16slice CT and 3D TOF PET. Patients' preparation included overnight fasting; fluid intake restricted to water only, tobacco avoidance, recent creatinine and urea tests, on-site measurement of blood sugar, excluding from the study the patients having more than 7 mmol/l blood glucose levels. FDG was injected manually through intravenous line in activity of 5.0mCi for sole brain scanning. After FDG administration all patients rested in a dimmed quiet room for 60 minutes and were then scanned. Acquisition was performed with a standard manufacturer's protocol for brain scanning using LowDoseCT 120Kev, 50mAs and PET in a single 10min frame with 256mmFOV, 2mm pixel size. We used a standard manufacturer's iterative reconstruction protocol brain-ctac, generating two datasets 3D RAMLA (PEVIEW) and LOR-RAMLA (Brain CTAC) without SUV calculation. The images were assessed visually and semiquantitatively.

#### 5.3.1. Visual assessment

The early image set, obtained for each patient was individually read by two nuclear medicine physicians and scored in a three-step scale (scores from 0 to 2). The datasets were anonymized and mixed. After the blind read, the two readers were made aware of the clinical data and the sequence of the scans and then subjectively assessed lesion delineation on early images and their contribution to the diagnosis. Score 0 was applied to any lesion not visualized on PET images, or hypometabolic with activity less than the surrounding tissues. Score 1 was reserved for patients with equivocal findings. Positive score 2 was applied to any lesion with focally increased metabolic activity exceeding that of grey matter or for lesions in the white matter with activity, clearly exceeding surrounding white matter activity although not reaching gray matter activity.

#### 5.3.2. Semiquantitative analysis

Region of interest (ROI) was placed over the hottest part of the lesion if hypermetabolic or over the lesion identified on CT, if not active. ROI was drawn as a spherical model in respect to the position in all three planes. Maximum pixel counts value was used for the calculations. Reference ROI was mirrored in the contralateral region. Lesion ROI maximum count/pixel were compared to contralateral ROI, ROI over white matter (parietal, avoiding lateral ventricles) and ROI over cerebellum. Differences of indices by tumor groups were tested for significance by Student's t-test for unpaired data. The data were assessed additionally by receiver operating characteristic analysis (ROC). For the purposes of ROC analysis the patients were classified as a positive fraction (having tumor) and a negative one (tumor free).

## 6. Results

Visual analysis revealed a recurrent tumor mass in total group of 15 (57.7%) patients: ten (62.5%) with gliomas (Case 1) and five (50%) with meningiomas. (18)F-FDG PET/CT scans showed lack of tumor recurrence in four (25%) cases with gliomas (Case 2) and five (50%) with meningiomas. In two (12.5%) patients with gliomas the features of glucose metabolic activity succeeded to distinguish postradiation necrosis from tumor recurrence (Case 3). The semiquantitative indices demonstrated significant differences among patients with and without recurrent tumor or postaradiation necrosis.

## 7. Discussion

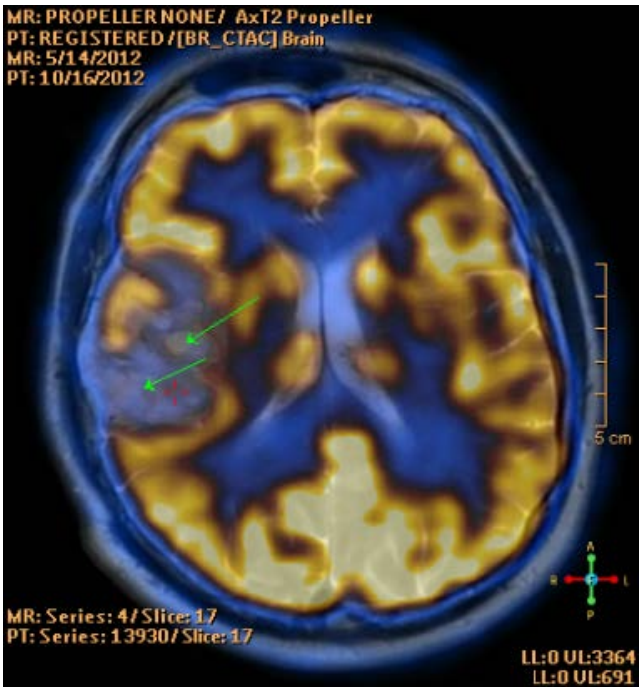
MRI and CT are routine diagnostic methods in neuro-oncology, but sometimes they may find certain difficulties, especially in the diagnosis of treated brain tumors (7, 20). In addition, PET scanning is used to detect brain tumors, based on the evaluation of cerebral glucose metabolism (2, 4, 10, 15). Accordingly, brain tumors are characterized by specific pattern of FDG uptake (4, 9, 11, 13). PET is also considered a golden standard for the pre-operative tumor grading and follow-up, especially in patients with clinical deterioration (5, 8, 9, 14). In agreement with previous reports, we performed (18)F-FDG PET/CT in 26 treated patients clinically suspected for tumor recurrence. The postoperative scans showed functional brain dysfunction in 57.7% of all cases, corresponding to tumor growth. In 34.6% of patients, although the deterioration of clinical symptoms, PET images were negative for tumor recurrence. These findings are in agreement with previously reported data, supporting the accuracy of (18)F-FDG PET/CT in postoperative delineation of possible tumor recurrence (12, 18, 19).

It is known that alterations in clinical course might be also due to morphological changes in tumor site, as a consequence of neurosurgery, radio- and/or chemotherapy (1, 6, 8, 12). Accordingly, several data exist that (18)F-FDG PET/CT is a suitable method for the differentiation of recurrent tumor from postradiation necrosis (8, 12, 14, 19). In accordance, we succeeded to distinguish brain tissue necrosis from postoperative tumor growth in two patients with high-grade gliomas. We found the same low accumulation of radiotracer in treated tumor site, as reported in earlier studies (1, 6, 8, 18).

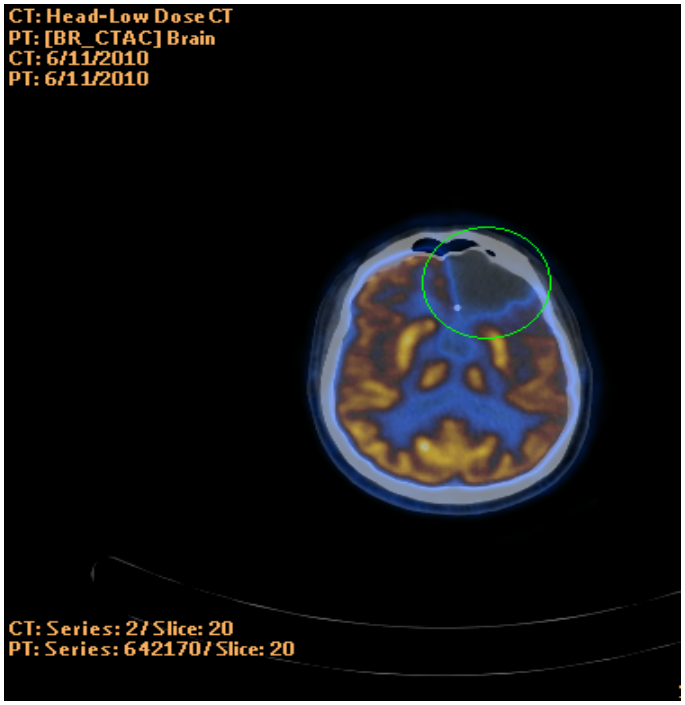
Nowadays, the development of computer technologies enables the reconstruction of morphological and functional images, obtained from MRI/CT and PET techniques (3, 16). Evidence exist that the co-registration of (18)F-FDG PET and CT scans facilitates the immediate comparison of location, characteristics, and distribution of brain dysfunction and improves the diagnostic accuracy (6, 17). In regards, our comparative (18)F-FDG PET/CT scanning confirmed the previous observations and clinical usefulness of this hybrid method in postoperative detection and differentiation of brain neoplasms.

In conclusion, based on our own findings and literature review, we support the notion that brain (18)F-FDG PET/CT images provide high accuracy in tumor delineation and its differentiation from postradiation necrosis. In addition to structural neuroimaging, the use of visual assessment and semiquantitative indices has a significant diagnostic value for the early and precise evaluation of treated patients with brain neoplasms and clinical deterioration.

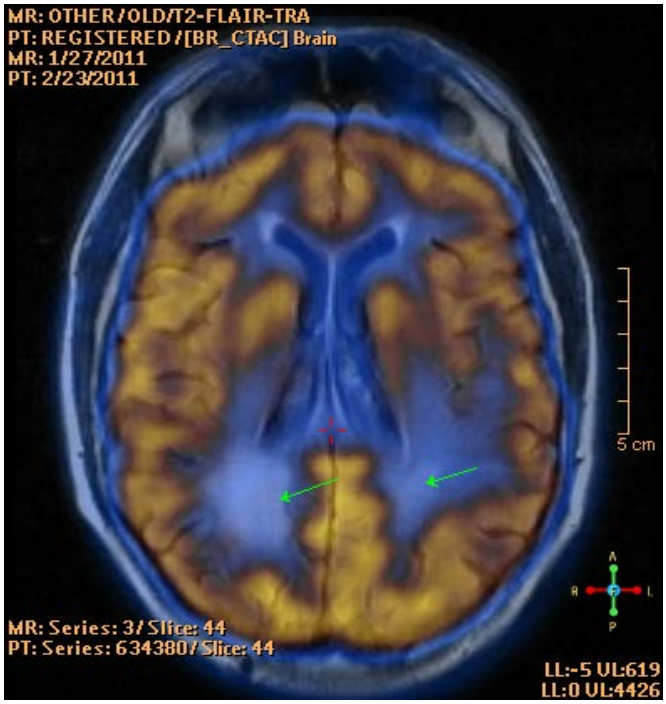
**Case 1:** 60-years old male with oligodendroglioma. Postoperative (18)F-FDG PET shows zone of hypometabolism in parts of right parietal and temporal lobes and in a small posterior-frontal region. Radionuclide uptake is higher than white matter in early and late scans. Neuroimaging findings correspond to recurrent low-grade glioma.




**Case 2:** 40-years old male with high-grade oligodendroglioma. (18)F-FDG PET after tumor operation, radio-, and chemotherapy shows a zone with very low metabolic activity in left frontal lobe excluding tumor recurrence.



**Case 3:** 49-years male with glioblastoma multiforme. (18)F-FDG PET after operation, radio- and chemotherapy shows hypometabolic zone in right occipital region and relatively increased activity corresponding to postradiation necrosis.

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# Femoral neck fractures - epidemiology and social burden

Filipov O

*Department of Geriathic orthopedics, Vitosha Hospital, Sofia, Bulgaria*

Keywords: femoral neck fracture, intracapsular fractures, osteoporotic fractures, hip, epidemiology

The incidence of femoral neck fractures, one of the most common traumatic injuries in elderly patients increases continuously among the ageing population on the planet [1,2].

The UN Human Rights Commission in 1999, has proposed to use the term "older people" instead of the word "elderly" [3]. Older people are the fastest growing age group in the world and the annual number of hip fractures will grow with the continued ageing of population. Even if age-related incidence of hip fractures continues to grow with unchanged rates, the number of hip fractures worldwide is expected to increase from 1.7 million in 1990 to 6.3 million in 2050. Assuming that the age-related incidence will increase by only 1% per year, the number of hip fractures in the world will reach the figure of 8.2 million in 2050 [2].

Femoral neck fractures and pertrochanteric fractures are of approximately equal incidence [4,5] and together make up over 90% of the proximal femur fractures and the remaining 5-10% are subtrochanteric. According to more recent research, half of the proximal femur fractures are intraarticular fractures of the femoral neck [6,7].

Most of the hip fractures occur after a fall. It is estimated that the lifetime risk of hip fracture was 23.3% for men and 11.2% for women [8].

The femoral neck fractures are rare among young people – they are only 2% in patients under 50 years of age [9]. The incidence increases with age, and after 50 years is doubled for each subsequent decade, and is 2-3 times higher in women than in men [5,10]. 80% of hip fractures occur in women and 90% in people older than 50 years [2]. They are twice or three times as common for white women as for black women [4,5]. The overall annual age-standardized rates of both femoral neck fractures and trochanteric fractures are higher among white women than among black women (4.33 vs. 1.91 and 4.23 vs. 1.54 per 1,000, respectively) [6].

Age-adjusted rate of hip fractures is highest in Scandinavian and North American populations and is lower in Southern European countries. Hip fracture risk is lower in Asian and Latin American populations and is lower in rural areas than in urban areas [2].

The increase in incidence of hip fractures with increasing the age is a result of an age-related decrease of bone mass in the proximal femur as well as of the age-related increase in the incidence of falls.

Study on femoral neck fractures in New England revealed that the incidence among white women aged 65-69 years was 2,2 per 1000 per year. This rate increased up to 31.8 per 1,000 per year at the age of 90-94 years. With white men aged 65-69 years,

the rate was 0.9 and rising up to 20.8 for the 90-94 age group [11]. In the UK, a rate of 1.6 (per 1000 per year) was registered for women aged 65-69, and 32.8 for age group 90-94, and 0.7 and 14.0 for men respectively [12].

Dhanwal [13] reported that, there are wide racial and geographical variation in the incidence of femoral neck fractures worldwide, with the highest incidence in industrialised countries, compared with developing countries. The rate among the black population is lower than that in white population in all age groups [11]. Among the population of Asia, lower incidence rates of femoral neck fractures are registered. In Japan, the frequency is 99 (per 100,000 per year) in men, and 368 in women. In 1990, the age-standardized rates of hip fracture in China were 87/100 000 for women and 97/100 000 for men. The highest incidence rate in Asia is registered in Singapore: 152 (per 100,000 per year) in males, and 402 in women. In Latin America, the frequency is also lower. In 2005 in Mexico, rates of 98 (per 100,000 per year) in men, and 169 in women were reported. In Argentina, the frequency is 137 in males, and 405 in women. In Africa, the incidence is 43,7 (per 100,000 per year) in men, and 57,1 in women [13].

The incidence rate in North America is the highest in the world: 201 (per 100,000 per year) in men and 511 in women [14]. The rate in Europe varies from Northern to Southern Europe, with the highest incidence in Sweden and Norway (399 per 100,000 per year in men and 920 in women) and the lowest in France and Switzerland (137 per 100,000 per year in men and 346 in women). These variations are explained by the differences in ethnic and climatic characteristics, as well as by the differences in living standards [13].

Increased frequency of these fractures has been observed over the years, combined with periods of declines. In the United States, the age-adjusted fracture incidence rate has increased over the period from 1986 to 1995 and then gradually reduced during the period 1995-2005. At the same time, in Denmark, the incidence of fractures of the proximal femur is decreased dramatically by 20% between 1997 and 2006 among the population aged over 60 years, which is related to the use of anti-osteoporotic therapy [15].

In Germany, the incidence continues to increase. Marked trend toward a slight decrease in the period 1997-2004 has been observed in Finland. In Sweden, the age-adjusted incidence rate among the population over 50 years of age has decreased, but the absolute rate of these fractures has increased among women over 90 years of age [16,17].

Institutionalized geriatric patients are exposed to a higher risk of femoral neck fracture. The annual incidence in New Zealand among the elderly living in their own home is 348 per 100,000 and is 10 times higher, 3975 respectively, among those older people living in an institution or a nursing home [18].

Patients with impaired cognitive status are at increased risk of femoral neck fracture. A significant increase in the incidence was observed when comparing the population of a mental hospital with the rest of the population in Sweden. The relative hip fracture risk was seven times higher for women and 12 times higher for men with mental disorders [19].

## 1. Economic aspects

Worldwide, the number of older people is expected to double by 2040, and the increase of hip fracture incidence rate is likely to become a substantial burden for the public health systems [20]. The proximal femur fractures are the most devastating result of osteoporosis. They require surgical treatment, often lead to disability and are associated with high mortality rate [20].

Currently, the hip fractures represent a major economic burden on the health care systems in the world. In the United States, adjusted first-year costs associated with hip fracture for patients aged 65 years or older were US $ 15,196, compared with the costs of US $ 6701 for vertebral fracture [21]. In 1997, an assessment of direct and indirect annual costs for hip fracture treatment in the world were $ 131,5 billion [22]. In 2005 in the United States are registered 2 million fractures with patients over 50 years old, costing a total of $ 17 billion for medical care. From all registered fractures, 14% were fractures of the proximal femur, but they take up a huge share of the 72% of the total value for the treatment of fractures. The total allocation of costs according to the type of treatment was 57% for hospitalized patients; 13 percent for outpatient treatment and 30% for long-term inpatient and institutional treatment. From the total cost of fracture treatment, 89% account for patients over age of 65 [23].

## 2. Conclusion

The increasingly aging population of the world will have to face the challenge of coping with the growing number of femoral neck fractures and with the increasing economic burden they represent for the healthcare system in the conditions of economic uncertainty in the future.

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Symposium 12: Hepatitis B in Europe: The burden and its management

# Management of HBV infection in Croatia – access to those in need for treatment

Vince A

*Department of Viral Hepatitis, University Hospital of Infectious Diseases, Zagreb, Croatia*

## 1. Epidemiologic background

The epidemiologic estimates are that around 25.000 people are chonically infected with hepatitis B virus (HBV) in Croatia. The prevalence ranges between 0.2% in pregnant women and 3% in intravenous drug users. Most of the infections are with HBeAg-negative strains of virus which coincides with 87% of genotype D among patients.

## 2. Clinical guidelines and reimbursement

Most of the patients are followed-up and treated at the Infectious Diseases Departments in 4 biggest cities in Croatia; Zagreb, Split, Rijeka and Osijek.

Croatian Health Insurance Fund currently reimburses treatment with 4 drugs: lamivudine, telbivudine, tenofovir and pegylated interferon alpha-2a (PEG IFN-α2a). Entecavir has not been registered in Croatia so far. Treatment is based upon the Clinical gudelines on viral hepatitis which are being updated every 4 years.

Current indications for treatment include HBV DNA>2000 IU/ml, fibrosis score>2 or fibroelastography>8kPa. First line therapy includes PEG IFN-α2a for 48 weeks, or tenofovir for patients not eligible for IFN treatment. However, lamivudine was the first-line treatment option for many patients in the past 14 years, prior to telbivudine and tenofovir introduction. Most of the patients treated with lamivudine and developing resistance are now being switched to tenofovir.

## 3. Results of treatment

The resistance pattern to lamivudine was consistent with the literature data: 20% of patients developed resistance after 12 months of treatment and 50% after 4 years of treatment. According to the data provided by the Croatian Reference Center for Viral Hepatitis, genotypic resistance of HBV to lamivudine and other nucleoside analogues was detected in 131/257 tested patients (50.9%). The most frequent pattern of resistance mutations in lamivudine-treated patients experiencing virological failure were M2014V/I/S and A181T as well as compensatory mutations L80V/I and L180M. In patients with no genotypic resistance, lamivudine therapy is usually continued. Tenofovir has been introduced in Croatia in 2011. There are now about 100 patients being treated with tenofovir, 90% of them switched from lamivudine or telbivudine or after the exacerbation of viremia following PEG IFN-α2a treatment. Median age of the patients is 56 years and 75% of patients are men. The normalization of ALT levels is usually observed

within 3 months of treatment but the inhibition of HBV replication is somewhat slower (it takes up to 12 months). One patient developed severe myalgias, otherwise no serious side-effects were noticed. IFN therapy demanded reinduction of therapy with nucleoside analogues in 67% of treated patients.

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Symposium 13: Hot topics in *Staphylococcus aureus*

## Control of Hospital MRSA

Gould IM

*Department of Medical Microbiology, Aberdeen Royal Infirmary, Aberdeen, Scotland*

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With its wealth of mobile genetic elements, *Staphylococcus aureus* has undergone rapid evolutionary change during the antibiotic era. Much of this evolution has been driven by antibiotic use and the many successful clones of MRSA attest to this. Fortunately most of these clones are not very virulent as the multiple resistance determinants carry a fitness cost. USA 300 has, however, demonstrated a different evolutionary approach with less resistance but fitness genes such as ACME and PVL making it very successful, firstly in the community and now in hospitals.

While hospital MRSA is traditionally seen as primarily an infection control problem, antibiotic stewardship has a major role to play in its control. Use of cephalosporins in particular, and quinolones and macrolides where strains are resistant, are major drivers of MRSA in our hospitals and reducing their use is a very effective MRSA control measure in addition to hand hygiene, admission screening, isolation or cohorting and body decontamination. Mupirocin and chlorhexidine are the most commonly used agents for such decolonisation or suppression although there are many possible alternatives. Other body washes include octenidene, triclosan, hexachlorophene and polyhexanide. Alternatives to mupirocin include also, povidone iodine, topical antibiotics like neomycin, polymicin, bacitracin and gramicidin, pleuromutilins like retapamulin and taurine derivatives. Oral antibiotics and topical quinolones cannot be recommended because of fears about development of resistance. Agents in development include fatty acids alkaloids, phenolics and peptides derived from naturally occurring substances, lysostaphin, probiotics and teatree oil. Photodynamic and phage based therapies are also being researched.

Current debates in MRSA control are many and the answers lie in understanding local epidemiology, prevalence and strain type so that control measures can be appropriately tailored. Specific, targeted control measures are probably, in general, better than broad reaching “universal measures”.

Symposium 14: Collateral effects of antibacterial drugs

## Risk factors for antibacterial drug adverse reactions

Nechifor M, Luca C, Gales C

*Dept. Pharmacology, Infect. Dis.Clinic Hospital, Dept. Histology "Gr. T. Popa" University of Medicine and Pharmacy Iasi Romania*

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The most important risk factors for antibacterial drug adverse reactions (ADRs) are: chemical structure and mechanism of action of drugs, age, off label use of antibacterial antibiotics, sex, genetic structure, number of antibiotics simultaneously administered and patient metabolic state. A complex relationship between these factors in ADR incidence and severity was observed.

Objectives: In this study was evaluated the incidence of ADRs to antibacterial antibiotics in different age groups of in children and adults of both sexes hospitalized in an infectious diseases clinic hospital.

Methods: The age groups were: 0-3; 4-10; 11-15; 16-65years. In the study were included 1303patients (673F and 630M).

Results: The number patients which ADRs during hospitalization was 237 (17,3%). A number of 49 patients have had two or more ADRs. The of ADRs incidence was higher in adult women (22.4%) compared to men (13.6%). No differences between incidence of ADRs in boys and girls until the age of 10 years. The most common ADRs were allergic reactions. There were no reported deaths due to ADRs.

Conclusions: The incidence of side effects was significantly higher in women only after age 15 years. We believe that this could involve the sex hormones and pharmacokinetic features of women in producing ADRs.

# New aspects of antibiotic prophylactics and therapy in a large university hospital surgery clinic

Metodiev K

*Medical University, Varna, Bulgaria*

"Clinical studies conducted over the years indicate that antibiotic use is unnecessary or inappropriate in as many as 50% of cases in the United States"

(Neil Fishman, American Journal of Infection Control, 34, 55-63, 2006)

However, the situation in Europe is, if not the same, even worse in some countries!

Objective reasons:

1. Escalating Antibiotic Resistance;
2. Slowed New Drug Development;
3. Ethical Considerations

Subjective reasons:

1. Insufficient knowledge:
   - Principles of Rational Antibiotic Prescribing;
   - Comparative Clinical Efficacy of Antibiotics;
   - Pharmacoeconomics: «Cost/Efficacy»;
2. Lack of Restrictive Measures
3. Lack of Routine Monitoring
4. Pressure from the Pharmaceutical Industry;

Some examples from the daily clinical practice in a hospital:

- Surgical site infections (SSI's) are a major contributor to patient injury, mortality and health care costs
- Mortality rates are 2 to 3 times higher in patients in whom an SSI's develops, compared with uninfected patients, and hospital readmission rates are significantly increased
- SSI's increase length of hospital stay by an average of 7 days and charges of approx. 3.000 Euro.
- The attributable costs of an SSI may be more than 30.000 Euro for patients undergoing orthopedic or cardiac surgery

The effectiveness of surgical antimicrobial prophylaxis (SAP) for the prevention of SSIs was established in the 1960s and has been repeatedly demonstrated since.

Many different antimicrobial regimens are effective for preventing SSI.

Which one of them to choose?

The optimal Antimicrobial agent for SAP should be effective against the pathogens that are most likely to be encountered during the type of operation that is to be performed;

It should also be:

- safe,
- inexpensive,
- bactericidal;

Considering the broad range of evidence-based recommendations for clinical practice, appropriate SAP would appear to be a relatively straightforward practice to implement.

Despite a lot of published guidelines for antimicrobial prophylaxis, its use is often suboptimal:

- inappropriate timing of antimicrobial administration
- inappropriate selection of antimicrobial drugs
- excess duration of prophylaxis.

WHO Criteria for Rational Prescribing of Antimicrobial Drugs:

- Efficacy: Antimicrobial Spectrum, Pharmacokinetics, Dosing Regimen, Duration of Treatment;
- Safety: ADR's, Drug Interactions
- Cost: €; £; $; Japan Yens; BG-leva!

A. Efficacy:

Adequate antibiotic tissue concentrations at time of incision maintained for a sufficient period of time!

Which means:

1. Preincisional i.v. application of AMD *(30-60 min)* according to $t_{max}$;
2. Adequate Dose of AMD (to exceed $MIC_{90}$ values of the potential pathogen - *Vd)*
3. Adequate Dose Regimen of AMD *(1-2 x* $t_{½}$*)*

B. Safety:

Minimal toxicity and minimal effect on patient's microflora, by means of:

» AMD with less probabilities of toxic effects

» Adequately short course of application

Guidelines for SAP were available by 2008 in several university hospitals in Bulgaria (even up to date) BUT THEY REMAINED ON PAPER ONLY?

Guidelines in real life are not God's lines!

The successfully implementation of a rational sap should be based on 4 cornerstones:

1. education
2. restriction
3. monitoring
4. feedback

Local guideline for performing SAP, as suggested by leading national specialists to some of the University Hospital Clinics of Surgery

I. Indications for sap:

a) Principles of Rational Antibiotics use for SAP;

b) Determination of the Risk Index for SSI;

c) Defining the necessity of SAP for specific OP's:

- Mandatory;
- At the discretion of the operating surgeon: (additional risk factors present);
- Obsolete.

II. Implementation of SAP:

- Precise instructions for SAP : How to be done!

III. Antibiotics for SAP: Doses and Dosing Regimens

- (which drugs, which doses, which dose regimens)

C. Implementation of SAP:

Perioperative i.v. application: usually 30 – 60 min. before incision, (exceptions: Ceftriaxone: 60 – 90 min- & Metronodazol: 10 – 20 min.):

- Single application (examples):
  - hernioplasty with mesh;
  - appendectomy;
  - dermoidal cysts;
  - anal and perianal OP's.
- Double application (examples):
  - prolonged OP's *( > 3 hours);*
  - excessive blood loss during OP ≥ 1500 ml.;

    - excessive intra OP infusions ≥ 15 ml/kg.;
    - elevated OP Risk (*AsA gr. ≥ III)*
- Multiple application (duration > 24 ≤ 48 hours), acc. to ASA :
    - OP's on rectum;
    - OP's on gastric cancer;
    - OP's on extrahepatic pathways;

Based on our recent experience, prerequisites for a rational SAP implementation into any hospital could be:

- Availability of an Expert Antimicrobial Team,
- Providing Training of the surgical team, including the nurses!
- Building good relationships with the surgical team – to prevent ignorance or resistance
- Performing regular monitoring of SAP
- Providing regular feedback with discussion
- Support by the Hospital Management

Results:

2008/2009: < 20% rational SAP

2010/2011: > 80% rational SAP

In monetary terms:

15,000 Euro savings per year in a single clinic of surgery only from adequate SAP in JUST one of the hospitals under study

PROPHYLACTIC APPLICATION:

AMD are being applied before tissue contamination, i.e. Perioperative Antimicrobial Prophylaxis with single shot AMD application,

Duration: up to 24 hours;

Most often in:

- Clean and Clean-contaminated OP's.

PRESUMPTIVE APPLICATION:

AMD are being applied in OP's with higher probability of tissue contamination, i.e. Perioperative Antimicrobial Prophylaxis with multiple AMD applications,

Duration: up to 48 hours;

Most often in:

- Colorectal surgery

THERAPEUTIC APPLICATION:

AMD are being applied AFTER tissue contamination, i.e. Perioperative Antimicrobial Treatment

Duration: ≥ 48 hours;

Most often in:

- Contaminated OP's.

**In summary:**

The optimal duration of prophylaxis still remains controversial, especially for cardiovascular, orthopedic and colorectal surgery, where many surgeons prefer to continue prophylaxis until all drains and tubes have been removed.

The bulk of published evidence however suggests, that short duration of prophylaxis - as little as one dose in many studies - is equally effective as long duration administration in preventing SSI's.

There is also evidence, that prolonged administration of AMD's for SAP can be harmful to patients, by promoting resistant bacteria and potential increasing the incidence of antibiotic–associated complications.

# Pediatric infections- current problems of antimicrobial treatment, collateral effects and etiologic related solutions

Grigore C[1], Iurian S[1], Iurian S[1], Grigore N[1]

[1]*University Hospital Sibiu, Romania*

## 1. Background

The most frequent infections in children are the upper respiratory tract infections, gastrointestinal infections and urinary tract infections. The laboratory search for the etiologic agents and performs antimicrobial sensitivity tests for bacterial isolates.

Most cases of upper respiratory tract infections are viral in origins and need no antimicrobial treatment. The cases with bacterial etiology have a better clinical outcome under antimicrobial therapy. The majority of gastrointestinal diseases need no antimicrobial treatment because the main objective in these cases is normalization of hydration status, but in severe cases antibiotics may be used against the bacterial pathogens. Urinary tract infections` most frequent pathogenic agents are Gram negative bacilli: mainly Enterobacteriaceae. The clinicians relay on the laboratory antimicrobial sensitivity tests for choosing the empiric treatment, according to the epidemiologic statistics, adjusting the treatment to the particularities of the patient and also for following the multi resistant strains.. Laboratory has to offer alternatives in different situations of multi resistant bacteria and also in cases of drug intolerance, frequent in pediatric diseases.

## 2. Material and method

We analyzed the pathogens found in cultures of pharyngeal specimens, middle ear specimens, stool specimens and urine specimens, and we analysed the antimicrobial sensitivity tests according to the EUCAST standard.

## 3. Results

The beta-hemolytic streptococci, etiological agents of pharyngitis, mainly group A, but also C, G, were found 100% sensitive to Penicillin, 90% to Erytromycin and only 50% to Sulfamethoxazol Trimethoprime.

Streptococcus pneumoniae, was found 50% Penicillin resistant and 19% Intermediate, 63% Erytromycin resistant, 61% Sulfamethoxazol Trimethoprime resistant, 24% Ceftriaxone resistant, 20% Cefotaxime resistant and 100% sensitive to Vancomycine.

Campylobacter spp and Salmonella spp were the most found etiologic agents in acute bacterial diarrhea, For Salmonella spp the antimicrobial sensitivity test results showed 40% resistance to Ampicillin, 10% resistance to Ceftazidime, Nalidixic Acid, Ciprofloxacin, and Sulfamethoxazol Trimethoprime.

Shigella was 90 % resistant to Ampicillin and Sulfamethoxazol Trimethoprime and was not found resistant to Nalidixic Acid, Ceftazidim and Ciprofloxacin.

Yersinia enterocolitica was 100% resistant to Ampicillin and 100% sensitive to Ceftazidim and Sulfamethoxazol Trimethoprime and 20% resistant to Ciprofloxacin and Nalidixic Acid.

E coli, the most found etiologic agent of urinary tract infections, was found ESBL-positive in 9% of the cases and 73% resistant to Ampicillin and mostly sensitive to Ceftazidime, Cefuroxime, Ceftibuten, Nalidixic Acid, Gentamicin and less sensitive to Sulfamethoxazol Trimethoprime.

Klebsilla, the second most found etiologic agent in urinary tract infections was 21% ESBL- positive and have a higher resistance to all the antimicrobial drugs.

## 4. Conclusions

The streptococcus pharyngitis benefits 100% of the Penicillin treatment. Despite of the correct etiologic treatment there are failures and the alternative schemes are beta lactam antibiotics in combination with beta lactamaze inhibitors, or Macrolide.

For the Streptococcus pneumoniae we remarked the increased resistance to Penicillin and Macrolide and consequantly the difficulties in finding an appropriate treatment.

For Salmonella infections we see a high resistance to Ampicillin, but Sulfamethoxazol Trimethoprim is a good choice for the etiologic antimicrobial treatment.

E coli, as an etiologic agent of the urinary tract infections, shows an increased resistance to Ampicillin and Sulfamethoxazol Trimethoprim, probably because of the frequent use of these antibiotics in pediatric cases, comparing to a good sensitivity to Nalidixic Acid.

Klebsiella spp show an increase resistance to antimicrobial drugs in the last time (ESBL positive 21%), comparing to E coli, (9% ESBL positive).

This increased resistance to many of the usual antibiotics is a collateral effect of their high use in childhood. Similarly, the repetitive use of the same antibiotic in a patient can lead to the selection of multi-resistant strains.

Symposium 15: Emerging Infections in SE Europe

## Rabies - Emerging infection in south-east Europe

Hostnik P[1], Maurer Wernig J[1], Malovrh T[1], Grom J[1], Toplak I[1], Rihtarič D[1]

*[1]University of Ljubljana, Veterinary faculty, Ljubljana, Slovenia*

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Rabies is one of the most deadly diseases in the world today. An overview of the rabies situation in the 11 countries (Slovenia, Hungary, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, FRY Macedonia, Kosovo, Albania, Romania, Bulgaria) of South-east Europe covering 869.979 $km^2$ is presented. In period from 2009 to 2013 a total 72.743 field samples were tested, of which 66.127 (90,9%) samples were collected in Slovenia, Croatia and Hungary. In 2013, 9.520 samples were tested and 516 cases of rabies were registered in South-east Europe with 26,6 % in domestic animals and 73,4 % in wild animals. Rabies was found in a low number of dogs and cats in 8 countries. Some of the countries from South-east Europe have not recorded human deaths from rabies for more than 60 years, but last human case was registered in Romania in 2010. The red fox (Vulpes vulpes) is the principal disease reservoir in South-eastern region of Europe, however, cases of rabies in the dog (Canis familiaris) are regularly reported. However, persistence of rabies in wildlife requires constant surveillance and maintenance of lowest possible number of rabid animals in order to prevent domestic animal and human rabies cases. Oral vaccination campaigns for foxes is being carried out in Slovenia since 1988, in Hungary 1991, but after 2010 also in Croatia, Serbia, Kosovo that are being conducted with the support of the European Union. Numerous studies showed that in this region only cosmopolitan lineage RABV1 and EBLV1 are present, but no Asian, Arctic or African strains were identified. In South-east Europe, at least four different variants have been described, belonging to the groups Central Europe (Slovenia, Hungary), Eastern Europe (Balkan countries), Serbian fox group and also North-eastern Europe variant, that is present in Romania. This last variant has been particularly associated with raccoon dogs (Nyctereutes procyonoides). The oral vaccination of foxes, that was launched in Croatia, Serbia, Kosovo in 2010, has resulted in a progressive reduction of rabies in animal cases. Uncontrolled, free roaming dog populations continue to pose a problem, especially Romania. Active and passive surveillance program, covering the whole territory, has not yet been fully implemented in all countries, so the real number of rabies cases is not accurate everywhere.

Symposium 16: Current experience on management of infections from Bulgaria

## Bioterrorism, definition, risk infections, current situation, future threats

Metodiev K

*Medical University, Varna, Bulgaria*

Terrorism is one of the ugliest forms of human hatred and confrontation.

Terrorist acts can vary from attacks in public places by using bombs or other explosive materials (we are witnessing such bloody episodes rather often recently) to individual or massive use of biological and agricultural agents.

Biological weapons, used for **bioterrorism**, are: microorganisms, bacteria, viruses, fungi or toxins. Their application must inflict harm on the opponent.

**Definition:** bioterrorism attack is the deliberate release of viruses, bacteria, toxins or other harmful agents used to cause illness or death in people, animals, or plants.

A number of viral and bacterial agents, such as small pox, Ebola virus, HIV, West-Nile, SARS, Marburg virus, Lassa virus, Hanta viruses, hepatitis, influenza, plague, glanders, Bacillus anthracis, Yersinia pestis, Francisella tularensis, Brucella species, Vibrio cholerae, TBC, Ricketsial agents, etc. are recognized as the etiologic reason for terrifying infections.

The history of bioterrorism goes back as far as human warfare, in which there have always been efforts to use germs and disease as weapons.

It is well-known that the production of such biological agents is easy and inexpensive, this is the reason they had been named ***"the poor man's atomic bomb".***

A biological attack must allow a rapid distribution (air-spray will be the best form), be able to affect quickly a big number of population, be hard to detect, treat and prevent.

Bioterrorism agents are separated into three categories: A, B, or C — depending on how easily they can be spread and the severity of illness or death they cause:

> Category A agents are considered the highest risk and the highest priority.
> Category B agents are the second highest priority.
> Category C agents are the third highest priority and include emerging pathogens that could be engineered for mass spread in the future.

Category A agents/diseases:

Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum* toxin)
Plague (*Yersinia pestis*)
Smallpox (variola major)
Tularemia (*Francisella tularensis*)
Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]).

Category B agents/diseases:

Brucellosis (*Brucella* species)
Epsilon toxin of *Clostridium perfringens*
Food safety threats (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*).

Category C agents/diseases:
Emerging infectious diseases such as Nipah virus, hantavirus and some other emerging pathogens from specific regions.

**In summary,** basically any microorganism inducing a severe infection could be successfully applied as a bio-weapon.

There are three necessary steps to achieve the goals of the medical response (no matter civil or military) against bioterrorism:

- Preparatory step: before the moment of attack
- Operational step: after the registration of the attack
- Conclusive step: elimination of the consequences

The Preparatory step includes:

Evaluation of the situation in the country, neighbouring region and world-wide, permanent analysis of normal infectious morbidity in the respective geographic region, evaluation of the resources (man power, materials, finance, organization and time for reaction, etc.), personnel education and qualification, upgrading of computer models for medical response management, application of specific prophylaxis.

The Operational step requires:

Immediate determination of the affected area and designing its borders, location of the staff under the attack, specifying the objects capable of transmitting the used bio-agent in the affected region, epidemiologic investigation of the population in the affected region, collection of any other information or data associated with the attack.

The identification of the causative agent has two levels:

- Performed by the Military Medical Unit and the Regional Civil Defense Organization (localization of the affected area, evaluation of the number of

infected subjects, performing of life-saving procedures on the spot, organization of evacuation to the nearest hospital, isolation, initial diagnose, treatment),

- Elimination of consequences, precise diagnosis in national reference laboratories, treatment in specialized hospitals and clinical units for severe infections, prognosis, epidemiologic analysis, possible issues of the bio-attack.

The following list summarizes some characteristics of a disease outbreak that suggest the possibility of intentional use of an infectious agent:

- Outbreak of a rare disease
- Outbreak of a disease in an area that normally does not experience the disease
- Occurrence of a seasonal disease at an inappropriate time of year
- Unusual age distribution of people involved in the outbreak
- Unusual epidemiologic features of an outbreak (e.g., a typical pathogen transmitted solely by food ingestion now found to be transmitted from person to person)
- Unusual clinical symptoms not typically seen with a known pathogen (especially respiratory symptoms)

Medical service assistance support includes the following:

a) Threat assessment
b) Epidemiology investigations
c) Technical advice and assistance for the specialized forces:
    - *Identification of agents used*
    - *Sample collection*
    - *Sample analysis*
    - *Onsite safety and protection activities*
    - *Medical management planning*

Operational support to national bureau for management of emergency situations:

- *Mass immunization*
- *Mass prophylaxis (preventive treatment)*
- *Mass fatality management*
- *Pharmaceutical support operations through national organization*
- *Contingency medical records*
- *Patient tracking*
- *Patient evacuation*

- *Definitive medical care provided through the National Disaster Medical System*

Response to bioterrorism incident or threat:

Government agencies which would be called on to respond to a bioterrorism incident would include law enforcement, hazardous materials/decontamination units and emergency medical units, if they exist.

New ideas: how to detect and to prevent:

Attempts to discern the origin of the outbreak: Researchers are experimenting with devices to detect the existence of a threat:

- Tiny electronic chips that would contain living nerve cells to warn of the presence of bacterial toxins (identification of broad range toxins)
- Fiber-optic tubes lined with antibodies coupled to light-emitting molecules (identification of specific pathogens, such as anthrax, botulinum, ricin)
- New research shows that ultraviolet avalanche photodiodes offer the high gain, reliability and robustness needed to detect anthrax and other bioterrorism agents in the air.

Preparation and planning for action in peace and wartime crisis situations associated with biological weapons is required to coordinate and synchronize the national programmes with the NATO-standards, which is of first priority in the struggle against bioterrorism world-wide.

It is admitted that the recent NATO scientific programmes for ARW in the field of bioterrorism are also a very good tool for education and research.

The chance to exchange experience with other well-known specialists in the field, like our colleagues from foreign university hospitals and medical societies, will give better results and contributions to successful issues.

What we do during any other and the present forum is: discussing and presenting certain guide-lines to the specialists working in this field.

However, GUIDE-LINES ARE NOT ALWAYS GOD'S LINES!!!

It is necessary to exchange opinions and experience and this is the major goal of similar meetings, regional, national and international.

The chance to communicate with our colleagues during the sessions, at the round-table discussions and between the lectures, is our hope for better understanding of the serious problem **"What bioterrorism is and how to struggle against it".**

By all means, every discussion on the topic "bioterrorism" in particular will be a big step on the long road to the victory over infections.

# Meningitis and traumatic brain injury: treatment, complications and prevention

Ridian N, Kyuchoukov G, Kiryakov I, Shopov M

*Saint Anna Hospital, Clinic of Neurosurgery, Varna, Bulgaria*

## 1. Objective

Neuroinfections are considered a true neurosurgical emergency requiring rapid diagnosis and appropriate surgical or conservative treatment to achieve good outcomes. Meningitis can lead to severe intracranial complications such as arachnoiditis, hydrocephalus, empyema, epidural or subdural intraparenchymal abscessus; conditions which need major surgery, expose patients to higher risks of disseminated infection and longer hospital stay.

The aim of this study is to evaluate the risk of meningitis among patients with brain injury and skull base fractures treated in our clinic for the last four years.

## 2. Methods

In this series of 210 patients with skull base fracture treated in our clinic for the last three years with prophylactic antibiotic therapy, meningitis was diagnosed in 3 patients (1,4%). All the patients received Ceftriaxone 2g i.v. for 7 days. A “prolongation protocol”of three days was applied in 11 cases which required surgery (the cases when CSF leak doesn't resolve spontaneously). All the patients with diagnosed meningitis suffered polytrauma and all of them died due to MODS (multiple organ dysfunction syndrome).

## 3. Results

We report low rate of meningitis (1,4 %) in cases of skull base fractures when prophylactic antibiotic therapy applied. 11 cases underwent surgery to shunt the CSF to other anatomical cavities- mostly lumboperitoneal shunting performed. Meningitis in polytrauma patients developed despite the prophylaxy but causa mortis was different then meningitis. All of them had a penetrating injury. The bacteria isolated were Klebsiella, Enterobacter and Acinetobacter.

## 4. Conclusion

There is no support for prophylatic antibiotic use in skull base fracture cases. Among neurosurgeons is admitted the policy of antibiotic prophylaxy when surgery duration is more than 2 hours. When talking about skull fractures this policy is considered controversial, although there’s communication with paranasal cavities for longer time. Brodie concluded there is a benefit with antibiotic prophylaxis whilst Villalobos didn't found a lower rate of meningitis when antibiotic prophylaxy in cases with skull base fractures. Up to date there is no study (evidence based) for the use of prophylactic antibiotics in traumatic brain injury patients and skull base fractures. Since posttraumatic meningitis has a great mortality and severe complications can follow, in our clinic the above mentioned protocol has been admitted and is being applied in such cases. More appropriately designed studies

are needed to establish the effectiveness of antibiotic prophylaxis following the diagnosis of skull base fracture.

# Emerging infections of terminal pregnancy

Masseva A, Shopova E, Marinov B

*University Hospital "Maichin Dom" Sofia*

## 1. Summary

The purpose of this study was to determine the most common causes of vaginal infection at the time of termination of pregnancy/delivery, and their relationship to the status of the neonate. The results show that 30% of births at term have a fungal infection, by contrast 46.7% of pregnant women giving birth before term are affected by mixed aerobic-anaerobic infection. Development of mixed infection is favored by the absence of lactic flora and its inability (more than 55%) to produce hydrogen peroxide. Examination of vaginal pathogens was focused on group B streptococci due to their pathogenicity for preterm (23.1% morbidity of early-onset GBS disease), and the term newborns (15,8% morbidity in the group of colonized mothers).

## 2. Introduction

The status of the vaginal ecosystem is crucial for the woman's reproductive health. The complex interaction between hormonal levels, stratified flat epithelium and microorganisms colonizing the vagina are yet to be clarified, although they have been studied for many years (in the context of various pathological conditions). The imbalance between vaginal bacterial species is considered to be a major cause of the development of ascending intrauterine infection. Intrauterine infection is considered a major cause of birth before term, and cause significant morbidity and mortality not only in preterm infants.

## 3. Objective

The objective of this study was to assess the status of the main components of the vaginal ecosystem: lactic acid bacteria and pathogenic organisms in terminal pregnant and preterm parturient women and their relation to the condition of the newborn.

## 4. Material and Methodology

This was a prospective study conducted for 1.5 years at the University Hospital of Obstetrics and Gynecology 'Maichin Dom' – Sofia. The study included 93 pregnant women at over 26 week of gestation (wg).

There were two main groups:

- research (test) group of pregnant women at 26 to 35 wg with signs of preterm birth (PTB group);
- control group of terminal pregnant women indicated for elective Caesarian section – Terminal group(T group) – 33 pregnant women.

The research (test) group was further divided by the integrity of the membranes into:

- parturients with intact membranes– PTB with Int. M – 30 pregnant women;
- parturients with preterm premature rupture of membranes (PPROM ) – PTB with PPROM – 30 pregnant women.

Additional 40 PTB women (100 in total) and 57 full-term parturients giving birth per vias naturales (90 in total) were added to the above groups to assess the colonization with Group B Streptoccocus (GBS) in pregnant women within the overall population of parturients, the relationship of this pathogen to PTB, and the neonatal morbidity rate.

For the preterm group, vaginal samples were collected after the start of tocolytic therapy and prior to antibiotic administration. For the control group, samples were collected at presentation of pregnant subjects.

For examination of cervico-vaginal fluid were used specific and routine microbiological techniques. Vaginal smears stained Gram were assessed in Nugent score system. Further smears were cultured for vaginal microbes.

Lactobacillus production of hydrogen peroxide was determined using a quantitative method.

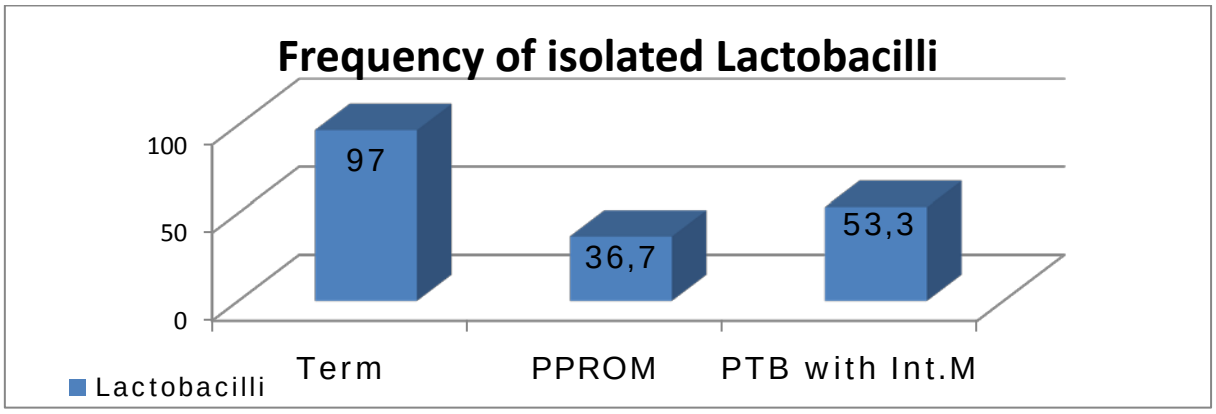
The fast and specific Granada medium (broth) was used for detection of GBS at Delivery Room, and at the Microbiologic Laboratory this test was completed following the good microbiological practice standards.

We used Mycoplasma IST (Bio Merieux) tests for quantitative assessment for the presence of Ureaplasma urealyticum and Mycoplasma hominis, as well as to test their sensitivity to antibiotics.

## 5. Results and Discussion

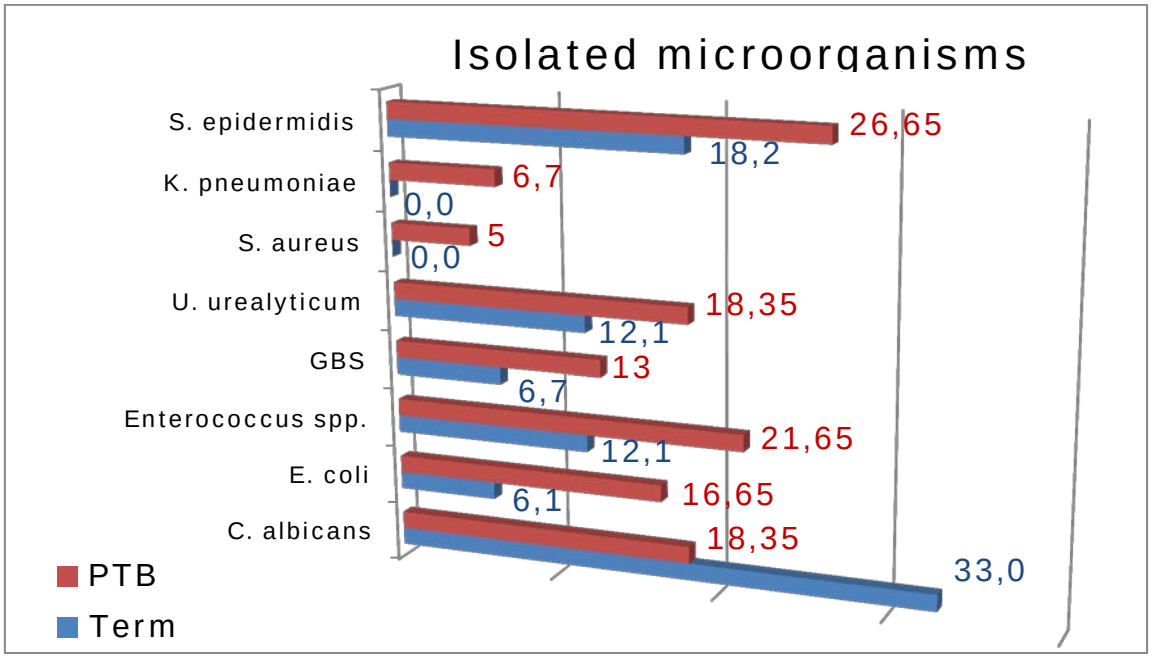
Figure 1 displays the results for the presence of lactobacilli in vaginal section of pregnant study subjects. There is a significant difference between the full-term and preterm groups (figure 1), which shows a correlation between PTB and the lack of lactobacillic defense.

Figure 1




Cultivation of the collected material in a variety of growth media, and under various conditions, found the resident, conditionally pathogenic and pathogenic vaginal microorganisms (MOs) displayed in Figure 2.

Figure 2




A significant proportion (33.3% in Int.M, and 36.7 in PPROM) of the isolated MOs was not found alone, but in combinations between each other.

According to the microbiological test, 46.7% of preterm group patients had evidence of a non-fungal vaginal infection versus 12.1% of those in the full-term group. A fungal vaginal infection was detected in an inverse fashion – 3-fold higher frequency in the full-term group.

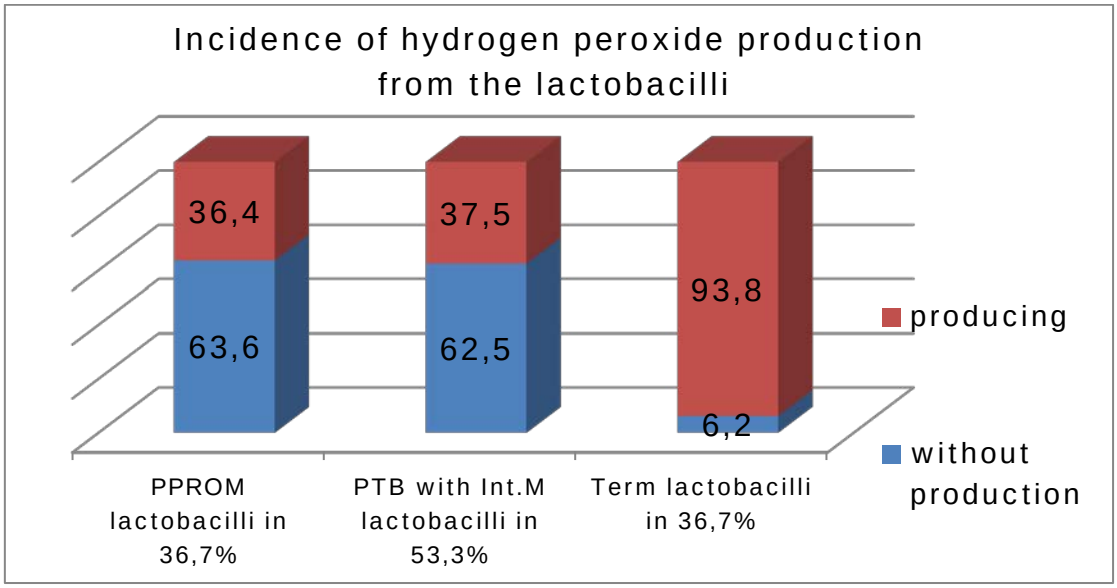
The search of the source of infection in PTB led to the vagina in 90% of the cases. Significantly less common was the hematogenous dissemination in general infections (e.g. listeriosis) or for another infection focus (oral infections).

According to microbiological (MB) test data, full-term pregnant subjects had dominating lactic-acid bacteria, normal leukocyte level, transient cocci flora, and vaginal infection in 12.1% of cases. The vaginal infection in full-term subjects mainly characterized as a mixed one, with almost permanent presence of yeast-like fungi as one of the causes.

The predominating role of lactobacilli in the vaginal secretion of preterm group pregnant subjects was not present in about 55-60% of cases. This was especially so for PPROM women. Overall for the PTB group, the infection was defined with predominating mixed microbial isolates, however in 75% of cases, it was mixed aerobic-anaerobic pathogen flora.

The formation of lactic acid as a result of glycogen metabolism is a defense mechanism connected to glycogen-tropic feature of lactic-acid bacteria. It is inherent to the Lactobacillus family. Another not less important defense is the production of hydrogen peroxide which is toxic for a number of pathogenic MOs in the vagina. The ability to produce hydrogen peroxide is species-specific and not all lactobacilli have it. Figure 3 shows the ability to produce $H_2O_2$ in our groups.

Figure 3

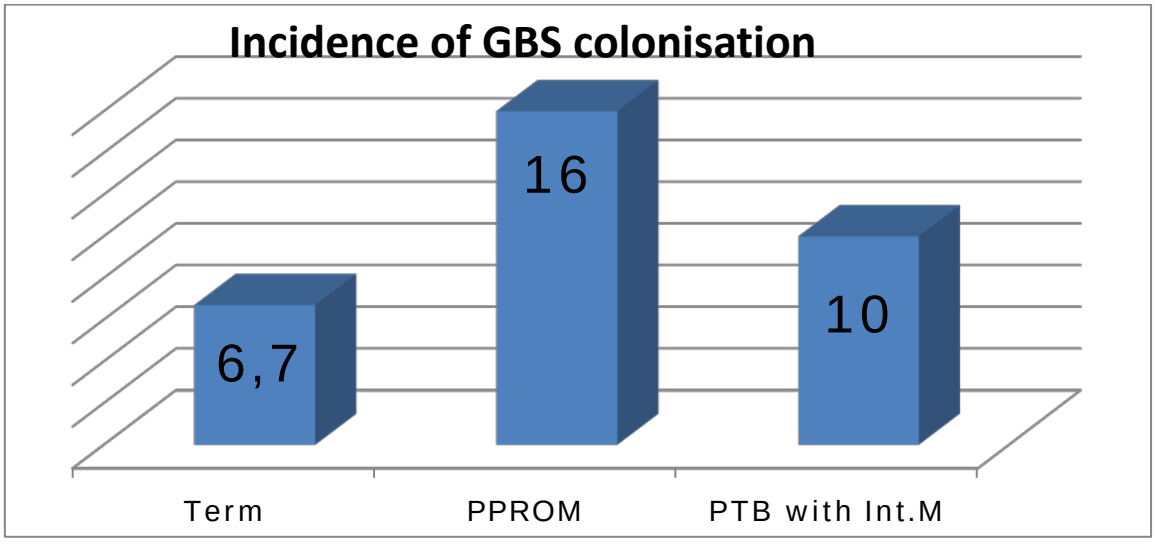



Approximately two thirds of the lactobacilli isolated in the preterm group subjects were not found to produce hydrogen peroxide. 20% of the Int.M. pregnant women had qualitatively and quantitatively ($H_2O_2$ production) whole lactic acid flora, another 33.3% had only quantitative priority of lactobacilli, while the remaining 46.7% of subjects in the group had no lactic acid colonization. For the PPROM pregnant subjects, 13.3% of them had adequate lactic acid defense, 23.3% had only lactobacilli (insufficient growth), and the remaining 63.3% had no lactobacilli. The absence of lactobacilli and the lack of hydrogen peroxide production are major risk factors for the development of infection and occurrence of PTB.

To a significant extent, this infection is due to the conditionally pathogenic β-hemolytic B group streptococcus. The vast prevalence of GBS and its risks for the neonate drives the GBS carriage screening at the time of birth.

GBS carriage (colonization) in the expanded screening group is shown in figure 4. Despite the 2-fold higher frequency of colonization in the preterm group, no statistical difference was demonstrated between the groups.

Figure 4



The screening detected β-hemolytic streptococcus in 10% of all parturients. 15.8% of the neonates of carrier mothers had an infectious disease requiring treatment.

In one of the six neonates of pathogen carriers in the full-term delivery mothers Streptococcus agalactiae was isolated from two peripheral secretions (ear and nasal). The full-term neonate did not develop any infectious disease symptoms, and the positive peripheral samples were assessed as 'neonatal colonization'.

S.agalactiae positive samples were found in four neonates of the thirteen GBS carriers in the preterm group. In one case, a peripheral sample (ear secretion) was positive, and the premature neonate did not develop any clinical manifestation of an infectious disease.

In two premature neonates, S. agalactiae was isolated from tracheal aspirate; the neonates presented signs of congenital pneumonia and were given antibiotic treatment.

One of the preterm neonates developed clinical manifestation of an early neonatal sepsis; the stomach aspirate and hemoculture samples were positive.

The practical use of a highly specific medium 'Granada' in the planned screening demonstrated the following features:

- using this medium helped to find 17 of all 19 GBS positive vaginal secretions (89.5% test sensitivity);
- no false positive result recorded;
- longest period for obtaining a positive result - 12.5 hrs (48-hour sample monitoring window);
- positive test result shown in color.

A microbial cause of the congenital maternal-fetal infection (MFI) was identified in 48.3% of the prematurely born children. In 75% of the cases, the microorganisms matched those isolated in the maternal vagina. In 30% of the neonates, the positive samples came from real infection foci (not from peripheral secretion) and provided evidence of a disease requiring adequate treatment, i.e. we have proved microbiologically congenital MFI in 30% of the neonates.

## 6. Conclusions

1. The vaginal secretions in the full-term group characterize by a domination of lactic acid flora and fungal infection in 30% of cases. For the preterm groups, lack of control of lactobacilli was found in over 60% of the pregnant women, with 46.7% of them presenting with vaginal infection caused by a mixed non-fungal pathogenic flora.
2. The lack of hydrogen peroxide-producing lactobacilli is a major risk factor for PTB and should be looked for especially in pregnant women at high risk for PTB.
3. At the 'Maichin Dom' Specialized Hospital, the proportion of patients carrying hemolytic group B streptococcus varies from 6.7% to 13%, and the genital mycoplasma colonoziation found was 2-fold higher; despite these rates, both organisms (GBS and U.urealitycum) are not a direct cause of PTB.
4. The highly GBS-specific medium 'Granada' is suitable for screening of parturients, especially before delivery, in the clinical practice.

Symposium 17: Invasive Fungal Infections, an update

## Fungal infections in immunosuppressed patients

Samonis G

*The University of Crete, Division of Medicine, Heraklion, Crete, Greece*

Invasive fungal infections (IFIs) represent a major threat for immunosuppressed patients, especially for those suffering of hematological malignancies. Chemotherapy, radiation therapy, administration of several immunosuppressive agents, antimicrobial agents, transplantations, surgical procedures and the use of devices are among the predisposing factors for the development of IFIs. *Candida* spp have been recognized as cause of such infections some decades ago, while *Cryptococcus* spp infections had been also observed in patients with lymphomas. *Aspergillus* spp were recognized later on as threatening organisms for leukemic patients, while *Zygomycetes* and *Fusarium* spp have become apparent rather recently as major risks. Although many new diagnostic methods and tools have been developed, diagnosis of IFIs is often problematic and many times is only a post-mortem event. Hence, prophylaxis for these infections is of paramount importance, especially in patients with prolonged and profound immunosuppression. During the last decade new antifungal drugs have enriched our armamentarium although the fight against fungi started five decades ago with amphotericin B. In the 80's a step ahead had been achieved with the first azoles ketoconazole and miconazole, which had minimal or no impact on IFIs. These drugs, however, heralded the production of other azole compounds such as fluconazole, which played a major role against *Candida,* and itraconazole, which was effective also against *Aspergillus*. In the 90's the new lipid and liposomal formulations of amphotericin B gave new possibilities to treat a wide spectrum of fungal diseases. These formulations are still in use as effective therapeutic modalities. The production of other azoles such as voriconazole, that made the difference in the treatment of aspergillosis, and posaconazole, that has a very broad spectrum, offered new weapons against IFIs. The latest class of echinocandins, that includes caspofungin, micafungin and anidulafungin, broadened further the antifungal armamentarium. However, despite the development of effective drugs the immunosuppressed patient remains a sensitive host. Immunosuppression minimizes the activity of antifungal agents. Hence, the infectious diseases community is looking forward to new compounds as well as to the results of ongoing clinical trials assessing the activity of combination antifungal treatments. It must be emphasized, however, that restoration of the immune system, if possible, remains the cornerstone for the treatment and prophylaxis of fungal infections of patients with immunosuppression.

Symposium 18: Urinary tract infections

## Genitourinary tract infection in postmenopausal women

Hoyme UB

*Klinik für Frauenheilkunde und Geburtshilfe, St. Georg Klinikum, Eisenach, Germany*

Urinary tract infection (UTI) is the most common bacterial infection in postmenopausal women. The anatomy as well as the physiology of the female urogenital tract change significantly after menopause.: the glycogen content of the urethral and vaginal epithelium decreases due to the lack of estrogen stimulation, the squamous epithelium of the vagina (urethra/bladder) and the columnar epithelium of the cervix atrophy and are exposed to changed local microbial flora. An elevation of vaginal pH (premenopausal <4.5) is indicative for the change of the ecosystem. This leads to an increasing risk of acquisition of an urinary tract infection. The main microbiological reservoir for any UTI is the vaginal environment, in clinically healthy pre- and also some postmenopausal women typically but not necessarily characterized by lactobacilli, however, at the same time also harboring all bacteria in various concentrations that can be normally cultured from mouth, skin and rectum of healthy individuals (e.g. E. coli spp., enterococci, S. aureus, corynebacteria).

Consequently, the adequate prevention as well as diagnosis and treatment of vaginal/cervical dysbiosis or infection by the gynecologist is one of the cornerstones in reducing predisposition for UTI. Other gynecological risk factors are descensus vaginae et uteri, carcinoma colli and surgery with and without transurethral drainage. About 70% of all gynecological nosocomial infections occur in the urinary tract. In addition, incontinence, cystocele/residual urine, premenopausal UTI history, diabetes mellitus, institutionalization/detioration/frequent catherization are also predisposing.

Genitourinary symptoms are not necessarily related to UTI; atrophic vaginitis is the relevant differential diagnosis. Asymptomatic UTI in postmenopause is no indication for antimicrobial therapy. Diagnosis of UTI in postmenopausal women follows the common guidelines for these infections. E. coli is the predominant pathogen.

Therapy is similar to that of premenopausal women, however, the concept of short term administration is not well established. Fosfomycintrometamol (1x3g), Nitrofurantoin macro (2x100mg 5 days) and Pivmecillinam (2x200 mg 7 days) are recommended as first line treatment by the recent German guideline. Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin and Cefpodoximproxetil are second line, Cotrimoxazol and Trimethoprim only in case of an E. coli – resistance < 20 % in the specific population.

In prevention probiotic lactobacilli, antimicrobial prophylaxis, immunization, immunestimulation and with contradictive results estrogen locally and systemically as well as alternative methods (e.g. cranberry juice) are recommended.

Complicating factors like urinary tract obstruction, stones and neurogenic bladder should be ruled out.

# Imaging of urinary tract infections in adults

Savov O[1], Mladenov D[2], Bismack E[1]

[1]*Department of Urology, "St. Theresa Hospital", Nuremberg, Germany*
[2]*Department of Urology, University Hospital, Sofia, Bulgaria*

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The typical patient symptomatology and urinary evaluation for the presence of bacteria and white blood cells present the primarily basis for diagnosis of urinary tract infection (UTI) in the adult. Radiological evaluation is usually not required unless the uncomplicated UTI is not recurrent. Imaging should be reserved for those patients in whom conventional treatment has failed, or for those who have recurrent or unusually severe symptoms. Patients with multimorbidity conditions predisposing to infection, or complications thereof, such as diabetes mellitus or immunocompromised states, may also benefit from early imaging. If pyonephrosis is suspected, imaging and urgent drainage is also warranted. Intravenous urogram and ultrasound have traditionally been used in the assessment of these patients, allowing detection of calculi, obstruction and incomplete bladder emptying. These imaging techniques have limitations in the evaluation of renal inflammation and infection in the adult. CT became a more sensitive modality for diagnosis and follow-up of complicated renal tract infections. Contrast-enhanced CT allows different phases of excretion and can define extent of disease and identify significant complications or obstruction. Nuclear medicine has a limited role in the evaluation of UTI in adults. Its main role is in the assessment of renal function, often prior to surgery. Magnetic resonance imaging has an increasing role. It is particularly useful in those with iodinated contrast allergies, offering an ionising radiation free alternative in the diagnosis of both medical and surgical diseases of the kidney.

Symposium 19: New approaches to fight infections

## Hepatitis B in resource limited setting

Tutuncu E

Approximately 350 million people are chronically infected with hepatitis B virus (HBV) worldwide. Unfortunately, the most endemic regions have limited resources. HBV is most prevalent in China, Southeast Asia, Sub-Saharan Africa and the Amazon basin of South America where health care resources are frequently restricted. Hepatitis B is a significant cause of life-threatening liver disease (e.g. cirrhosis, liver failure and hepatocellular carcinoma) and more than 600000 deaths are attributable to HBV related liver disease each year. These deaths predominantly occur in underprivileged regions, particularly in Sub-Saharan Africa and Southeast Asia (1,2,3).

Most of low-income countries are dependent on external support for health-care services from international organizations and/or funds and priority has been given to HIV/AIDS, tuberculosis and malaria, hence the burden of HBV infection is largely underestimated (2). This is mostly due to lack of accurate prevalence data on chronic hepatitis B and its long-term complications.

In resource-limited settings, the prevalence and mortality rates related to chronic hepatitis B are the consequence of a combination of factors, such as the lack of surveillance data, poor education and unawareness of the public, inadequate prevention and screening strategies, imperfect vaccine coverage, and insufficient or even nonexistent access to treatment (1).

Since the chronic hepatitis B infection is mostly asymptomatic, accurate assessment of disease prevalence is not possible unless population based screening has been performed. Surveillance of chronic hepatitis B varies widely and is generally inadequate in resource-limited settings. The lack of accurate prevalence data on hepatitis is recognized as inhibiting more effective prevention and control at both international and national levels (4). Therefore large scaled surveillance studies should be performed to define the dimensions of the problem.

Awareness in general population regarding hepatitis B is quite low in resource limited countries. Furthermore, proper education and awareness is lacking even among healthcare workers. Knowledge of the risks and routes of transmission is essential to prevent the spread of the infection. Consequently, both the healthcare providers and the public should be educated. Public awareness can be raised by using mass communication and the media. Development of guidelines for screening, follow-up and treatment of chronic hepatitis B can help to standardize the approach for diagnosing and treating hepatitis B patients.

Detection and evaluation of hepatitis B patients requires laboratory tests. Most of the serological markers of hepatitis B, such as HBeAg, antiHBe, HBV DNA, and even HBsAg are often not carried out in resource constrained countries because of a lack of facilities. The existing infrastructure for the HIV programs could be adapted for both HIV and HBV screening and treatment programs (5).

Strategies for prevention of the spread of hepatitis B should be implemented. The use of sterile medical devices and single use syringes, screening of all donated blood for transfusion and screening all pregnant women before labor are important aspects of prevention of transmission of hepatitis B. It should be noted that, although the most effective method of prevention for hepatitis B is vaccination, the coverage of infant vaccination programs are unsatisfactory in these regions and this approach is not effective enough for prevention of the population in older age groups. Vaccination policies and programs need to be expanded and strengthened. The coverage of infant vaccination should be increased and catch-up vaccination schedules should be implemented for older age groups.

Although effective treatment for HBV is widely available in developed countries, high cost of medical care and antiviral drugs precludes the use of newer and more potent antivirals in resource limited settings and access to therapy is very limited. Screening of certain risk groups such as HIV-positive subjects, pregnant women, blood donors may help to define chronic hepatitis B patients. Although liver biopsy is the gold standard for defining patients who are in need of treatment, the availability of this approach is very limited in resource limited settings. Evaluation of liver disease should be performed by means of physical examination, AST/ALT ratio, platelet count and ultrasound where it is feasible. Patients with advanced liver disease (e.g. compensated or decompensated cirrhosis) and HIV co-infected patients should be prioritized for treatment.

Either entecavir or tenofovir should be used as first-line monotherapy for chronic hepatitis B patients if available (5). Monotherapy with lamivudine, emtricitabine and telbivudine should be avoided because of the risk of resistance and difficulties with access to monitoring for viral resistance. If entecavir and tenofovir are not available, an expert panel consensus report by Wiersma et al. recommends the use of adefovir and lamivudine, or adefovir and telbivudine combinations (5). Patients on therapy should be followed-up regularly to monitor compliance, response to treatment and for HCC surveillance.

It can be concluded that education, prevention, screening and treatment policies are required to control hepatitis B in resource limited settings. To achieve this goal, strategic planning and coordination are essential.

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## Insects borne diseases

Kastanakis S

*Internal Medicine Department, Chania General Hospital, Chania, Crete, Greece*

The possibility of a zoonotic infection can be confirmed from history, animal contacts, travel history, geographic considerations, clinical and laboratory data e.t.c.

Insects as vectors are capable of spreading diseases caused by many different types of micro - organisms including bacteria, viruses, protozoans, e.t.c.

In these instances it is the micro - organism that is the pathogen (disease causer) and the insect involved as the vector. The more well known insect borne diseases are the follow: Malaria (Mosquitoes), Yellow Fever (Mosquitoes), Dengue Fever.

(Mosquitoes), Japanese B Encephalitis (Mosquitoes, Ticks), Filariasis (Mosquitoes, blackfllies), Lyme Disease (Ticks), Leishmaniasis (Sandflies), Sleeping Sickness (Tsetseflies), Chagas Disease (Assasin Bugs), Thyphus Fever (Ticks and Lice), Plague (Fleas).

This is why it is important to provide to the international travelers health information concerning the distribution of the zoonotic diseases and their modes of spread, protective immunizations, medications and general instructions for food and drink.

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# Chemotherapy and additional biological concepts on bisphosphonate treated patients during dental implant therapy

Abadzhiev M, Nenkov P

*Medical University, Faculty of Dental Medicine, Varna, Bulgaria*

During an implantology treatment on patients with history of i.v. bisphosphonate intake, is vital antibiotics preparation and the primary healing process to be guaranteed. Usage of osteotropic antibiotic as Clindamycin is a gold standard assuring lack of post-operation infection and prevention of complications. Clindamycin is a semisynthetic derivative of lincomycin, a natural antibiotic produced by the actinobacterium Streptomyces lincolnensis. It is obtained by 7(S)-chloro-substitution of the 7(R)-hydroxyl group of Lincomycin. The synthesis of Clindamycin was first announced by BJ Magerlein, RD Birkenmeyer, and F Kagan on the fifth Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in 1966. The mechanism of action of clindamycin is mainly bacteriostatic. It is bacterial protein synthesis inhibiting ribosomal translocation. It does so by binding to the 50S rRNA of the large bacterial ribosome subunit. Because of its osteotropic affinity it is highly preferred.

Lack of dehiscence assure us smooth osteointegration process and lack of bone loss and of course prevents form exposing the bone in the area of inplantation and/or osseo augmentation. That is why common requirements for soft tissue management, suitable implantology system, proper operation technique, atraumatic preparation, and last but not least proper suturing materials and technique. Most of the listed above are closely related to the personal skills of the operator but according to the suturing materials PTFE (polytetrafluoroethilene) is essential due to its ability to compensate pressure during the healing edema and in this way preventing from tearing the soft tissues.

Primary healing process – tremendous advancement has been made in understanding the process of wound healing. The cell types and the order in which they appear in the wound have been elucidated. The high concentration of platelets rich in growth factors (PRGF) in the operation field contribute to a light and with lack of troubles post-operative process, guaranteeing perfect conditions for the osteointegration process.

Advantages of primary wound closure:

- Primary wound closure simplifies wound care for the patient, who simply needs to keep the suture line clean.
- A wound closure primarily heals much more quickly and with less pain than a wound allowed to heal with dressings alone.
- All vital, underlying structures are covered. Including augmentation materials and implants.

The high concentration of platelets rich in growth factors (PRGF) in the operation field contribute to a light and with lack of troubles post-operative process, guaranteeing perfect conditions for the osteointegration process.

The process of tissue repair is based on a complex cascade of biological events controlled by a long list of biologically active growth factors and proteins. The spatial and temporal action of this family of mediators on the tissue-damaged area regulates the mechanisms and phases that govern tissue repair and regeneration. In case, for example, of bone regeneration the purpose of the initial local growth factor expression is to stimulate the migration of osteoprogenitor cells to the wound site and subsequently, in a controlled fashion, to direct their differentiation to the osteogenic cell line. Throughout this process, another set of factors will regulate the dynamic equilibrium between cell inhibition and proliferation, as well as angiogenesis and extracellular matrix formation.[1]

Usage of photodynamic therapy contribute for cell destruction of the microorganisms and prevent complications and accelerate wound healing[2].

Another method used in our biological concept is ozone therapy. There are several known actions of ozone on human body, such as anti-microbial, immunostimulating, antihipoxyc, analgesic, detoxicating, bioenergetics and biosynthetic (activation of the metabolism of carbohydrates, proteins and lipids) etc. Anti-microbial effect of ozone as a result of its action on cells by damaging its cytoplasmatic membrane due to ozonolysis of dual bonds and also ozone-induced modification of intercellular contents because of secondary oxidants effects. This action is non-specific and selective to microbial cells, it does not dameage human cells because of their major antioxidative ability. Ozone is very efficient in antibiotics resistant strains. Its anti-microbial activity increases in liquid environment of acidic pH. In viral infections the ozone action lies in the intolerance of infected cells to peroxides and change of activity of reverse transcriptase, which takes part in synthesis of viral proteins. [3]

One of overall three bisphosphonate cases will be presented in details. In all cases patients undergo implantology treatment after a history of i.v. bisphosphonate intake ceased at least three years prior to implnatology treatment.

In the case presented 60 year old female patient receive one implmant in the area of the second upper right premolar (15) tree years after i.v. bisphosphonate treatment with Bonviva® 3mg. every second mount per year performed as osteoporosis treatment. The bisphosphonate medicine was stoped 3 wears before the dental intervantions. As premedication before implantological treatment was prescribed Clindamycin 1800mg.dayli 24 ours before implantation,ozone therapy and hyperbaric-chambers day before and in the first three days after implantation. This protocol was combined with PRGF activation of the implant surface and covered by PRFG fibrin membrane, postimplantation photodynamic therapy using PhotoSan® for 4 minutes two time a day till the suture removal. Considering this biological concept as successful in this first implant another implantation followed in the upper left region of two implants keeping the same protocol shortly followed by seven instantaneous implants on the lower jaw. A multiple extractions were performed on the lower jaw. During the extractions PRGF clothes were inserted in the tooth sockets combined with free mucosa graft from the palate in order to cover the sockets. Again the biological concept protocol was followed.

It could be concluded the despite the bisphosphonate intake a biological approach towards the tissues could contribute the whole picture of smooth and aseptic healing process. Treating tissues properly by appropriate medication, tissue and additional procedure management could lead to expected results and avoid unexpected complications. Actually this is everyone’s goal and having in mind the

lack of approved concept for bisphosphonate patients and piecemeal articles on this subject this biological concept, showing 100% success rate till now could be a remedy for many patients expecting implantology treatment. It is vital to consider that these are only three bisphosphonate cases but we have to observe much more similar patients, they should be monitored frequently in order to detect any late complications. The practitioner should know whether the bisphosphonate is taken per os or i.v. Careful consideration should be taken prior to any bisphosphonate and dental treatment. General practitioners and other specialist should consult dentists before bisphosphonate treatment in order to rehabilitate dental status and especial surgical manipulation such as extraction, apical resections, parodontal surgery or insert dental implants if it is needed, wait till the end of the osteointegration period and after that to start the medicine intake. Interdisciplinary thinking is of great importance in order to avoid extremely unpleasant osteonecrosis complications.

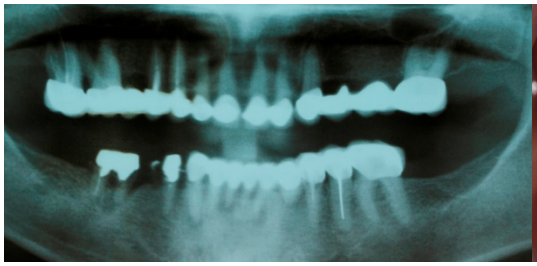

Fig.1 Panoramic X-ray before treatment

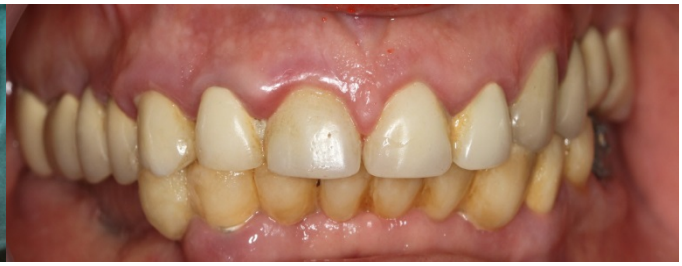

Fig.2 Clinical view before treatment

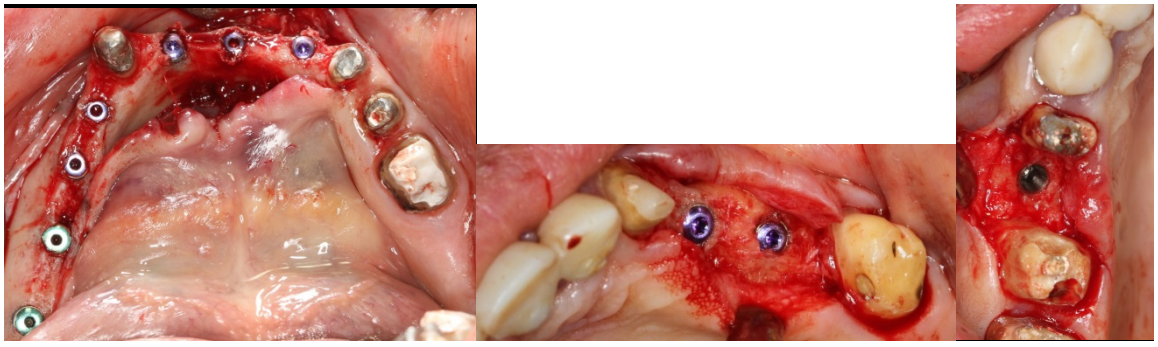

Fig.3, Fig.4, Fig.5 The surgical procedures

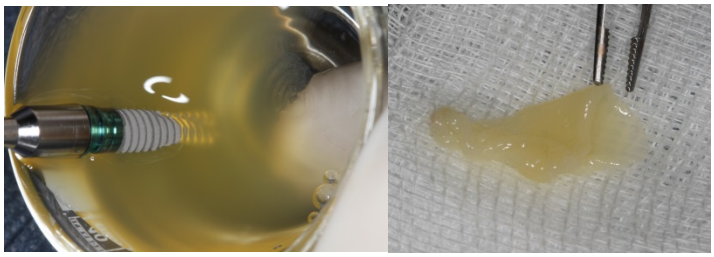

Fig.6, Fig.7 The PRGF activation and pure fibrine

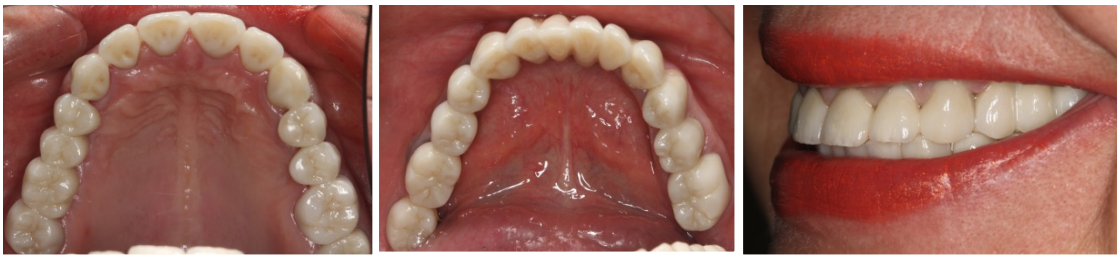

Fig. 8, Fig.9, Fig.10 Clinical view after treatment

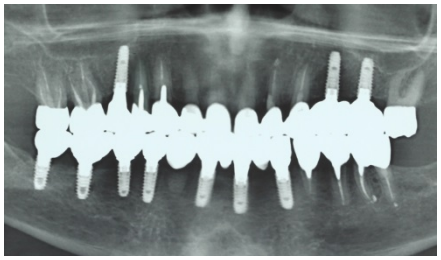

Fig.11 Panoramic x-ray after treatment

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Symposium 20: Antibiotic consumption – current issues

## The impact of pharmacodynamic/pharmacokinetic knowledge on antibiotic use

Prostran M

*Department of Pharmacology, Clinical Pharmacology and Toxicology*
*Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

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Antibiotics are among the most commonly used of all prescribed drugs, and at the same time, these drugs are the most commonly misused of all drugs. The consequence of the inappropriate use of antibiotics has been the emergence of resistance. Antibiotic-resistant pathogens kill around 50,000 people a year in the western world of the U.S.A. and Europe. According to the WHO that number of people is rising. Several years ago, Race (2008) reported that ESCAPE bacteria: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter species* effectively "escape" the effects of antimicrobial drugs. The ESCAPE bacteria cause the majority of U.S. hospital infections. In various European countries resistance of *Klebsiella pneumoniae* was less than 20% in 2006 *vs* 40% in 2012 (Italy) and less than 5% in 2006 *vs* 10% in 2012 (Spain). Two million people a year will contract a drug-resistant bacterial infection in the U.S.A. Direct health-cost is around $20 billion according to the CDC, not to mention additional billions due to lost productivity. It can be concluded that antimicrobial resistance is a global problem, constantly increasing while the resistant strains are spreading rapidly. For example, linezolid, a synthetic antimicrobial agent of the oxazolidinone class with the unique mechanism of action, was approved in 2000 in the U.S.A. Resistance in enterococci and staphylococci due to point mutations of the 23S rRNA was reported for the first time in 1999, even before the drug reached the market! Resistance has a major impact on disease severity, mortality and health-care costs. Organizations such as the Centers for Disease Control and Prevention (2013) have published a number of steps to optimize the proper, rational use of antibiotics and to prevent drug resistance. Some of these steps are listed below:

- Antimicrobial therapy of the diseases caused by viruses, and at least 90% of infections of the upper respiratory tract and many GI infections is ineffective and, therefore, useless
- Rather than embarking on a course of empirical antibiotic therapy for fever of unknown origin, the physician should search for its cause
- Dosing errors, which can be the use of either the excessive or a subtherapeutic dose, as well or wrong frequency of the administration, are very common

Generally speaking, we need to use antibiotics less and to use them prudently. Prudent antibiotic use means not using them when benefit is minimal (*e.g.* in many respiratory infections), to use narrow-spectrum antibiotics whenever possible, to use optimal dosage and regimens. Also, we should prevent infections, educate health-care workers and general population as well, to apply surveillance and to invest money in research. Almost two million deaths occur per year from bacterial

infections. Therefore, the rise of antibiotic-resistant infections is considered one of the greatest global threats to human health. The rapidly evolving resistance development by *Staphylococcus aureus* has the potential to re-create a pre-antibiotic world with mortality rates of 80% for those patients systemically infected. Unfortunately, for years many drug companies felt it wasn't worth their while to reasearch new treatments. The number of approved new antibiotics to treat systemic infections has been steadily declining for decades. Comparatively very expensive initial investment must go into the R&D (research and development) of antibiotics, with little guarantee of long term revenue due to the brief nature of the drug's prescription. According to estimates from London School of Economics report, even if antimicrobials make it to the market, oncology drugs are on average three times as profitable. Unlike an oncology drug, antimicrobials are usually taken for a short period of time, a week or two, limiting sales. The most commonly prescribed antibiotics, including amoxicillin and macrolide azithromycin, are now available as low-cost generics. While most big pharma companies invest elsewhere, some small companies are stepping into the antibiotic breach. Small and medium-size companies are now responsible for 73% antibiotics under development in the U.S.A. Although antibiotics are never going to be huge blockbusters, these drugs are desperately needed. In the U.S.A., several proposals have been made to expedite the D and regulatory review of antibiotics while, at the same time, ensuring that safety and efficacy requirements are met. The Infectious Diseases Society of America-IDSA developed a related drug development pathway called the Limited Population Antibacterial Drug (LPAD) approval mechanism. This approach calls for smaller, faster and less costly clinical trials to study antibiotics that treat resistant bacteria which cause serious infections.

So what is the best approach in antibiotic use? Is it optimizing the use of currently available antibiotics? It should be pointed out that inappropriate therapy is more likely if antibiotic resistance is present, and antibiotic-resistant microorganisms are more commonly associated with inappropriate therapy. To predict microbiological and clinical efficacy we use minimum inhibitory concentration (MIC) of antibiotics in combination with plasma concentration achieved during therapy. The goal of antibiotic therapy is to achieve complete bacterial eradication and to minimise the risk of resistance selection. The dosing regimen for a particular antibiotic is influenced by its pharmacokinetic (PK) profile and the susceptibility of the target pathogen. The bactericidal activity of an antibiotic can be time- or concentration-dependent. Efficacy also depends on the persistence of the antibiotic effect after serum levels have fallen below the MIC for the target pathogen (*post-antibiotic effect - PAE*). Also, prolonged exposure to suboptimal concentrations of antibiotics can lead to incomplete bacterial eradication and emergence of resistance. Penetration into the target tissue is also very important. Time-dependent killing means that bacteria are killed at the same rate and to the same extent once the treshold concentration is reached (T > MIC): penicillins, cephalosporins, carbapenems, macrolides. Some antibiotics kill best when concentration persists above MIC for longer duration of the dosing interval: kill is decreased by reducing the time above the MIC. Clinical implication is that a drug which is optimised by T > MIC should be dosed more frequently or if possible, should have its $t_{1/2}$ prolonged by other drugs, so that the drug concentrations persist above MIC or $EC_{95}$ as long as possible. Concentration-dependent killing means that the rate and extent of killing increases progressively with higher antibacterial concentrations ($C_{pmax}$/MIC or $C_{max}$/MIC): aminoglycosides, fluoroquinolones. Persistence of concentration above

MIC has less relevance for these drugs, meaning that these drugs can be dosed more intermittently. Another parameter used to integrate PK and PD is the area under the inhibitory curve (AUIC): for this group of drugs, dosing schedule has no effect on efficacy, but the cumulative dose matters.

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# Early application of antibiotics and its influence upon clinical manifestation in the early stage of Lyme disease

Begović Kuprešanin V

*Clinic for infectious and tropical diseases, MMA, Belgrade, Serbia*

Lyme disease (LD) is a multysystematic infective disease from the group of zoonoza, caused by spirochaeta Borrelia burdoferi (Bb), which develops by a bite of an infected tick from the Ixodes genus.

The disease appears in the form of different symptoms and signs of organs -skin, ankles, heart and nervous system being most commonly infected. All infected persons do not develop the disease, and it manifests itself from mildest to very serious forms with a possible invalidity and death.

The aim of the study has been to establish, in persons with an early diagnosed LB, the disease symptoms and signs, as well as how far the organs were attacked, in accordance with previously defined criteria, and to establish whether there existed any significant differences in symptoms and signs of the disease between those who had received an early antibiotic therapy after having been bitten by a tick and those who had not.

The examinations was carried out on 2070 patients of both genders, aged from 9 months to 86 years, divided into two groups. One group, A (н=591) received an early therapy, and the second, B (н=1479) did not. An early antibiotic therapy means the application of antibiotics in persons with a likely infection up to 5 day after being bitten by a tick, and who, at the time of the therapy application, did not show any symptoms nor early signs of Lb. The antibiotics which were applied were cephalosporin, macrolids, tetracycline, semi-synthetic penicillin, for seven or fourteen days, or benzyl penicillin only once.

The results of the study show that, in patients without an early antibiotic therapy, the disease developed in statistically more significant number of persons (537/1479) than in persons who received the therapy (10/591), that is 36.3%: 1,7%.

By the application of five groups of antibiotic, the conclusion was drawn that, for an optimal prevention, only two antibiotics were sufficient: doxycycline and ampicillin. These antibiotics showed statistically high efficiency.

It is mutually quantified and it went from the lowest - 93.7% (cephalosporin) to the highest - 99.4% (macrolids) regardless of the duration of application.

The number of the diseased after an expert removal of ticks was statistically significantly lower when compared with the persons in which the tick was inexpertly removed and who did not receive Ab therapy (7.6% : 28.76%).

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Symposium 21: Hot topics in infective endocarditis

# Diagnosis of infective endocarditis: What is new?

Lejko Zupanc T

*Department of Infectious Diseases, University Medical Centre Ljubljana*

## 1. Introduction

Infective endocarditis (IE) is still an infection with high morbidity and mortality and remains both a diagnostic and a treatment challenge. Imaging plays a key role and echocardiography remains the cornerstone of the methods in use. Although echocardiography improved diagnostics of infective endocarditis there are still cases especially of endocarditis involving intracardiac devices, prosthetic valves and homografts which are difficult to diagnose by echocardiography. Echocardiography depicts structural changes and abnormalities in the heart, but it does not uncover the underlying pathophysiological processes at the cellular or molecular level. This problem is addressed with introduction of new molecular imaging methods as $^{18}$F-fluorodesoxyglucose ($^{18}$F-FDG) PET-CT). But there are distinct limitations with $^{18}$F-FDG PET-CT which should not be neglected. New molecular microbiological techniques can also improve aetiological diagnosis of IE.

## 2. Positron emission tomography ($^{18}$F-FDG-PET-CT)

A few reports, mostly in prostethic valve endocarditis and cardiac device infections (CDIE) have shown promising results of PET-CT imaging (1). In native valve endocarditis, however, the use of PET-CT may be limited. In a a prospective cohort study 47 patients with definite IE undergoing PET/CT were compared with matched controls (94 patients with definite IE not undergoing PET/CT). The results were compared with those of conventional diagnostic techniques and clinical follow-up. PET/CT revealed at least 1 lesion in 35 patients (74.5%). The validity values for the efficacy of PET/CT in the diagnosis of septic lesions were as follows: sensitivity, 100%; specificity, 80%; positive predictive value, 90%; and negative predictive value, 100%. PET/CT was the only initially positive imaging technique in 15 true-positive cases (55.5%). The systematic use of PET/CT was associated with a 2-fold reduction in the number of relapses (9.6% vs. 4.2%, P = 0.25) and enabled significantly more infectious complications to be diagnosed (18% vs. 57.4%, P = 0.0001) (2). In a study by Ricciardi twenty-five out of 27 patients (92%) had a confirmed diagnosis of IE (18/25 PVE and 7/25 NVE); 16 had a positive echocardiography evaluation and 16 had positive $^{18}$F-FDG-PET-CT findings. Echocardiography showed a higher sensitivity as a diagnostic tool for the detection of IE compared to $^{18}$F-FDG-PET-CT (80% vs. 55%). However, a greater number of PVE had positive $^{18}$F-FDG-PET-CT results compared to those with positive echocardiography findings (11/13 vs. 9/13), and overall 89% (16/18) of confirmed PVE resulted $^{18}$F-FDG-PET-CT positive. Analyzing only the cases who underwent transoesophageal echocardiography, $^{18}$F-FDG-PET-CT showed a sensitivity of 85% in PVE (vs. 69% for echocardiography and 77% for the Duke criteria). All seven patients with NVE had a positive echocardiography and negative $^{18}$F-FDG-PET-CT

findings ($p<0.001$) (3). Adding an abnormal FDG uptake around a prosthetic valve as a new major criterion increases the sensitivity of the modified Duke criteria at admission from 70 to 97% (4). In patients with pyrexia of unknown origin, PET-CT has also been useful to reveal IE despite negative transoesophageal echocardiography. Despite reports of diagnostic sensitivity and specificity of 89 and 86%, respectively, in patients with CDIE not all studies confirm this finding. In one study a total of 21 patients with infection underwent FDG PET/CT. Findings demonstrated superficial skin infection in 1 patient, pocket site infection in 15, and CDIE in 13 patients. In patients with pocket site infection, the sensitivity and specificity of FDG PET/CT were 86.7% and 100%. The sensitivity and specificity of FDG PET/CT in patients with CDIE were 30.8% and 62.5%. Most false-negative results occurred in patients who had undergone previous antimicrobial treatment. This study in contrast to others indicated that FDG PET/CT is highly accurate for the diagnosis of skin and pocket CIED infection but low for infective endocarditis (5).

The 18F-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT) offers an excellent negative predictive value. Consequently, it is a reliable tool for excluding an infectious phenomenon in case of negativity. In case of persistent fever of unknown origin after cardiac surgery and in combination with other bacteriological examinations and medical imaging, we can rely on FDG-PET/CT to confirm or eliminate deep infections and prosthetic endocarditis. For this reason, FDG-PET/CT should be considered among the examinations to be performed in case of suspected infection after cardiac surgery.

## 3. Broad-range eubacterial PCR

Broad-range eubacterial PCR amplification followed by direct sequencing to identify microbial pathogens in heart valve material from 29 patients with histologically confirmed infective endocarditis and 23 patients free of infective endocarditis was performed. Microorganisms cultured by conventional techniques matched those identified by PCR in 21 cases. PCR alone identified the causative agent in three cases and corrected the initial bacteriological diagnosis in three cases. Among the 29 cases of histologically confirmed infective endocarditis, PCR findings were positive in 27 cases and were consistent with the bacterial morphology seen at Gram staining (26 cases) or with the results obtained by immunohistologic analysis with an anti-C. burnetii monoclonal antibody (one case). Ten clinical diagnoses of possible infective endocarditis were ruled out by histopathological analysis of the valves and subsequently by PCR. PCR was negative in 13 of the 14 patients in whom infective endocarditis was rejected on clinical grounds. In total, PCR contributed to the diagnosis and management of infective endocarditis in 6 of 29 (20%) cases (6).

In our retrospective study of 36 patients with definite IE by Duke's criteria who needed surgery and were hospitalized at the Department of Infectious Diseases, University Medical Centre Ljubljana the causative agent was identified in all patients; *Staphylococcus aureus* was the most prevalent pathogen. Blood cultures were positive in 30 (83.3%), negative in 3 and in 3 cases they were not withdrawn before a surgical procedure. In 6 (16.7%) cases the etiology was determined by additional microbiological tests (conventional culture, sonication culture, broad-range polymerase chain reaction) from material obtained by surgical procedure. Blood cultures showed concordance in all cases where multiple microbiological tests were done.

## 4. Conclusions

Several modalities (imaging and microbiological) can contribute to diagnostics in difficult cases of infective endocarditis or improve efficacy of treatment. The recommendations for the use of these modalities are based mostly on small case series so a larger prospective study is needed for better assessment of their diagnostic accuracy.

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# Early cardiac surgery in specific subpopulations of patients with infective endocarditis

Krajinović V

*University Hospital for Infectious Diseases, Zagreb, Croatia*

Despite advances in diagnosis and treatment of patients with IE, the mortality is still high (20% for in-hospital mortality, reaching 30% at one year after discharge). The management of patients with infective endocarditis (IE) remains one of the greatest challenges of clinical medicine.

Adequate antimicrobial treatment of IE is crucial for patient's survival. Since the 1960s, valve replacement and valve repair have become common procedures for management of IE in selected cases. In addition, number of patients operated on during the active phase of IE (early surgery) has increased during the last decade from 30% to 60%[1]. There are several explanations for this: advances in surgical techniques, epidemiological profile change and several studies demonstrating the beneficial effect of surgery in complicated IE. A reduction in mortality due to IE has been ascribed to treatment with effective antibiotic therapy and timely surgical intervention when appropriate[2]. While further advances in prevention, diagnosis, and antibiotic therapy are expected, optimization of surgical approaches seems to offer the best immediate opportunity to reduce mortality.

Management of patients with IE requires a thorough evaluation including early surgical consultation to identify patients who may benefit from surgery. Evaluation for valve cardiac surgery (CS) is recommended for decompensated heart failure, severe valvular insufficiency, valvular perforation or dehiscence, paravalvular abscess or fistula, persistent bacteremia and prevention of embolic risk (large vegetations). Although indications for cardiac surgery are well established, there are no enough randomized clinical studies to confirm the benefit of surgery[3]. Also, the benefits of surgery are not seen uniformly in all patients with IE, but are most realized in a targeted population[4].

Although IE has been the subject of many papers during the last 10 years, there are few studies concerning the subset of patients with severe IE requiring admission to the intensive care unit (ICU). Despite many useful informations from articles published as a result of the International Collaboration on Endocarditis-Prospective Cohort Study (ICE-PCS), intensivist remained frustrated because published studies generally did not identify specific problems of IE in the ICU and may not be relevant to critically ill patients[5].

Severe sepsis and septic shock are often seen in patients with IE (up to 30%). These patients are almost always admitted and treated in ICU. When there is an indication for CS of patients with IE complicated with severe sepsis and/or septic shock, surgery is not easy to perform, and influence of surgery on outcome of patients with IE and different stages of sepsis is unknown. Here we present the study of influence of CS on outcome of specific subpopulation of patients with IE with an accent on CS of IE complicated with different stages of sepsis (sepsis, severe sepsis, septic shock, multiorgan failure).

We prospectively analyzed 294 patients with IE hospitalized at University Hospital for Infectious Diseases in Zagreb between January 2000 and December 2011. We devided our patients in two groups. One group was with patients with sepsis (199 pts or 67%) and the other one was with 95 patients (33%) with severe sepsis/sepstic shock (SSSS). There was no difference among treatment groups regarding treatment (medically and surgically). Surgical treatment has a positive effect on outcome (in-hospital, one-year and five-year mortality) especially in the SSSS group of patients.

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# Chronic use of statins and infective endocarditis

Santini M

*University of Zagreb School of Medicine, Croatia*
*University Hospital for Infectious Diseases „Dr. F. Mihaljević", Zagreb, Croatia*

Infective endocarditis (IE) is a severe, most commonly bacterial infection of the endocardium. The epidemiology of this disease has changed significantly during the past few decades: the incidence grew from 2 to 12 cases per 100 000 persons per year, the mean age of patients rose from 58.6 to 60.8 years and the number of hospitalisations increased by 26% in people aged 65 and older [1]. Today the main risk factors for infective endocarditis include >60 years of age, male gender, poor dentition and dental infections, structural hearth disease, injection drug use, prosthetic heart valves, history of IE, presence of intravascular devices, chronic haemodialysis and HIV infection [2]. Mortality averages between 15 and 30% [3].

A large number of people with risk factors for cardiovascular disease take statins, 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, which lower the risk for atherosclerosis [4]. However, research has shown that statins have other, so called pleiotropic effects which include anti-inflammatory, antioxidant, anticoagulation, and even antimicrobial effects. This has led to further research about the influence of statin therapy on the severity of clinical presentation and outcome of treatment of sepsis. The results of studies (mostly observational) done so far are divided – half of the studies show that statins have positive influence on the outcome of treatment of sepsis, while the other half doesn't find that statins have any influence in this setting [5].

The question which arises is whether prior statin therapy has any effect on the incidence of IE.

To answer this question, we have carried out a retrospective, cohort, observational study at the University Hospital for Infectious Diseases „Dr Fran Mihaljević", Zagreb, Croatia, in patients aged 50 years and older with community acquired sepsis and bacterial isolates from blood cultures taken at hospital admission. The study covered the 5-year period from 1st January 2008, until 31st December 2012. Predictor variables were the age, gender, Charlson comorbidity index, and prior statin therapy. Outcome variables included the diagnosis of IE and SOFA score.

This study included 1161 patients, 647 (55.5%) were female, with mean age 71.7±10.7 years and mean Charlson comorbidity index 5.2±2.8. There were 997 (85.9%) patients with no prior statin therapy, and 164 (14.1%) patients with prior statin therapy. We compared patients who had prior statin therapy (statin group) with the patients without prior statin therapy (non-statin group). The two groups did not differ in age, gender or Charlson comorbidity index. IE was diagnosed in 95 (9.5%) patients in non-statin group and in 27 (16.4%) patients in statin group, p=0.007. SOFA score equalled 3.1±3.7 in non-statin group, while it was 2.6±2.8 in statin group, p=0.92.

The incidence of IE was higher in the statin group, which can be explained by higher incidence of chronic cardiovascular diseases in these patients, which are

themselves a risk factor for IE. However, our results suggest that prior statin therapy doesn't have significant protective effect for acquiring IE.

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Symposium 22: AB stewardship: do it now!

# Antibiotic consumption in Serbia

Radonjić V

*Medicines and Medical Devices Agency of Serbia, Belgrade*

Keywords: Antibiotics, WHO ATC/DDD methodology, macrolides, azithromycin, co-amoxiclav

## 1. Objectives

Medicines and Medical Devices Agency of Serbia as regulatory institution ensure quality, efficacy and safety medicinal products in Serbia, and as mandatory duty collect and estimate data about medicines consumption with the aim to make posible to see volume and contribution of particular medicines in total medicines consumption in Serbia. Comparation with EU countries, other non-European-Union (EU) south-eastern European countries (SEE) and newly independent states (NIS) made possible participation in ESAC project, which is of great importance because there is no reliable data on antimicrobial SEE and NIS countries.

Extent of patient exposure to medicine correlates with the occurrence and number of reported adverse reactions in post-marketing drug monitoring.

## 2. Methods

Valid 2011 total antimicrobial use data of Serbia were analysed according to the WHO Anatomical Therapeutic Chemical (ATC)/Defined Daily Doses (DDD) methodology and expressed in DDD/1000inhabitants/day (DID). Wholesales data on antibacterials (ATC group J01), antimycotics (J02) and antifungals (D01BA) were provided by the Medicines and Medical Devices Agency of Serbia, covering 100% of the population. Number of adverse drug reactions reports submitted to National Pharmacovigilance Centre.

## 3. Results

Total (outpatients and hospital care) antibacterial use was 25.6 DID. The top 5 antibacterial subgroups (ATC level 3) were: penicillins, ATC group J01C (11.1 DID, 43.2% of all antibacterials); macrolides, lincosamides and streptogramins, ATC group J01F (5.0 DID, 19.5%); other beta-lactam antibacterials, ATC group J01D (3.6 DID, 14.2%); quinolones, ATC group J01M (2.6 DID, 10.1%) and tetracyclines, ATC group J01A (2.3 DID, 9.0%). The top 5 antibacterials (ATC level 5) were: amoxicillin (6.6 DID, 25.8%); amoxicillin and enzyme inhibitor (co-amoxiclav, 3.8 DID, 14.8%); azithromycin (2.7 DID, 10.4%); cephalexin (2.3 DID, 8.8%) and doxycycline (2.2 DID, 8.7%). Serbia reported considerable use of pipemidic acid (0.9 DID, 3.7%) and of the oral third-generation cephalosporin cefixime (0.5 DID, 2.1%). Use of sulphonamide and trimethoprim in Serbia was low (0.2 DID, 0.8%). Use of nitrofurantoin was not reported. In 2011, the highest number of total reported adverse reactions to medicines in group J, was for: penicillins, ATC group J01C (29%), other beta-lactam

antibacterials, ATC group J01D (21,5%), quinolones, ATC group J01M (17%) and macrolides, lincosamides and streptogramins, ATC group J01F (14%). Based to this corellation between extent of patient exposure to medicines with number of reported adverse events is identified.

## 4. Conclusion

We present a standardised and validated data set of systemic antimicrobial use in Serbia. Particular for Serbia is the high use of macrolides (mainly of the long-acting macrolide azithromycin) and pipemidic acid, which is common to many former Yugoslavian countries. There was an increase in the proportional use intermediate acting (mainly claritomycin) and long-acting macrolides (mainly azithromycin) in terms of pharmacokinetic profiles and adverse drug reactions, could be considered a positive trend. On the other side increasing AMR pay attention to inappropriate use. Benchmarking by comparisons between countries has proven to be important stimulus to quality improvement. Opportunities for quality improvement are reduce total use of antibiotics and reduce use of co-amoxiclav and azithromycin.These data offer opportunities for antimicrobial quality target setting. Sustainable surveillance data will facilitate auditing of antimicrobial use and evaluation of the implementation of guidelines and public health policies to promote its judicious use.

Collecting, processing data on medicinal products marketing and consumption, as well as pharmacovigilance as regulatory affairs of Agency are important for improving pharmacotherapy and patient care.

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# Antimicrobial surgical prophylaxis in Slovenia

Lejko Zupanc T

*Department of Infectious Diseases, University Medical Centre Ljubljana*

Surgical site infections are one of the most common type of healthcare-associated infections (HCAI), accounting for 19.6% of all HCAI, based on data from the European Point Prevalence study 2011/12 (1). Perioperative antibiotic prophylaxis is considered an effective measure for preventing surgical site infections (SSI) as demonstrated by. Botwater et al. who analyzed data from 21 meta-analyses based on randomized controlled trials in 250 hospitals and 48,909 patients (2). The authors demonstrated that perioperative antibiotic prophylaxis reduced the risk of SSI in a broad range of surgical procedures, irrespective of the extent of wound contamination (relative risk = 0.19-0.82). These results imply that up to 80% of SSI could be prevented by appropriate perioperative prophylaxis.

However, antibiotic prophylaxis contributes considerably to total antibiotic use in hospitals (and subsequently to antibiotic resistance and cost). The recent point prevalence survey of European hospitals in 2011/12 indicated that surgical prophylaxis was the indication for 16.3% of antimicrobial prescriptions. Three-quarters of patients received the surgical prophylaxis for longer than necessary. Overall, 59.2% surgical prophylaxis was continued for more than one day, 15.8% for one day and only 25.0% for less than one day (1). Many studies have demonstrated deviations in perioperative prophylaxis guidelines in up to 88% of patients indicating the imperative for improvement in many European hospitals.

In Slovenia data from the European Point Prevalence study 2011/12 showed that antimicrobial agents were prescribed to 1761 of 5628 patients (31,3%) (1, 3). Antimicrobials were prescribed for treatment in 76 % and for prophylaxis in 17 %. In 55.6 % cases surgical prophylaxis lasted more than one day although there were significant difference between hospitals. These data show an improvement regarding previous point prevalence studies conducted in Slovenia in previous years (2006, 2008, 2009) although not all Slovenian hospitals participated (4). The duration of prophylaxis was longer than one day in 84% in year 2006, 80% in year 2008 and 69% in year 2009. Single dose was used accordingly in 6% (2006), 18% (2008) and 14% (2009). A recent retrospective cross-sectional survey in UMC Ljubljana studying appropriateness of surgical prophylaxis in several surgical departments showed that most surgical departments in UCLJ follow the AP guidelines in more than 80 % but there was still a lot of possibility for improvement. The most common inconsistency in AP application in UMCL was prolonged duration of AP. There was no significant decrease in hospitalization length when prolonging the AP (5).

Ministry of Health of Slovenia has introduced appropriateness of surgical prophylaxis as a quality indicator which should be regularly reported but a review of hospital web sites across Slovenia did not give a comprehensive picture on this indicator. There is an impression that this quality indicator is not reported according to definitions and that the duration of prophylaxis is too long.

Conclusions

Prevalence of antimicrobial prescribing in Slovenia hospitals is belove European average but the duration of antimicrobial prohylaxis is too long in 55.6% and is similar to European hospitals. Activities aimed at improving of antimicrobial prescribing should be directed also at antimicrobial prescribing of surgical prophylaxis.

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Symposium 23: Novel anti-Gram positive agents: Facts and promises

## Procalcitonin to guide duration of treatment

Tsangaris I

*2nd Critical Care Department, Athens University Hospital "Attikon", Athens, Greece*

Sepsis is among the most common causes of death in hospitalized patients, and hospital mortality of septic patients unacceptably high even in the modern management era. Treatment protocols for sepsis necessitate the rapid institution of broad-spectrum antibiotics, often empirically, as delayed antimicrobial therapy is associated with both increased morbidity and mortality. Although this is an evidence-based practice, the choice and dosing of the antibiotics, and their duration remains largely arbitrary and this lack of standardization results to increased cost, rising resistance and poor clinical outcome.

The diagnosis of sepsis can be a clinical challenge. More than 150 biomarkers have been tested as potential sepsis diagnostic markers; among them, procalcitonin (PCT), a calcitonin precursor, seems to be the most promising. However, and despite the initial enthusiasm, the role of PCT as a diagnostic marker of sepsis, especially among critically ill patients or certain patient groups is not universally accepted and is still a subject of controversy. PCT correlates with the extent and severity of infection and may have prognostic implications. Given that PCT levels increase upon bacterial infection and decrease upon recovery, it was proposed that the use of PCT measurements could guide antibiotic therapy.

This was first attempted at 2006 at a population of patients with community acquired pneumonia and was demonstrated that a PCT based algorithm could reduce duration of therapy from 13 to 6 days. Further studies in the field of emergency medicine confirmed these findings in patients with lower respiratory tract infections. At 2008 the first study in patients with severe sepsis and septic shock demonstrated that a PCT guided algorithm could reduce the use of antibiotics in this setting. Since then, numerous studies employing different cut-offs and assays, different research protocols and patients populations have been published.

At 2010 we sought to investigate the effect of a PCT-based algorithm in the ICU setting. We performed a systematic review and meta-analysis of the randomized controlled trials reporting on the outcomes of critically ill septic patients managed with or without a PCT-based algorithm. By synthesizing data from the relevant published studies, we found that the application of PCT-guided algorithms for the management of critically ill septic patients seems to be associated with 1) decreased antibiotic exposure; 2) similar mortality rates and ICU or hospital length of stay; and 3) comparable rates of superinfection and persistent or relapsed infection. The main finding of our meta-analysis (that is, decreased antibiotic exposure) was confirmed in a variety of outcomes (that is, duration of antibiotic treatment for the first episode of infection, total duration of antibiotic therapy, and antibiotic-free days) and was also retained in the prespecified sensitivity analyses. Last year Prkno raised the same question for the population of critically ill

patients with severe sepsis and septic shock, also concluding that a PCT-guided treatment reduces the duration of antimicrobial therapy in severe sepsis patients, without increasing 28-day and in-hospital mortality rates.

The limitations of the randomized control trials are many. Perhaps the most important is that in most cases the control groups did not use protocols with a track record of reducing duration of parenteral antibiotic therapy. For example, it has been clearly shown that locally applied practice protocols may decrease antibiotic use without compromising patients' outcomes in ventilator associated pneumonia. Overall, it is unclear how incorporation of these state of-the-art components might have affected meta-analysis findings if the comparison had been performed between protocols and PCT than between routine practice and PCT. On the other hand current evidence to limit duration of antibiotic treatment to 7 to 10 days is rather low and has been included only as a grade-2C recommendation in the recent guidelines of the Surviving Sepsis Campaign, in which is stated that "decisions to continue, narrow, or stop antimicrobial therapy must be made on the basis of clinician judgment and clinical information". Furthermore most studies excluded difficult-to-treat microorganisms, infections that are known to require prolonged antibiotic therapy, and severely immunocompromised and neutropenic patients. Finally refusal to stop antibiotics despite the opposite suggestion of the PCT's protocol was observed in a significant proportion of patients, indicating the difficulty of implementing this practice in real life.

On the other hand there are many negative studies coming, keeping the debate open. In a recent study of patients with intrabdominal infection and septic shock, Jung investigated whether an absolute drop down to at least 0.5 ng/ml or a significant reduction of at least 80% compared to the peak value was associated with the success of the initial treatment for septic shock related to an intra-abdominal infection. They found that a PCT threshold of 0.5 ng/ml was specific but neither sensitive nor accurate and that a PCT decrease of at least 80% from its peak was not associated with the patient's response to treatment. Duration of antibiotics in this subpopulation could not be recommended based on PCT level whatever the threshold used. In a recently presented German study with PCT monitoring at day 1, 4, 7, 10 and 14 no difference in mortality could be demonstrated for the PCT guided group compared to controls. It should be emphasized however, that in this trial the exposure to antibiotics was 823 vs 862 days (per 1000 ICU days) for the PCT guided and controls respectively and the difference was significant.

Where are we going? Leaders in the field believe that future clinical trials should be multicentric, ideally in different regions of the world and use less strict entry criteria, that better reflect real life conditions. But the most important modification should be made at the control arm of these studies. Instead using standard of care these studies should use best care practices, according to best available evidence. In conclusion there is accumulating evidence to suggest that the implementation of PCT-guided algorithms appears to be effective at reducing antibiotic use without compromising patients' safety. Further research is necessary before the adoption of this strategy in different settings and patient populations. Nevertheless it should be clear that the interpretation of any biomarker based algorithm or strategy should never be made *in vacuo* and certainly the combination of clinical

assessment, detection of physical signs and evaluation of a battery of biomarkers and imaging studies is necessary to minimize potentially catastrophic decisions.

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# Scientific Abstracts - Poster Presentations

## P1: Therapy of chronic Hepatitis B infection with Tenofovir

Stojanović D[1], Dupanović B[1], Andrić B[1], Dragaš S[1], Vujošević D[2], Terzić B[1]

[1]*Clinic of Infectious Disease, Podgorica, Montenegro*
[2]*Institute of Public Health, Podgorica, Montenegro*

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Keywords: Hepatitis B, therapy, tenofovir

Background: Chronic Hepatitis B virus infection is a chronic inflammatory reaction in the tissue of liver, which lasts for at least six months. It is estimated that two billion people worldwide have been infected with hepatitis B virus with more than 350 million with chronic infection. Those with chronic hepatitis B infection have up to a 15 to 40% risk of developing cirrhosis and hepatocellular carcinoma in their lifetime.

Objective: To present effectiveness of Tenofovir in the therapeutic response of chronic Hepatitis B infection.

Methods: The study presents 21 patients with chronic hepatitis B infection treated with Tenofovir in the Clinic for Infectious Diseases - Clinical Centre of Montenegro, during the period from June 2012 to June 2014. Determination of HBV DNA viral load was performed in the Laboratory for Molecular Diagnostic at the Institute of Public Health Montenegro.

Results: Data analysis showed that among 21 patients with chronic hepatitis B treated with Tenofovir, 17 (80%) were men and 4 (20%) were women. The average age of these patients was 36.2 years. After three months of treatment with tenofovir, twelve patients (57%) had negative HBV DNA in the blood. Reduction of HBV DNA viral load was registered in all patients. After twelve months of treatment HBV DNA was negative in twenty (95%) patients. In the first month of treatment three patients had elevated serum liver enzymes (5x), nausea, but in these patients use of the tenofovir was discontinued.

Conclusion: In our study Tenofovir proved to be effective in the therapeutic response to chronic Hepatitis B infection.

## P2: Hepatitis B virus vaccination and evaluation of management of occupational exposure to bloodborne viruses in a teaching hospital

Gregorčič S[1], Kordiš P[1], Pirš M[2], Poljak M[2], Seme K[2], Matičič M[1]

[1]*Department of Infectious Diseases and Febrile Illnesses, University Medical Centre Ljubljana, Slovenia*
[2]*Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia*

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Keywords: healthcare workers, occupational blood exposure, HBV immunization

Objectives: Occupational exposure to blood-borne viruses (OBE) poses risk for transmission of hepatitis B virus (HBV) to healthcare workers (HCW). Hepatitis B immunization and appropriate post-exposure management are important in prevention of infection. Aim was to evaluate impact of updated anti-HBs testing strategy and supplementary written information (brochures) on HBV vaccination, post-vaccination serostatus awareness and post-exposure management provided to HCW in the largest teaching hospital in Slovenia.

Methods: HCW of various profiles from surgical wards, non-surgical wards and laboratories completed anonymous questionnaire; 251 during 2007/2008 and 214 during 2012/2013 study periods. Statistical analysis was performed using chi-squared test.

Results: Majority of HCW from both groups received HBV immunization; anti-HBs assessment following immunization was significantly higher in 2012/2013 when also the proportion of those who were never assessed was significantly smaller (Table 1). More than a third of participants from each group was unaware of their anti-HBs titer. Proportion of HCW experiencing OBE and proportion of performed HBV screening in HCW and index patient following reported OBE, were comparable. Reporting of OBE has increased in 2012/2013 with borderline statistical significance (p=0.053). 62% were aware of information brochures which did not influence their knowledge on anti-HBs status (p=0.348), nor their decision to report OBE (p=0.494).

Conclusion: Rate of vaccination against hepatitis B is satisfactory and modified strategy on routine post-vaccination anti-HBs assessment has been successful. Unawareness of anti-HBs status and inadequate post-exposure measures are worrying. Enhanced educational programs are needed to increase awareness about the risk and prevention of OBE among HCW.

| | HCW | | p value |
|---|---|---|---|
| | 2007/2008 n (%) | 2012/2013 n (%) | |
| All participants | 251 (100) | 213 (100) | |
| **HBV vaccination performed** | 229 (91.2) | 201 (94.4) | 0.721 |
| Post-vaccination anti-HBs titer tested | 106 (46.3) | 136 (67.7) | **0.000** |
| Latest anti-HBs titer: | 221 | 186 | |
| <10 IU/mL | 15 (6.8) | 9 (4.8) | 0.398 |
| >10 IU/ml | 71 (32.1) | 95 (51.1) | 0.000 |
| unknown | 83 (37.5) | 79 (42.5) | 0.335 |
| Anti-HBs titer never tested | 52 (23.5) | 4 (1.6) | **0.000** |
| **OBE experience** | 124 (49.4) | 108 (50.7) | 0.711 |
| Reporting of OBE | 63 (50.8) | 67 (62.0) | **0.053** |
| HBV, HCV, HIV screening of HCW following reported OBE | 43 (68.3) | 53 (79.1) | 0.211 |
| HBV, HCV, HIV screening of index patient following reported OBE | 46 (73.0) | 45 (67.2) | 0.396 |
| Anti-HBs titer following reported OBE: | 59 (85.7) | 62 (89.6) | |
| <10 IU/mL | 5 (8.5) | 2 (3.2) | 0.204 |
| >10 IU/ml | 28 (47.5) | 28 (45.2) | 0.706 |
| unknown | 27 (45.8) | 32 (51.6) | 0.505 |

HCW - healthcare worker; HBV - hepatitis B virus; anti-HBs - serum concentration of hepatitis B surface antibody; OBE - occupational exposure to bloodborne viruses; HCV - hepatitis C virus; HIV: - human immunodeficiency virus.

## P3: HCV-induced hepatitis flare in a patient with non-Hodgkin B-cell lymphoma treated by rituximab including chemotherapy [R-CHOP] regimen

Ülçay A[1], Karagöz E[1], Karaahmetoğlu G[1], Turhan V[1], Acar A[1], Görenek L[1]

*[1]GATA Haydarpasa Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*

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Keywords: Hepatitis C virus, chemotherapy, non-Hodgkin lymphoma

Hepatitis C is known to be one of the main leading causes of liver failure worldwide. About 60-85% of people who are exposed to HCV will go on to develop chronic hepatitis C. It is globally accepted that reactivation of both HBV and HCV occurs after immunosuppressive treatments especially after chemotherapy. In addition, mortality rates related to reactivation in patients with oncologic and hematological malignancies who are receiving intensive chemotherapy regimens is higher than that observed in the general population. HBV reactivation was reported in patients receiving chemotherapy to vary between 14-72%. On the other hand, HCV reactivation is observed rarely and the result of HCV reactivation has modest results. According to various studies in NHL patients, the seroprevelance of HCV in NHL patients is higher than the general population. Today, cytotoxic drugs, corticosteroids, rituximab and hepatotoxic regimens are administered to NHL patients. Especially, utilization of rituximab regiments for NHL patients may result with flares in HCV patients. Here, we report a case of a HCV infected patient with NHL and after 4 months of this rituximab regimen, an acute flare up of HCV occurred. Peg Interferon alfa-2b 100 mcg/week subcoutanously + Ribavirin 1000 mg/day perorally treatment was commenced. After 4 weeks of antiviral therapy, rapid virologic response was observed and her HCV RNA levels were undetectable. Antiviral therapy was completed in about 48 weeks. After treatment there was no recurrence in HCV, serological virologic response occurred and oncological monitoring has still been underway with remission.

## P4: Seroprevalance of HBV, HCV and HIV in a training hospital

Selek MB[1], Bektöre B[1], Karagöz E[2], Baylan O[1], Özyurt M[1]

*[1]GATA Haydarpaşa Training Hospital, Medical Microbiology department*
*[2]GATA Haydarpaşa Training Hospital, Infectious Diseases and Clinical Microbiology department*

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Keywords: Viral hepatitis, seroprevalence, ELISA

Objective: Viral hepatitis is one of the most important liver diseases in Turkey and in the World. On the other hand, a rapid increase in the number of HIV cases across the globe has been observed over the years in the world. In this study, we aimed to investigate the seroprevalence of HBV, HCV and HIV in patients admitted to hospital.

Material and method: Patients who admitted to our hospital between 2012-2013 were evaluated retrospectively to find out the seroprevalence of these diseases. HBsAg, Anti-HCV, Anti-HIV were analyzed in serum samples via ELISA.

Results: 15146 samples were analyzed for HBsAg and 270 of these samples were positive (1.78%). For Anti-HCV, 12776 samples were analyzed and Anti-HCV positivity was detected in 170 patients (1.33%). Anti-HIV was analyzed in 11559 patients and 22 patients were found to be positive (0.19%).

Conclusion: The seroprevalence of HBsAg, anti-HCV and anti-HIV were similar to national data. These viral diseases can be transmitted via body fluids and blood. They pose threat for health care workers especially for surgical department staff. When these rates are considered, it is necessary to screen the patients. In future, we expect to see a significant decrease in HBV seroprevalance due to national immunization program.

## P5: Boceprevir in genotype 1 chronic hepatitis C: first experiences in Serbia

Simonović Babić J[1], Bojović K[1], Fabri M[2], Kostić V[3], Jovanović M[3], Mijailović Ž[4], Svorcan P[5], Janković G[6]

*[1]Faculty of Medicine, University of Belgrade and Clinic of Infectious and Tropical diseases, Clinical Center of Serbia, Belgrade , Serbia*
*[2]Faculty of Medicine, University of Novi Sad and Clinic of Infectious diseases, Novi Sad, Serbia*
*[3]Faculty of Medicine, University of Niš and Clinic of Infectious diseases, Niš, Serbia*
*[4]Faculty of Medicine, University of Kragujevac and Clinic of Infectious diseases, Kragujevac, Serbia*
*[5]Faculty of Medicine, University of Belgrade and Department of gastroenterology and hepatology, Belgrade, Serbia*
*[6]Faculty of Medicine, University of Belgrade and Clinic of gastroenterohepatology, Clinical Center of Serbia, Belgrade, Serbia*

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Keywords: Hepatitis C, boceprevir, antiviral therapy

Introduction: The triple therapy which consists of boceprevir plus pegylated interferon and ribavirin (P/R) is the standard of care for treatment of hepatitis C virus (HCV) genotype 1(G1) infection in treatment of naïve and experienced patients. The aim of this study is to analyze the efficacy and tolerability of this regime in real clinical practice in Serbia.

Method: From July 2012 to October 2012 twenty experienced patients with advanced fibrosis and HCV G1 infection, were included in the triple antiviral regime in six referral centres in Serbia. All patients were treated in response guide therapy (RGT) regimen according to the boceprevir treatment protocol. During the 4-week lead-in period all patients received peginterferon plus ribavirin. After the lead-in period boceprevir was added in dosage of 800 mg three times a day orally. The subsequent treatment varied according to virologic response and fibrosis. During the therapy HCV RNA level was measured at week 4, 8, 12, 24 of the treatment for assessment of virologic response profile. All patients who completed therapy were assessed at the end of the treatment and at the end of an additional 24-week treatment free period for a sustained virologic response (SVR).

Results: Total of 20 patients with advanced fibrosis were treated. Among patients with an undetectable HCV RNA level at week 8 the rate of SVR was 100%. No patient with decrease in the HCV RNA level < 1 log 10 IU/ml at treatment week 4 achieved SVR. The overall rate of SVR was 55%. The safety profile of the treatment regimen was good. Anemia was reported in 25% of patients. There was no life-threatening treatment adverse event.

Conclusion: Boceprevir in combination with P/R achieved high SVR rates in patients that were “most difficult to treat” who failed on dual therapy and is effective for use in patients with cirrhosis.

## P6: Acute hepatitis C infection: influence of the viral load on the success of antiviral treatment

Mitrović N[1], Bojović K[1,2], Simonović Babić J[1,2], Mitrović J[3], Švirtlih N[1,2], Delić D[1,2]

*[1]Clinic for infectious and tropical disease, Belgrade, Serbia*
*[2]University of Belgrade, Faculty of Medicine, Serbia*
*[3]Primary health care center "Dr Simo Milošević", Belgrade, Serbia*

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Keywords: acute hepatitis C; viral load; treatment

Introduction: Acute hepatitis C virus (HCV) infection often develops into chronic form but application of antiviral therapy (standard or pegylated interferon) increases chances of the recovery. The aim of the study was to determine influence of HCV viral load on the success of antiviral therapy.

Methodology: Study was conducted on 20 patients with confirmed acute hepatitis C. All patients were treated with standard interferon alpha in Clinic for infectious and tropical disease, Belgrade, Serbia. Before the onset of therapy HCV RNK PCR was performed and we analyzed influence of the viral load on the success of treatment. Full clinical and biochemical recovery in the time frame of minimum six months was considered as success of therapy. We used X2 and Fisher's exact test, $p<0.05$ considered statistically significant.

Results: From 20 patient enrolled in the study in 5 (25%) initial HCV RNK PCR was negative. Average viral load was 140,379 UI/mL (641±9,923,130 UI/mL). Low viral load (<600,000 IU/mL) was detected in 14 (70%) patients, and high viral load (>600,000IU/mL) in 6 (30%). From 20 patients treated with antiviral therapy 16 (80%) fully recovered, while 4 (20%) got chronic form of disease. Among group of patients with low viral load 13 (92.8%) recovered, while in group with high viral load chronic form of disease developed in 3 (50%) patients. This difference was statistically significant ($p=0.028$).

Conclusion: The success of antiviral treatment in patients with acute hepatitis C with standard interferon is greater in patients who initially have low viral load (<600,000IU/mL).

## P7: Significant life-style changes after successful treatment of chronic hepatitis C in people who inject drugs in Slovenia

Matičič M[1], Kordiš P[1], Kastelic T[1], Oblak T[1], Meglič-Volkar J[1], Rajter M[1], Prah J[1], Kotar T[1], Strle F[1], Bogovič P[1], Maver M[1], Kastelic A[2], Čuk Rupnik J[3], Rebolj-Kodre AM[4]

*[1]Clinic for Infectious Diseases and Febrile Illnesses, University Clinical Centre Ljubljana, Slovenia*
*[2]Coordination of Centers for Prevention and Treatment of Illegal Drug Addiction, Slovenia*
*[3]Center for Prevention and Treatment of Illegal Drug Addiction Logatec, Slovenia*
*[4]Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Slovenia*

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Keywords: Hepatitis C, people who inject drugs, treatment

Objectives: Only a small proportion of people who inject drugs (PWID) is treated for hepatitis C virus (HCV) infection although sustained virological response (SVR) is comparable to non-PWID. Stigma related to their life-style represents important barrier. Aim was to identify possible life-style changes after SVR among PWID in Slovenia and compare them to healthy non-PWID.

Methods: Cross-sectional cohort controlled study compared PWID (active, former and/or on opioid substitution treatment - OST), treated for HCV with SVR during 2000-2012, to non-PWID healthy controls. In 2013/2014 they filled-out anonymous questionnaire on housing, education and employment, both before HCV treatment and by the time of study, controls timely matching those periods. Chi square of difference between ratios and multilinear regression determining impact of named characteristics on life-style changes were performed.

Results: Out of 130 invited PWID, 109 (84%) responded. 96 met inclusion criteria, 64% males, median age 37 years. Median period between reaching SVR and study was 3.5 years. After achieving SVR, 84% reported on improving housing situation, 64% on finding employment and 56% on improving formal education, pre- and post-treatment ratios of all parameters showing significant differences ($p<0.001$). Compared to housing, employment and education of controls in comparable periods, PWID significantly more often changed the three examined parameters (Table 1). Except for sex ($p=0.02$, $\alpha=0.05$), selected characteristics (age, HCV genotype, OST, psychological/psychiatric support) showed no significant effect on overall change in PWID life-style.

Conclusions: After SVR, significant improvement in certain lifestyle parameters was found in PWID, justifying better access to HCV treatment.

**Table 1.** Baseline and 3.5-year follow-up characteristics of the study population (people who inject drugs) and controls.

| Period | Baseline | | Follow-up | | p-values (PWID) | p-values (Controls) |
|---|---|---|---|---|---|---|
| Group | PWID N=96 | Controls N=113 | PWID N=96 | Controls N=113 | | |
| Housing (n, %) | | | | | <0.001 | 0.07 |
| Homeless | 5 (5 %) | 0 | 2 (2 %) | 0 | | |
| Alone/ with a partner | 41 (43 %) | 87 (77 %) | 73 (76 %) | 97 (86 %) | | |
| With parents/in a community | 46 (48 %) | 25 (22 %) | 21 (22 %) | 16 (14 %) | | |
| Other | 4 (4 %) | 1 (1 %) | 0 | 0 | | |
| Employment status (n, %) | | | | | 0.001 | 0.12 |
| Fully employed | 35 (37 %) | 99 (88 %) | 52 (55 %) | 93 (82 %) | | |
| Precarious workers | 23 (24 %) | 3 (3 %) | 16 (17 %) | 6 (5 %) | | |
| Unemployed | 37 (39 %) | 11 (10 %) | 27 (28 %) | 14 (12 %) | | |
| Education (n, %) | | | | | <0.001 | 0.98 |
| Primary school | 14 (15 %) | 8 (7 %) | 5 (5 %) | 8 (8 %) | | |
| High school | 76 (79 %) | 59 (52 %) | 73 (76 %) | 56 (50 %) | | |
| College | 3 (3 %) | 9 (8 %) | 3 (3 %) | 9 (8 %) | | |
| University | 2 (2 %) | 36 (32 %) | 15 (16 %) | 39 (35 %) | | |
| Interrupted education | 1 (1 %) | 1 (1 %) | 0 | 1 (1 %) | | |

PWID - people who inject drugs.

## P8: Prevalence of anti-HCV antibodies in the general population of Slovenia: the results of five World Hepatitis Day screening campaigns

Matičič M[1], Maver M[1], Kmet N[1], Vidmar Vovko D[1], Meglič-Volkar J[1], Rajter M[1], Prah J[1], Kotar T[1], Videčnik Zorman J[1], Gregorčič S[1], Lešničar G[2], Baklan Z[3], Remec T[4], Pal E[5], Poljak M[6]

*[1]Clinic for Infectious Diseases and Febrile Illnesses, University Clinical Centre Ljubljana, Slovenia*
*[2]Department of Infectious Diseases and Febrile Conditions, General Hospital Celje, Slovenia*
*[3]Department of Infectious Diseases and Febrile Illnesses, University Clinical Centre Maribor, Slovenia*
*[4]Department of Infectious Diseases, General Hospital Novo mesto, Slovenia*
*[5]Department of Infectious Diseases, General Hospital Murska Sobota, Slovenia*
*[6]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia*

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Keywords: HCV screening, seroprevalence, general population

Objective: In two million population of Slovenia hepatitis C virus (HCV) seroprevalence in general population has been estimated 1 to 2.5%. So far, only 3000 individuals have been diagnosed. Anonymous free-of-charge HCV screening campaigns of general population may be effective in increasing awareness and identifying undiagnosed individuals to get effective treatment.

Methods: Observational multicenter study was performed analyzing five national general population HCV screening campaigns performed on World Hepatitis Day in years 2007-2011. Participants were screened for anti-HCV antibodies. Demographic characteristics and risk factors for HCV infection were assessed through anonymous questionnaire. Fisher's exact test was used for comparison of binominal categorical data and logistics regression analysis to assess impact of risk factors on infection.

Results: Among 3743 individuals screened, average age was 37.6 years (SD 1.5), 56.9% were females, 65.5% presented high school or college education. 71 (1.9%) tested anti-HCV positive. The major risk factor for infection was intravenous drug use (53.5 % vs. 3,6 %, p<0.001, OR 32.5), followed by cocaine snorting (57.7% vs. 10%, p<0.001, OR 12.23), HIV/HBV co-infection (18.3% vs. 1.1%, p<0.001, OR 22.67), risky sexual behaviour (38.0% vs. 22.8%, p=0.003, OR 2.13), accidental needlestick injury (29.6% vs. 16.6%, p=0.004, OR 2.21), tattooing/piercing (28.1% vs. 10.3%, p=0.000, OR 3.50), HCV-infected partner (8.4% vs. 1.9%, p= 0.000, OR 2.14). 49.3% of infected presented several risk factors.

Conclusions: HCV-seroprevalence in the studied sample is comparable to the estimated HCV-seroprevalence in general population of Slovenia being associated with well-known risk factors and justifies the performance of screening campaigns.

## P9: »Real-life« experiences with boceprevir/telaprevir triple therapy in hepatitis C genotype 1 difficult-to-treat patients: Results of Slovenian national study

Matičič M[1], Biasizzo H[1], Staražinič N[1], Meglič-Volkar J[1], Rajter M[1], Prah J[1], Kotar T[1], Vidmar L[1], Lešničar G[3], Selič-Kurinčič T[3], Baklan Z[4], Ekart K[4], Hafner M[5], Poljak M[2]

*[1]Clinic for Infectious Diseases and Febrile Illnesses, University Clinical Centre Ljubljana, Slovenia*
*[2]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia*
*[3]Department of Infectious Diseases and Febrile Conditions, General Hospital Celje, Slovenia*
*[4]Department of Infectious Diseases and Febrile Illnesses, University Clinical Centre Maribor, Slovenia*
*[5]Department for Gastroenterology, University Clinical Centre Ljubljana, Slovenia*

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Keywords: Hepatitis C, protease inhibitors, treatment

Objectives: Triple therapy of hepatitis C (HCV) genotype 1 patients with pegylated interferon, ribavirin and first-generation protease inhibitors (PI) boceprevir (BOC) or telaprevir (TVP) shows higher rates of sustained virological response (SVR) compared to PI-free treatment. The aim of this national study was to evaluate efficacy and safety of triple BOC/TVP therapy among Slovenian patients during period 2011-2013.

Methods: In a prospective, observational multicenter study 59 patients (29 with BOC, 30 with TVP) were enrolled consecutively on an intention to treat basis. Their clinical and virological data were analysed.

Results: Median age was 55 years (range 32-72 years), 61% were males, 52% genotype 1b, 86% presenting IL28B non-CC. 47% had METAVIR F≥3, 7% detected platelet count <100.000 and serum albumin <35 g/L. 90% failed previous therapy (30% relapsers, 35% partial responders, 25% null-responders). During treatment no patient died, 10% presented hepatic decompensation and 25% severe infections. Severe anaemia (Hb<90g/L) was observed in 20%; reduction of ribavirin was needed in 59%, 39% received erythropoetin and 15% blood transfusion. Treatment discontinuation rate was 12% for severe adverse events and 20% for detection of resistance to PI. SVR of entire cohort was 53%; 59% in BOC, 47% in TVP (difference not statistically significant); 39% in F4, 75% in F3; 94% in relapsers, 40% in partial responders and 29% in null-responders.

Conclusion: Our results confirm first-generation triple therapy to be effective in difficult-to-treat patients. Due to frequent serious adverse events treatment decisions should be made very judiciously and patients should be monitored very carefully.

## P10: Efficacy of boceprevir-based therapy in HCVG1 treatment-experienced patients with advanced fibrosis/cirrhosis: southeast European NPP study

Tchernerv K[1], Antonov K[2], Simonović Babić J[8], Vince A[14], Dušek D[14], Krastev Z[2], Nikolovska D[3], Kotsev I[4], Genov J[5], Donkova A[1], Idriz N[1], Jelev D[2], Jeleva N[2], Ivanova I[4], Dukova D[4], Mitova R[5], Katsarov K[6], Simonova M[6], Tomov D[6], Tsonev R[7], Balabanska R[7], Bojović K[8], Fabri M[9], Kostić V[10], Jovanović M[10], Mijailović Ž[11], Svorcan P[12], Janković G[13], Kurelac I[14]

*[1]Clinic of Gastroenterology, University Hospital, Sofia, Bulgaria*
*[2]Clinic of Gastroenterology, St. Ivan Rilski' University Hospital, Sofia, Bulgaria*
*[3]Clinic of Gastroenterology, Medical Institute Ministry of Interior, Sofia, Bulgaria*
*[4]Clinic of Gastroenterology, Medical University, Varna, Bulgaria*
*[5]Clinic of Gastroenterology, Medical University Hospital Queen Joanna, Sofia, Bulgaria*
*[6]Military Medical Academy, Sofia, Bulgaria*
*[7]Gastroenterology Department, Tokuda Hospital Sofia, Bulgaria*
*[8]Clinic of Infectious and Tropical diseases, Clinical Center of Serbia, Belgrade, Serbia*
*[9]Clinic of Infectious diseases, Novi Sad, Serbia*
*[10]Clinic of Infectious diseases, Niš, Serbia*
*[11]Clinic of Infectious diseases, Kragujevac, Serbia*
*[12]Department of gastroenterology and hepatology, KBC Zvezdara, Belgrade, Serbia*
*[13]Clinic of gastroenterohepatology, Clinical Center of Serbia, Belgrade, Serbia*
*[14]Clinical Department for Viral Hepatitis, University Hospital for Infectious Diseases, Dr. Fran Mihaljevic, Zagreb, Croatia*

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Keywords: hepatitis C, boceprevir, real life data

Objectives: To evaluate efficacy and tolerability of boceprevir based triple therapy (BOC) in treatment-experienced patients with advanced fibrosis/cirrhosis.

Methods: Prospective multinational survey including treatment-experienced patients with G1 HCV and cirrhosis or advanced fibrosis treated with P/R/BOC in the NPP early access program in Bulgaria, Serbia and Croatia.

Results: From August 2011 to October 2012, 149 patients (61.7% male, mean age 49 yrs, 45.6% G1b, 29.5% F4 metavir, 65.7% relapsers, 11.4% partial and 22.8% null responders) were enrolled in 14 sites in 3 SEE countries. Baseline viremia was 931.364 IU/mL (median), hemoglobin levels below 100 g/dL were present in 10.7% patients and platelets count <100.000 was present in 5.4% patients. Overall, in the ITT analysis, SVR (week 72) rate was 46.3% (55.3% in F3, 38.8% in F4; p = 0.01), while it was 49,0% in relapsers, 62.5% in partial responders and 32.3% in null responders respectively.

Hematologic side effects were the most frequent adverse events, causing therapy discontinuation in 7% of patients. Dysgeusia, asthenia/fatigue and emotional difficulties were commonly reported by the patients, sometimes causing lack of compliance (in 1.5% therapy was discontinued due to patients request). One patient (0.67%) developed life-threatening adverse event in Wk. 12 (agranulocitosis, staphylococcal sepsis and hepatic decompensation).

Conclusions: Triple therapy with boceprevir is effective in treatment-experienced patients. High SVR rates can be achieved even in those with evidence of fibrosis and cirrhosis, but response is poor in prior null responders. Despite the incidence of severe adverse events was lower in comparison to other real-world surveys, close monitoring of side-effects is needed.

## P11: Epidemiological surveillance of CA-MRSA infections in Slovenia between 2006 and 2013

Grmek-Košnik I[1], Ribič H[1], Rupnik M[1], Dermota U[1]

[1]*National Laboratory of Health Environment and Food*

Keywords: CA-MRSA, S. aureus, Panton-Valentine leucocidin

Community-acquired (CA) methicillin resistant S. aureus (MRSA) infections have been recognized as an emerging public health problem worldwide. They are defined as MRSA infections in patients who do not have risk factors for MRSA such as recent hospital admission, operations, antibiotic administration, dialysis, drains or a nursing home stay.

The infections are usually mild, often limited to the skin and subcutaneous tissues; however, they may be severe and even fatal like necrotising pneumonia.

In Slovenia, the disease has been notifiable by law since 2004. In the period from January 1st 2006 to December 31st 2013 six regional Institutes of Public Health and National Institute of Public Health took a part in a monitoring of CA-MRSA infections in Slovenia. During this period we analyzed 395 strains from 395 patients.

Only S. aureus isolates resistant to oxacillin and susceptible to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin or gentamicin were included for further analysis. The presence of the gene for Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin, exfoliative toxins, enterotoxins and the type of a staphylococcal cassette chromosome (SCCmec) was confirmed by polymerase chain reaction (PCR). We determined spa type.

Nowdays became evident that CA-MRSA strains spread between the community and the hospital and cause nosocomial infections. Continued surveillance as well as analysis of their virulence factors will therefore be important for monitoring this spread.

Epidemiological monitoring of MRSA in a community, hospitals and in groups at risk is important task for the future due to the possibility of a minor problem developing into a serious epidemic which could be difficult to control.

## P12: Antibacterial synergy between JEK 47 and oxacillin in a murine model of MRSA

Hendricks O[1, 5], Poulsen MØ [2, 5], Christensen JB[3], Kristiansen JE[4]

[1]*King Christian X Hospital for Rheumatic Diseases, University of Southern Denmark*
[2]*Institute of Clinical Research, Research Unit of Clinical Microbiology, Denmark*
[3]*Department of Chemistry, University of Copenhagen*
[4]*Center for Biomembrane Physics (Memphys), Denmark*
[5]*Institute of Regional Health Services Research, Denmark*

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Keywords: Antimicrobial resistance, non-antibiotics, modern drug design

Objective: Global rates of infections caused by Staphylococcus aureus continue to increase. Phenothiazines and thioxanthenes and their stereoisomers have extraordinary anti-microbial activities. In vitro studies have demonstrated an effect of stereoisomeric thioridazine (-) = JEK 47 on MRSA. JEK 47 is likely to be characterized by less cardiotoxicity compared to stereoisomeric thioridazine (+). Furthermore, JEK 47 has been reported to be the compound that is concentrated in human tissue cells at higher levels than the dextrorotatory form. Taken together, these facts suggest that JEK 47 should be superior in the context of antimicrobial treatment due to potentially fewer side effects than the (+) - form or the racemic form. Consequently, the aim of the actual study was to investigate the antibacterial activity of JEK 47 in a murine model of MRSA sepsis.

Method: We used a peritonitis model for non-lethal MRSA sepsis in order to investigate the effect of JEK 47 in vivo.

Results: Statistically significant fewer SA appeared in the infected animals treated with JEK 47 when the infected mice received the drugs in medium dose (p=0.0006). The protective effect of JEK 47 was similar with or without the addition of oxacillin. Significantly fewer SA was also observed in a group of mice receiving JEK 47 as pre-treatment (p=0.0009). Furthermore medium and high treatment doses appeared to prevent spleen hypertrophia.

Conclusion: The actual study provides new insight about the in vivo potential of JEK 47 in infections caused by MRSA.

**Total number of SA counts during the entire study period presented for each treatment group**

| Group No. | MRSA Challenge | Oxacillin Mg/kg | JEK47 Mg/kg bid. | No. of SA counts during treatment |
|---|---|---|---|---|
| **1** | - | - | - | 0 |
| **2** | + | - | - | 39 |
| **3** | + | 30 | - | 42 |
| **4** | + | - | 0,95 | 26 |
| **5** | + | 30 | 0,95 | 36 |
| **6** | + | - | 2,85 | 22 |
| **7** | + | 30 | 2,85 | 17 |
| **8** | + | - | 8,55 | 24 |
| **9** | + | 30 | 8,55 | 27 |
| **10** | + | 30 | 8,55 Pre-infection treatment | 15 |

## P13: Macrolide resistance of Streptococcus pneumoniae and Staphylococcus aureus strains isolated from patients of the University Hospital Bratislava - Stare Mesto, and Children`s University Hospital in Bratislava

Slobodníková L[1,2], Blažeková M[1,2], Koreň J[1,2], Záborská M[1,2]

[1]*Institute of Microbiology, Faculty of Medicine of the Comenius University*
[2]*University Hospital in Bratislava*

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Keywords: Macrolide resistance, Streptococcus pneumoniae, Staphylococcus aureus

Objectives: Resistance to macrolides among pneumococci and staphylococci emerged shortly after introduction of erythromycin to clinical practice and started to spread more extensively in 90-ties. The goal of the study was therefore a resistance survey of Streptococcus pneumoniae and Staphylococcus aureus strains isolated in our Institute during years 2012 a 2013.

Methods: The biological samples were processed according to the standard laboratory methods. Susceptibility to erythromycin, clindamycin, penicillin, oxacillin, tetracycline, co-trimoxazole, and vancomycin was tested according to EUCAST. PBP2a was detected by latex-agglutination test.

Results: 180 S. pneumoniae and 1461 S. aureus strains were isolated. The resistance of S. pneumoniae to macrolides increased from 28 % in the year 2012 to 38 % in the year 2013. Higher percentage of resistance to all monitored antibiotics was detected in strains isolated from children. Macrolide resistance of S. aureus reached 27 % to 40 %, depending on the origin of the strains. A high percentage of co-resistance to penicillin and macrolides in pneumococci, and oxacillin and macrolides in S. aureus was detected. The predominant type of macrolide resistance in both of the monitored bacterial species was MLSB type.

Conclusions: It is necessary to continue the monitoring of resistance to macrolides at the local levels, in the case of S. pneumoniae individually in the children and in the adults, and attract the attention of the clinicians to keep the strict rules of rational antibiotic therapy, and to the cautious usage of macrolides in order to preserve their antibacterial activity for the future.

## P14: Inhibitory effect of newly-synthesized chalcones on hemolytic activity of methicillin-resistant Staphylococcus aureus

Božić DD[1], Milenković MT[1], Ivković BM[2], Larsen AR[3], Ćirković IB[4]

*[1]Department of Microbiology and Immunology, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia*
*[2]Department of Pharmaceutical Chemistry, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia*
*[3]Department of Microbiological Surveillance and Research, Statens Serum Institute, Copenhagen, Denmark*
*[4]Department of Bacteriology, Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Belgrade, Serbia*

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Keywords: MRSA, chalcones, α-hemolysin

Objectives: Pathogenicity of methicillin-resistant Staphylococcus aureus (MRSA) is associated with broad spectrum of virulence factors, amongst which is α-hemolysin. It is generally considered that α-hemolysin plays a central role in the pathogenesis of staphylococcal infections. Conventional antimicrobial chemotherapy often fails due to occurrence of multiresistant strains. Accordingly, development of antimicrobial compounds that selectively target virulence factors might overcome therapeutic failure of antibiotics. The aim of this study was to investigate the effect of three newly-synthesized chalcones (1,3- Bis-(2-hydroxy-phenyl)-propenone, 3-(3-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propenone and 3-(4-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propenone) on α-hemolysin production of MRSA.

Methods: Antibacterial activity of chalcones was tested against 20 clinical isolates of MRSA by broth microdilution test according to CLSI guidelines (CLSI, 2007). Identification and methicillin resistance were confirmed by PCR for nuc and mecA genes. Strains were genotypically characterized (SCCmec, agr, pvl and spa typing).

Results: Minimum inhibitory concentrations (MIC) of the tested compounds were in the range of 25-150 μg/ml. Subinhibitory concentrations of chalcones inhibited hemolytic activity of MRSA strains, with almost complete abolishment of hemolysis at concentrations in the range of 1/2-1/4 x MIC. The most significant dose-dependent inhibition of rabbit erythrocyte hemolysis was observed in MRSA supernatants cultivated with subinhibitory concentrations of 1,3- Bis-(2-hydroxy-phenyl)-propenone (1/2-1/16 x MIC). Other two compounds inhibited α-hemolysin production in concentrations ranging from 1/2-1/8 x MIC and 1/2 -1/4 x MIC, respectively.

Conclusion: Newly-synthesized chalcones tested in this study showed potent antibacterial activity and inhibitory effect on α-hemolysin production of multiresistant and genetically diverse MRSA strains, and therefore may be considered as promising new antimicrobial agents.

## P15: Prevalence and genotypic characteristics of methicillin-resistant Staphylococcus aureus isolates in Serbia

Ćirković I[1], Stepanović S[1], Pelemiš M[2], Stevanović G[2], Švabić Vlahović M[1], Larsen AR[3]

*[1]Institute of Microbiology and Immunology, Faculty of Medicine, Belgrade, Serbia*
*[2]Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Faculty of Medicine, Belgrade, Serbia*
*[3]Statens Serum Institute, Copenhagen, Denmark*

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Keywords: MRSA, surveillance, resistance, genotyping, CC5-I clone

Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most significant hospital-associated (HA) and community-associated (CA) pathogens. The aim of the present study was to provide the first multicentre analysis of occurrence and characteristics of MRSA strains isolated in Serbia.

Methods: National reference laboratory (NRL) established network of 14 clinical laboratories from nine different cities in Serbia and started to collect data and isolates of invasive MRSA in June 2012. Phenotypic identification of isolates was confirmed by PCR for mecA gene and susceptibility to antibiotics was investigated by Vitek2 System. All MRSA strains were spa typed and related to MLST-CC groups as well as SCCmec elements were typed and the lukSF-pvl genes were detected by PCR.

Results: During six month period, a total of 77/172 (44.8%) MRSA isolates were reported to the NRL. Susceptibility testing of collected strains revealed that 95.7% of them were resistant to one or more antibiotics beside beta-lactam antibiotics. The majority of the MRSA isolates (53.1%) was associated with the CC5-I clone. In addition, various other MRSA clones were observed, such as the CC8-III (30.9%), the CC45-IV (7.4%), the CC22-IV (3.7%) and the CC30-II (0.6%). Seven (4.3%) PVL-positive MRSA isolates were found associated with CC80- IV, CC30-IV and ST152/ST377-V.

Conclusion: A high proportion of MRSA was noted in Serbia. The CC5-I clone was dominant in most Serbian hospitals. Several typical CA-MRSA clones (CC30-IV, CC80-IV and ST152-V) were found indicating repeated MRSA introductions into the hospitals from the community.

## P16: Trends of antimicrobial resistance development and the extended spectrum β-lactamase (ESBL) production in isolates from Enterobacteriaceae family

Koreň J, Záborská M, Slobodníková L

*Institute of Microbiology, Faculty of Medicine of the Comenius University and the University Hospital in Bratislava, Slovak Republic*

Keywords: ESBL, Enterobacteriaceae, antimicrobial resistance

Objectives: Emergence of ESBL-producing Enterobacteriaceae poses a worldwide concern. We focused to assess trends of these pathogens occurence in FMCU and UHB from year 2007 to 2013 and the development of antimicrobial resistance in the most frequent ESBL-producers.

Methods: Clinical samples were obtained from hospitalized patients. Brilliance ESBL chromogenic screening-medium, confirmatory ESBL disk diffusion and dilution tests were used. The latter was incorporated in susceptibility testing by quantitative colorimetric micro-method.

Results: The numbers of ESBL-producing isolates during the particular years were the following: 131 (6%), 169 (7%), 383 (13%), 314 (12%), 269 (9%), 293 (8%) and 511 (15%). Decreasing or growing tendency of resistance against third generation cephalosporins during the observed period was noticed: 48% Klebsiella pneumoniae in 2007 versus 37% in 2013; 11% versus 26% Escherichia coli; 12% versus 27% Proteus mirabilis. Resistance of the ESBL-producers against meropenem was 1% in 2007 and 2% in 2013 for K. pneumoniae isolates, 0% and 2% for E. coli, and no resistance in P. mirabilis. Against amikacin, 20% resistance was observed in K. pneumoniae isolates in 2007 vs 19% in 2013; 2% vs 6% in E. coli, and 1% vs 5% in P. mirabilis.

Conclusion: Two peaks of ESBL-producing Enterobacteriaceae isolates were noticed: in 2009, containig mostly pathogens of Klebsiella spp, and in 2013, containing isolates of E. coli, Klebsiella spp. and Proteus spp. ESBL growing pathogens E. coli and P. mirabilis recorded increasing resistance to cephalosporins, aminoglycosides and cotrimoxazole. Meropenem and amikacin preserved their antimicrobial activity in the majority of ESBL-producers.

## P17: ESBL(+) isolates from urine cultures in 2013 in Saint George General Hospital of Chania Greece

Papadogianni M, Papadomanolaki E, Aleuraki G, Zouridi A, Tsouri A, Kastanakis S

*Microbiology Department, Saint George General Hospital of Chania, Crete, Greece*

Keywords:

Objective: Our aim is to record all urine-originated ESBL (Extended Spectrum Beta lactamase) positive strains of the most frequently encountered Enterobacteriaceae in one year's time.

Materials and methods: We have studied all Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis strains that were isolated from urine cultures in 2013 and have examined them for ESBL production. The cultures were processed using the usual culture media and standard microbiological methods. Susceptibility testing and examination for ESBL production were performed according to the CLSI criteria using the Kirby-Bauer method and the automated system Vitek-2 (bioMérieux).

Results: There were 1400 Escherichia coli strains from 1380 patients (786 outpatients and 594 inpatients); 91 of them (86 from inpatients and 5 from outpatients) were ESBL (+) (6.5%). 235 Klebsiella pneumoniae strains from 230 patients (90 outpatients and 145 inpatients); 110 of them (113 from inpatients and 7 from outpatients) were ESBL (+) (46.8%). 221 Proteus mirabilis strains from115 patients; 10 of them (9 from inpatients and 1 from outpatients)were ESBL (+) (4.5%).

Conclusions: Klebsiella pneumoniae strains originated from urine specimens can express in high rates the ESBL phenotype thus making urinary infections due to these bacteria difficult to treat.

## P18: Prevalence of ESBL+ Klebsiella pnemoniae in intraabdominal infections (IAIs) in Croatia vs. Europe- SMART study- Impact on empirical IAI therapy: 2011

Budimir A[1,2,] Bošnjak Z[1,2], Francetić I[3,4], Plečko V[1,2]

[1]*University Hospital Center Zagreb, Department of Clinical and Molecular Microbiology*
[2]*School of Medicine University of Zagreb, Department of Medical microbiology and Parasitology*
[3]*University Hospital Center Zagreb, Department of Clinical Pharmacology*
[4]*School of Medicine University of Zagreb, Department of Medical microbiology and Parasitology*

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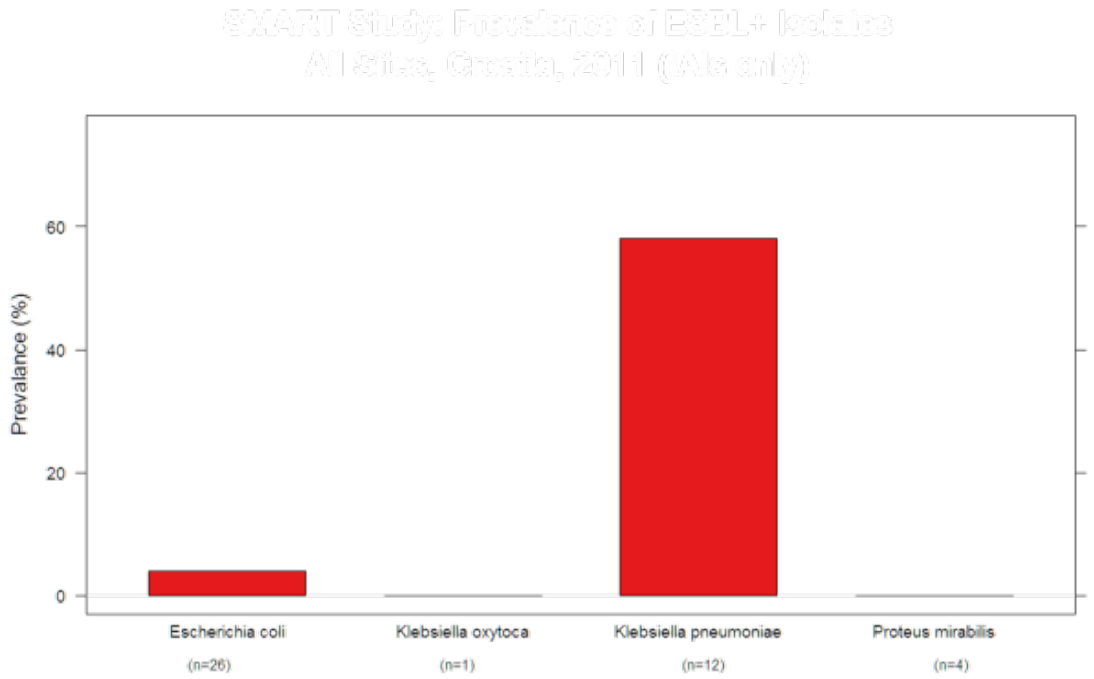
Keywords: Klebsiella pneumoniae, intraabdominal infections, susceptibility, ESBL

Objectives: The aim of this study was to investigate and compare different microorganisms isolated in intraabdominal infections (IAIs), and prevalence of ESBL-positive K. pneumoniae in Croatian and European strains. Results were part of The Study for Monitoring Antimicrobial Resistance Trends (SMART).

Methods: One hundred of consecutive IAI isolates were collected and tested in UHC Zagreb in 2011. Identification, based on standard microbiology techniques, and susceptibility testing of strains was performed and confirmed in UHC Zagreb. Susceptibility testing was performed by using MicroScan dehydrated broth microdilution panels. Sensitivity to antimicrobials and presence of extended spectrum beta-lactamase (ESBL) were determined according to EUCAST standards.

Results: Proportion of microorganisms causing IAIs in Croatia was different from isolates in the rest of Europe. Leading cause of IAIs in Europe was E. coli (49%), as well as in Croatia (31%), and the second most frequent was K. pneumoniae (11%), followed by Pseudomonas (8%). In Croatia, K. pneumoniae was present in 24% of cases and Pseudomonas in 14% of samples. The prevalence of K. pneumoniae producing ESBL in Croatia was 60% and in Europe 30%, respectively.

Conclusion: The distribution of isolates from IAIs in Croatia and Europe was different. Although the order of pathogen frequency was the same, different proportions from Europe and Croatia of particular pathogens were observed. In order to implement empirical antimicrobial therapy it is necessary to consider proportion and sensitivity pattern of isolates in certain country. This study showed that for some, common isolates in IAIs, current empiric therapy could be ineffective.



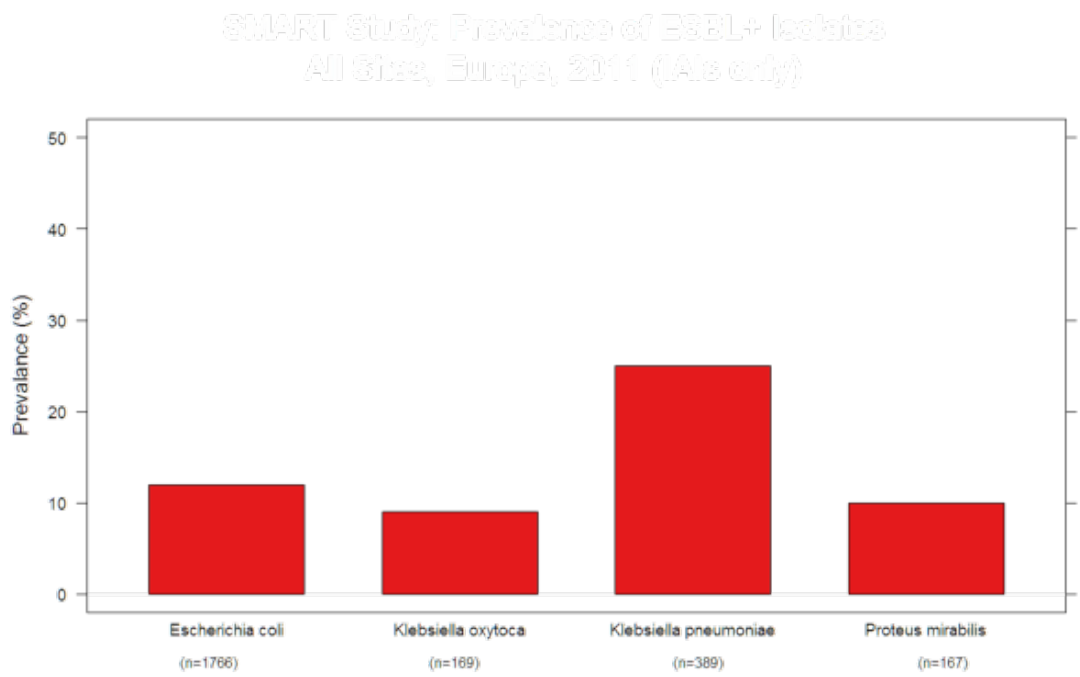




Figure 1,2: Prevalence of ESBL+ isolates in European strains and in Croatia

## P19: Risk factors and outcome of ESBL-producing Enterobacteriaceae bloodstream infection in year 2013: hospital-based study in Trnava University Hospital, Slovakia

Garabasova M[1], Streharova A[1], Betkova M[3], Michalikova L[2,4], Brnova J[2,4]

[1]*Department of Public Health, School of Health and Social Work, Trnava University, Slovakia*
[2]*Department of Laboratory Medicine, School of Health and Social Work, Trnava University, Slovakia*
[3]*Department of Nursing, Constantine the Philosopher University, Nitra, Slovakia*
[4]*Laboratory of Molecular Microbiology, St. Elisabeth University, Bratislava, Slovakia*

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Keywords: ESBL-Enterobacteriaceae, bloodstream infection, risk factors

Objectives: To analyze risk factors and outcomes of bloodstream infections (BSI) with ESBL-producing Enterobacteriaceae (ESBL-E).

Methods: This prospective observation study was conducted at the Trnava University Hospital (592-bed; approximately 25 000 patients per year). BSI caused by ESBL-E was defined as a bloodstream infection documented in at least one positive blood culture specimen from a patient with systemic inflammatory response syndrome diagnosed between January 2013 and December 2013.

Results: ESBL-E BSI were diagnosed in 105 of the 24 569 hospitalized patients (incidence 4.27 per 1,000 admissions) and ESBL-producing strains were identified in 63 (60%). The most frequent ESBL- E were Klebsiella spp. (43%), E. coli (35%), Proteus mirabilis (17%) and Enterobacter spp. (5%). The most frequent ESBL types of enzymes were TEM (65%). Risk factors for ESBL-E BSI were previous hospitalization [OR=2.73, 95%CI 1.17-6.37, p=0.02], especially previous ICU stay [OR= 3.51, 95%CI 1.09-11.32, p=0.03], prior use of any type of antibiotic therapy [OR=2.92, 95%CI 1.3-6.58, p=0.009] a special exposure to fluoroquinolones therapy [OR=3.19, 95%CI 1.1-9,39, p=0.03]. The patients with ESBL-E BSI had significantly more associated severe co-morbidity [OR=4.0, 95%CI 1.04-15.38, p=0.04] and were more often assessed as health care-associated infections. The hospital mortality rate in the ESBL-E BSI was more than twice as high as that in the non-ESBL group [30.2% vs. 14.3%; OR= 2.59, 95%CI 0.98- 4.89, p=0.06].

Conclusions: More judicious use of antimicrobial agents and better infection control in a high-risk group of patients may decrease the possibility of ESBL-producing Enterobacteriacae BSI in our facilities.

## P20: Colonization Sites of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae

Papst L[1], Beović B[1], Seme K[2]

[1]*Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia*
[2]*Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

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Keywords: ESBL, colonization, screening

Objectives: To determine the colonization sites of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, in order to optimize screening for colonization with these bacteria.

Methods: Patients colonized with ESBL-producing Escherichia coli or Klebsiella pneumoniae were included in a prospective study with a 2-year follow-up. Rectal swab, urine culture, throat swab, as well as other clinically relevant samples (wound swab, tracheal aspirate, sputum) were collected in each patient. Sets of follow-up samples were collected every 3 months for 2 years.

Results: 611 sample sets were collected in 114 patients. Out of 309 (50.6%) positive sample sets, 278 (90%) sets had rectal swab positive for ESBL, throat swab was positive for ESBL in 53 (17.2%) sets and urine in 112 (36.2%) sets. 11/31 (35.5%) lower respiratory tract samples and 14/42 (33.3%) wound swabs were positive for ESBL. In 31/309 (10%) positive sample sets, rectal swabs were negative for ESBL, but patients were positive for ESBL from other sites, most often from urine (29/31 sample sets). In 8 patients, urine was the only site positive for ESBL throughout the study period. 307/309 (99.4%) positive sample sets were positive for ESBL from rectal swab and/or urine.

Conclusions: According to our results, rectal swabbing is a relatively reliable method for routine screening for colonization with ESBL-producing Enterobacteriaceae. In some patients, however, urine can be the only site positive for ESBL. Screening with rectal swabbing together with urine sampling would detect almost all patients, colonized with ESBL-producing Enterobacteriaceae.

## P21: Bloodstream infections caused by antibiotic resistant gram negative bacilli

Lila G, Raka L, Mulliqi G, Bajrami R, Azizi E, Kurti A

*National Institute of Public Health of Kosova, Department of Microbiology*

Keywords: Bloodstream infections, antibiotic resistance, gram negative bacilli

Aim: This study aimed to identify the gram-negative bacilli present in blood samples and their antibiotic resistance.

Materials and methods: A retrospective study was conducted in department of Microbiology at National Institute of Public Health of Kosova in Prishtina. Samples were collected from different clinics of UCCK during March 2013 - March 2014. Species identification and antibiotic susceptibility testing was carried by standard microbiological methods and Vitek automatic system 2 (Bio-Merieux- France).

Results: There were 460 (37.5%) positive blood culture samples from which 210 (45.6%) were gram negative bacilli. Majority of them were Klebsiella spp 57 (27.1%), Enterobacter spp. 45 (21.4%), Acinetobacter spp. 37 (17.6%), Pseudomonas spp. 30 (14.2%), and E. coli 24 (11.4%). Majority of blood culture isolates were from the Neonatal Intensive Care 130 (61.9%). Klebsiella spp. has shown highest resistance towards aminoglycosides - gentamycin 100%, amikacin 80% and tobramicin 60%, third generation cephalosporins - cefotaxim 80%, ceftriaxon 60%, ceftazidin 25%, Acinetobacter spp. - gentamycin 100%, amikacin 95%, tobramicin 62%. Low rate of resistance was in carbapenems (imipenem and meropenem), except Acinetobacter spp. - imipenem 40%, meropenem 33%, and Pseudomonas spp. - imipenem 27% , meropenem 30%.

Conclusion: Results from this study show that gram negative bacilli has developed high resistance towards the tested antimicrobial. Antibiotic stewardship and adequate infection control practices should be reinforced in order to control the spread these gram negative bacteria.

## P22: Risk factors for colonization with imipenem resistant Pseudomonas aeruginosa during hospitalization in the intensive care unit

Plankar Srovin T[1], Blagus R[2], Seme K[2], Čižman M[1]

[1]*University Medical Centre Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia*
[2]*Faculty of Medicine, University of Ljubljana, Slovenia*

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Keywords: Pseudomonas aeruginosa, resistance, intensive care unit

Objectives: To determine the incidence and risk factors for colonization with imipenem resistant Pseudomonas aeruginosa (IRPA) among patients hospitalized in the ICU.

Methods: The prospective study was carried out in the ICU of the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, from April 2004 through June 2005, in patients whose expected length of stay was at least 5 days. Colonization was investigated by performing surveillance rectal swabs every week during ICU stay. Patients with isolated IRPA were considered as cases. All the other included patients were considered as controls. Demographics and known risk factors were retrieved and assessed by univariate and multivariate statistical methods.

Results: We included 109 patients: 6 were discharged/died before any repeated cultures were obtained. Of the rest 103 patients 15 (14.6%) acquired IRPA during their stay in the ICU. In univariate analysis the strongest risk factor for colonization with IRPA were hospitalization days (40.9 vs 23.7 days; $P<0.0001$). Other important risk factors were previous hospitalization, shock, decubitus ulcers, liver disease, higher patients per nurse ratio, days of arterial line, intubation days, hemodialysis, antibiotic days, consumption of antibiotics expressed in DDD, inotropes, red blood cell transfusion and carbapenem, cefepime and vancomycin treatment. The results of multivariate analysis are presented in Table 1.

Conclusion: The results suggest that the most important risk factors for IRPA colonization were antibiotic treatment, invasive devices and serious illness. Further efforts to minimize unnecessary prolongation of invasive devices and to optimize antibiotic use in the ICU are proposed.

Table 1. Multivariate analysis of risk factors for nosocomial colonization with imipenem resistant *Pseudomonas aeruginosa* in the ICU.

| Variable | *P value* | OR (95%CI) |
|---|---|---|
| Time at risk[a] | 0.008 | 0.48 (0.28-0.82) |
| Decubitus ulcers | 0.023 | 39,6 (1.7-944) |
| Higher patients per nurse ratio | 0.003 | 780 (9.5-64133) |
| Hemodialysis, days | 0.012 | 1.3 (1.06-1.7) |
| Intubation, days | 0.013 | 1.7 (1.1-2.7) |
| Red blood cell transfusion | 0.055 | 25.6 (0.93-723) |
| Imipenem treatment | 0.023 | 25.5 (1.6-414) |
| Consumption of cefepim expressed in DDD[b]/patient | 0.013 | 1.07 (1.03-1.3) |

[a] time at risk (time to the first resistant isolate in cases; days of hospitalization in controls) was included as a covariate, [b]defined daily doses

## P23: Pseudomonas Species: Clinical Samples Isolates and Antibiotics Susceptibility in Kosova

Bajrami R, Mulliqi G, Raka L, Lila G, Kurti A, Azizi E

*National Institute of Public Health of Kosova*

Keywords: Pseudomonas, resistance, nosocomial infection

Purpose: The aim of the study was to assess the occurrence of Pseudomonas species from various clinical samples in the University Clinical Center of Kosova and to evaluate the antimicrobial susceptibility patterns.

Methods: A retrospective study was carried out of all clinical samples presenting growth of Pseudomonas species through year 2013 at UCCK –tertiary care center with 2100 beds. Isolation and identification of microorganisms was prepared with standard microbiological technique and sensitivity to antibiotics with disk diffusion methods to Miller Hinton agar plate based EUCAST and Vitek 2 Automation System (bioMerieux, France).

The study was conducted by the Department of Microbiology of National Institute of Public Health of Kosova in Prishtina.

Results: The most common species of Pseudomonas found was Pseudomonas aeruginosa 108 (55.1%). Results were examined for 196 clinical samples that grew significant microorganisms. Out of 196 positive samples of Pseudomonas, the largest number was isolated from endotracheal aspirates 88 (44%) and wound swabs 34 (17.3%).

The majority of Pseudomonas isolates were from the Central Adult Intensive Care Unit and Neonatal Intensive Care Unit with 76 (38.7 %) and 32 (16.3%) isolates respectively. Pseudomonas isolates expressed highest susceptibility to Ciprofloxacin (86% susceptible), Cefepim (75%) and Meropenem (62%). The resistance rate for trimethoprim-sulfamethoxasole was 100%, cefoxitin 95%.

Of the 196 isolates, 130 (66.3%) were resistant to three or more agents and were considered multi-drug resistant.

Conclusion: This study confirmed a high incidence of nosocomial infection and revealed high rates of resistant Pseudomonas in Intensive Care Units.

## P24: One-year prevalence study of Pseudomonas aeruginosa infections in University Hospital Trnava

Michalikova L[1,2], Brnova J[1,2], Streharova A[3], Garabasova M[3], Kulková N[2]

*[1]Department of Laboratory Medicine, School of Health and Social Work, Trnava University, Slovakia*
*[2]Laboratory of Molecular Microbiology, St. Elisabeth University, Bratislava, Slovakia*
*[3]Department of Public Health, School of Health and Social Work, Trnava University, Slovakia*

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Keywords: Pseudomonas aeruginosa, risk factors, carbapenem resistance

Objectives: To analyze risk factors and outcomes of infections caused by Pseudomonas aeruginosa (PSA) and carbapenem-resistant Pseudomonas aeruginosa (CRPSA) in Trnava University Hospital.

Methods: This retrospective observation study was performed at the Trnava University Hospital (592-bed; approximately 25 000 patients per year) from June 2013 to May 2014. Through laboratory information system, all patients with positive cultures of PSA (N=392) were identified while colonization were excluded (N=170).

Results: Of 222 PSA infected patients with median age 68 (IQR=54-77), 59,9% were males. Ciprofloxacin resistance rate were the most frequently 68,9%, followed ceftazidime, meropenem, piperacillin/tazobactam and amikacin (45,5%; 42,3%; 36,5% and 7,6%, respectively). Infections due to CRPSA were present in 95 (42,8%) cases. Respiratory tract infection (RTI) (35,1%; N=78) and urogenital tract infection (UTI) (31,5%; N=70) were the most prevalent, followed by bloodstream (BSI) (15,3%; N=34) and surgical site infection (SSI) (7,2%; N=16). Overall in-hospital mortality reached 27% and was significantly higher in elderly (68,1% vs. 31,9%; p=0,04), BSI infection (64,7% vs. 35,3%; p<0,001), intensive care unit patients (ICU) (55,6% vs. 44,4%; p<0,002) and in patients hospitalized in Department of Lung Diseases (91,7% vs. 8,3%; p=0,048). Concerning the risk factors for CRPSA infections, age below 65 years (53% vs. 47%; p=0,03) and ICU stay (70,4% vs. 29,6%; p<0,001) were significantly more frequently represented.

Conclusions: In this study, high prevalence of carbapenem resistant PSA infections was found in hospitalized patients. Systematic prevention strategies to avoid spreading PSA especially in patient requiring nebulizer therapy and general improvement in infection control practice are urgently needed.

## P25: Evaluation the pattern of antibiotic resistance and incidence of broad-spectrum betalactamases type TEM and CTX at acinetobacter isolations separated from clinical specimen at educational hospitals of Sari city

Ahanjan M[1], Kholdi S[2], Rafiei A[2]

[1]*Department of Microbiology, Antimicrobial Resistant Nosocomial infection Research center, Mazandaran University of Medical Sciences, Sari, Iran*
[2]*Department of Microbiology, Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3]*Department of Immunology, Molecular & Cell-Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

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Keywords: Acinetobacter, Esbl, antimicrobial resistant, blaTEM, blaCTX

Background: Acinetobacter has emerged as a significant opportunistic pathogen responsible for nosocomial infections. Treatment of infections due to this organism is becoming a serious clinical concern and this bacteria are frequently resistant to multiple classes of antibiotics such as Family of beta-lactam drugs. B-lactamases enzymes represent the main mechanism of bacterial resistance to b-lactam antibiotics. This study was conducted to determine the prevalence of TEM-1and CTX beta-lactamases in acinetobacter isolates from Sari hospital.

Material and method: The study included 100 Acinetobacter isolates that were isolated from various clinical specimens susceptibility of isolates toward the antibiotics was determined by standard disk diffusion method. ESBL production was determined by then combination disk method using disks containing ceftazidim and cefotaxim alone and in combination with Clavulanic acid and TEM and CTX types of ESBL producing genes was detected by PCR test.

Result: Among all acinetobacter isolates, the highest resistance was seen for cefotaxime (100%), ceftazidim (100%), ceftraiaxone (96%) and whereas the highest susceptibility was observed for colistin (65%), gentamycin (37%), tobramycin (27%). Combined Disc Test showed that 24% of isolated were ESBL positive and among them 79.1% and 31.5% were positive for blaTEM and blaCTX genes.

Conclusion: According to the results most of the acintobacter isolate are drug resistant With regard to the number of isolates of β lactamas producer are 24% of the total samples. Thus other mechanisms such as secretory pump and purines can have a role in drug resistance.

## P26: Resistance to carbapenems of Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa strains, isolated in 2013 in Saint George General Hospital of Chania Greece

Papadogianni M, Papadomanolaki E, Aleuraki G, Tzagaraki K, Tsouri A, Kastanakis S

*Microbiology Department, Saint George General Hospital of Chania, Crete, Greece*

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Keywords:

Objective: Our aim is to record all urine-originated ESBL (Extended Spectrum Beta lactamase) positive strains of the most frequently encountered Enterobacteriaceae in one year's time.

Materials and methods: We have studied all Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis strains that were isolated from urine cultures in 2013 and have examined them for ESBL production. The cultures were processed using the usual culture media and standard microbiological methods. Susceptibility testing and examination for ESBL production were performed according to the CLSI criteria using the Kirby-Bauer method and the automated system Vitek-2 (bioMérieux).

Results: There were 1400 Escherichia coli strains from 1380 patients (786 outpatients and 594 inpatients); 91 of them (86 from inpatients and 5 from outpatients) were ESBL (+) (6.5%). 235 Klebsiella pneumoniae strains from 230 patients (90 outpatients and 145 inpatients); 110 of them (113 from inpatients and 7 from outpatients) were ESBL (+) (46.8%). 221 Proteus mirabilis strains from115 patients; 10 of them (9 from inpatients and 1 from outpatients)were ESBL (+) (4.5%).

Conclusions: Klebsiella pneumoniae strains originated from urine specimens can express in high rates the ESBL phenotype thus making urinary infections due to these bacteria difficult to treat.

## P27: Fungal isolates during 7 year period: 2007-2013

Tosic T[1], Jovanović M[1], Varagic-Stojanovic Z[1], Stosovic B[1], Jovanović S[2], Stevanović G[3], Urošević A[3], Pelemiš M[3]

*[1]Clinical Center of Serbia, Bacteriology Laboratory, Belgrade, RS*
*[2]Clinical Center of Serbia, Department for Microbiology, Belgrade, RS*
*[3]Clinical Center of Serbia, Clinic for Infectious and Tropical Diseases, Belgrade, RS*

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Keywords: Candida, bloodstream isolates

Objectives: The aim of our study was to investigate the isolation of Candida spp in blood cultures and cerebrospinal fluids and to evaluate their antifungal susceptibility during a 7-year period (2007-2013) in a tertiary care hospital.

Methods: The blood cultures were incubated in the automated blood culture system BACTEC 9240 (Becton Dickinson). Positive blood cultures were examined microscopically directly for yeast or pseudohyphe and subcultured on Sabouraud dextrose agar (Liofilchem Italy). Candida isolates were identified using automated VITEK 2 system (bioMerieux) or Api 20CAUX (bioMerieux). Antifungal susceptibility was carried out by automated VITEK 2 system (bioMerieux) using AST Y01 and AST Y07 test card.

Results: During the study period there were 67 candidemia cases. The 51.35% of candidemias occurred in the ICUs, the 40.54% in the medical wards and the rest of 8.11% in surgical wards. C. parapsilosis was the predominant species (35.15%), followed by C. albicans (32.43%), C. glabrata (10.81%), C. tropicalis (8.12%) and C. norvegensis, C. guilliermondii, C. kefyr, C. famata, Cryptococcus neoformans with (2.70%). All tested isolates were susceptible to amphotericin B and voriconasole. Among non-albicans strains increasing resistance to fluconasole was found: C. parapsilosis (8.33%), and C. tropicalis (8.33%).

Conclusion: Candidemia was more frequent in ICUs followed by medical and surgical wards. C. parapsilosis was the predominant cause of candidemias in our Hospital. Amphotericin B were active against all tested isolates.

## P28: Antibiotic use in Bosnia and Herzegovina: Results of the WHO/Europe-ESAC project

Spasojevic T[1], Versporten A[2], Grubisa N[1], Bolkhovets G[3], Bak Pedersen H[3], Sautenkova N[3], Goossens H[2]

*[1]Agency for Medicines and Medical Devices of Bosnia and Herzegovina, Banja Luka, Bosnia and Herzegovina*
*[2]Laboratory of Medical Microbiology, Vaccine and Infectious Diseases Institute, University of Antwerp, Antwerp, Belgium*
*[3]Health Technologies and Pharmaceuticals, Division of Health Systems and Public Health, the WHO Regional Office for Europe, Copenhagen, Denmark*

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Keywords: antibiotics, WHO ATC/DDD methodology, amoxicillin

Objectives: There wasn't any reliable data on antibiotic use in non-European-Union (EU) south-eastern European countries (SEE). We aimed to collect valid, representative, comparable total national wholesales data on systemic antimicrobial use in Bosnia and Herzegovina, a SEE country with a population of 3.836.377 (estimated by http://www.bhas.ba/).

Methods: Valid 2009-2012 total antibiotic use data of Bosnia and Herzegovina were analysed according to the WHO Anatomical Therapeutic Chemical (ATC)/Defined Daily Doses (DDD) methodology and expressed in DDD/1000 inhabitants/day (DID).

Results: Total (outpatients and hospital care) antibiotic use was 18.5 DID in 2009, 18.1 DID in 2010, 18.4 DID in 2011 and 18.1 DID in 2012. The top 5 antibacterial subgroups (ATC level 3) in 2012 were: penicillins (J01C) 8.9 DID, 48.1% of all antibacterials; other beta-lactam antibacterials (J01D) 2.3 DID, 12.7%; quinolones (J01M) 2.2 DID, 12.2%; sulfonamides and trimethoprim (J01E) 1.7 DID, 9.4% and macrolides, lincosamides, streptogramins (J01F) 1.5 DID, 8.3%. The top 5 antibacterials (ATC level 5) in 2012 were: amoxicillin (4.9 DID, 27.1%); amoxicillin and enzyme inhibitor (co-amoxiclav, 2.6 DID, 14.4%); ciprofloxacin (1.8 DID, 9.9%); sulfamethoxazole and trimethoprim (1.7 DID, 9.4%) and doxycycline 1.0 DID, 5.5%). Other beta-lactam antibacterials represented mainly the first-generation cephalosporin cephalexin (1.1 DID, 6.1%) and the second-generation cephalosporin cefuroxime (0.7 DID, 3.9%). Azithromycin and ciprofloxacin use increased from 2009 to 2011, but in 2012 decreased slightly.

Conclusion: Standardised and validated data set of systematic antibacterial use suggests good antibiotic prescribing practise (amoxicillin) but also offers opportunities for quality improvement (appropriate use of co-amoxiclav and azithromycin).

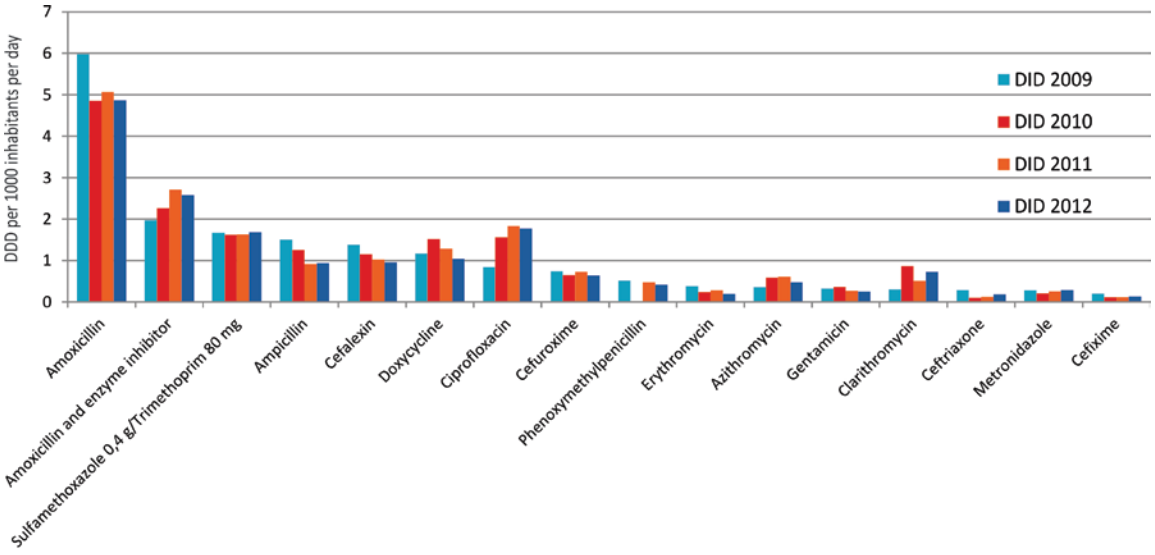


**Figure 1. The top 10 antibacterials at ATC5 level (2009-2012) out of total antibacterials, expressed in DDD per 1000 inhabitants per day**
DDD: defined daily doses

## P29: Preliminary results on pharmacists perception for antibiotic use in Albania

Hoxha I[1], Malaj A[1], Kraja B[1], Malaj L[1], Bino S[2]

[1]*Faculty of Pharmacy, University of Medicine Tirana, Albania*
[2]*Department of Infectious Disease, Institute of Public Health, Albania*

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Keywords: Pharmacists, antibiotics, Albania

Background: Use of antibiotics has been factored as directly proportional to its resistance. The role of health care professionals has been highlighted as crucial in controlling and preventing antibiotic misuse. Pharmacists play an indispensable part in promoting rational and correct dispensing of antibiotics. The aim of this study is to access the perceptions of community pharmacists on proper use/dispense of antibiotics in Albania.

Method: Community pharmacists from all regions of Albania were invited to complete a 21 question survey. The survey was firstly validated with a smaller group of 20 pharmacists, and then it was reformulated and tested in another group of 15 pharmacists. Pharmacists were assisted by professional trainers. Survey data results were analyzed by EpiInfo software.

Results: A total of 366 pharmacists were invited in this study, which only 48 of them refused to participate. The survey questions were focused on professional knowledge and attitude towards rational antibiotics use. Questions were focused on the commonly used antibiotics classes, specific effects of antibiotics and inappropriate dispense.

Conclusion: Gap of knowledge exists among community pharmacists in appropriate use and dispense of antibiotics in Albania. Pharmacists outline a large number of wrong practices in dispensing of antibiotics to patients. The situation deteriorates more because in Albania pharmacists can still sell antibiotics without prescription. Serious interventions from health authorities have to be implemented in order to stop the antibiotic misuse phenomena.

## P30: Successful treatment with Teicoplanin of patients with multiple recurrences of Clostridium difficile infection

Popović N[1], Milošević I[1,2], Nešić Z[3], Prostran M[3], Pelemiš M[1,2], Delić D[1,2], Stevanović G[1,2], Korać M[1,2]

*[1]Clinic for Infectious and Tropical Diseases, Clinical Center Serbia, Belgrade, Serbia*
*[2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia*
*[3]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

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Keywords: *Clostridium difficile*, Teicoplanin, recurrence

Objectives: High recurrence rates represent a significant problem in the treatment of Clostridium difficile infection (CDI). The rate of first recurrence reaches 25% while the risk of second and subsequent recurrences is even higher. We present two patients with third recurrence of CDI who were treated with Teicoplanin.

Methods: Patients were treated in the Clinic for Infectious and Tropical Diseases, Clinical Center Serbia. They were given Teicoplanin if they had third or later recurrence of CDI which occurred after adequate previous therapy.

Results: Both patients were women, with the age of 77 and 94 years who were previously treated for second recurrence of confirmed CDI with prolonged therapy of Vancomycin using a tapered regimen. During the last week of tapered regimen in the first patient and 7 days after the ending of therapy in the second diarrhea reappeared. Enzyme immunoassay test was positive for C.difficile toxin A and B in stool. One patient had moderate and the other severe form of the disease. They were given oral Teicoplanin 100mg twice a day for 14 days. The resolution of diarrhea occurred on the third day of treatment. No complications during therapy or recurrences during the 8 weeks follow up were observed.

Conclusion: Oral Teicoplanin was successful in the treatment of patients with CDI who developed third recurrence after treatment with Vancomycin using tapered doses. Patients had no subsequent recurrences. Future studies with more patients are needed to confirm the role of Teicoplanin in the treatment of patients with multiple CDI recurrences.

## P31: Comparison of treatment outcomes in patients with Clostridium difficile infection treated with Vancomycin and Vancomycin and Metronidazole combination therapy

Popović N[1], Milošević I[1,2], Prostran M[2,3], Delić D[1,2], Nešić Z[2,3], Marković M[1], Korać M[1,2]

*[1]Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade, Serbia*
*[2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia*
*[3]Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

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Keywords: *Clostridium difficile*; combination therapy; outcome

Objectives: Combination therapy of intravenous Metronidazole and oral Vancomycin is recommended treatment for severe complicated Clostridium difficile infection (CDI), but in everyday practice is often used even in patients with moderate disease. Our aim was to compare clinical cure rates, time to resolution of diarrhea and recurrence rates between patients treated with Vancomycin and Vancomycin and Metronidazole dual therapy.

Methods: The study enrolled patients with confirmed moderate and severe CDI treated in the Clinic for Infectious and Tropical Diseases in Belgrade.

Results: We analyzed 142 patients. Sixty-seven patients were treated with Vancomycin and 75 with combination therapy. There was no statistically significant difference in the mean age (68.7±10.7 and 68.3±12.3 years, respectively; p= 0.840) or baseline leukocyte count (12100±6900/mm3 and 12600 ± 6500/mm3; p= 0.674) between patients treated with monotherapy and combination therapy. Clinical cure rates were 95.5% in Vancomycin and 93.3% in the combination therapy group. Fatal outcome occurred in three patients (4.5%) treated with Vancomycin and five (6.7%) treated with both antibiotics (p= 0.722). Time to resolution of diarrhea was statistically significantly shorter in patients treated only with Vancomycin (5.5+/-2.9 vs. 7.3+/-3.6 days; p=0.026). No statistical difference in recurrence rates was observed between patients receiving Vancomycin and combination therapy (25% vs. 18.5%; p=0.406).

Conclusions: Patients with moderate and severe CDI treated with combination therapy did not have better outcome comparing to those treated with Vancomycin. Therefore, dual therapy should not be given to all patients and should be kept only for complicated disease.

## P32: The relation between the amount of inflammation and intensity of malabsorption in patients with pseudomembranous colitis

Marković M[1], Janković M[1], Popović N[1], Marković A[1], Pelemiš M[1,2], Stevanović G[1,2], Korać M[1,2], Milošević I[1,2]

*[1]Clinic for infectious and tropical diseases, Belgrade, Serbia*
*[2]Medical faculty, University of Belgrade, Belgrade, Serbia*

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Keywords: Inflammation, malabsorption, pseudomembranous

Objectives: Pseudomembranous colitis represents an inflammatory disease of the colon caused by the Gram-positive spore-forming bacterium Clostridium difficile. One of its' consequences is development of the malabsorption syndrome, which represents itself inter alia by low values of serum albumin, protein, iron, and the lengthening of prothrombin time (PT). We have tried to determine whether there is a correlation between the intensity of inflammation (represented by levels of C-reactive protein (CRP), fibrinogen, d-dimer and leukocyte count) and the duration of diarrhea prior to hospitalization (DPH) with aforementioned factors mirroring malabsorption.

Methods: By means of a retrospective study we analyzed data from a group of 34 patients treated at the Clinic for Infectious and tropical diseases in Belgrade during the period January – July 2014, using the IBM SPSS Statistics 20 software.

Results: CRP levels and leukocytosis correlate inversely with serum albumin ($p<0.05$) and iron concentrations ($p<0.05$), but there is no statistically significant association with PT or serum protein concentration. D-dimer levels had an opposite statistical correlation with serum albumin concentration ($p<0.05$) and PT ($p<0.05$), while there was no relation with iron or protein levels. We have found no important link between fibrinogen levels and malabsorption parameters. There was no correlation between duration of diarrhea and degree of malabsorption.

Conclusion: Malabsorption syndrome in patents with pseudomembranous colitis correlates with d-dimer, CRP and leukocyte count which reflect the severity of disease. There was no correlation between duration of diarrhea and degree of malabsorption.

## P33: Is leukocytosis an important marker of disease severity in patients with Clostridium difficile infection?

Milošević I[1,2], Popović N[2], Marković M[2], Ilić M[2], Jegorović B[2], Janković M[2], Marković A[2], Korać M[1,2]

[1]*School of Medicine, University of Belgrade*
[2]*Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade*

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Keywords: *Clostridium difficile*, leukocytosis, mortality

Objectives: Clostridium difficile is a leading cause of hospital-acquired antibiotic-associated diarrhea and pseudomembranous colitis. At the end of 2008 increase in the number of patients with Clostridium difficile infection (CDI) and more deaths were registered in Serbia, especially among older people. It is known that considerable number of patients, especially those with pseudomembranous colitis, have significant leukocytosis. The aim of study was to correlate leukocytosis with clinical findings, complications and mortality in patients with CDI.

Methods: 162 patients treated in Clinic for Infectious and Tropical Diseases in Belgrade, over a period of two years (2011-2012), were included. We correlated patients with significant leukocytosis (leukocyte count ≥ 30x109/L) and those with less than 30x109/L leukocytes according to clinical findings, laboratory analyses, duration of illness, incidence of complications (ileus, toxic megacolon) and outcome of disease.

Results: 28 (17.3%) patients had leukocyte count ≥ 30x109/L. Duration of diarrhea was significantly extended in patients with significant leukocytosis (12.3±6.1 vs. 6.8±4.5 days; P = 0.000). 32.1% of patients with significant leukocytosis had complications, versus 2.2% in another group (P=0,000). A significantly higher percentage of patients with a leukocyte count ≥ 30x109/ L had pleural effusion and ascites (P = 0.000). They also had significantly higher C-reactive protein values (178,2±89,2 mg/l vs. 93,6±77,1 mg/l; P = 0.000), and lower concentrations of proteins, albumins and iron. 28.6% of patients with significant leukocytosis (versus 6%) died, with statistically significant difference (P = 0.000).

Conclusions: Leukocytosis ≥30x109/L is an important parameter of disease severity in patients with CDI.

## P34: Prognostic factors for Clostridium difficile infection

Korać M[1,2], Popović N[2], Marković M[2], Ilić M[2], Jegorović B[2], Janković M[2], Marković A[2], Milošević I[1,2]

[1]*School of Medicine, University of Belgrade*
[2]*Clinic for Infectious and Tropical Diseases, Clinical Centre of Serbia, Belgrade*

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Keywords: *Clostridium difficile*, diarrhea, pseudomembranous colitis

Objectives: Clostridium difficile infection (CDI) is the most common cause of the hospital acquired diarrhea in Serbia. CDI is usually clinically presented as pseudomembranous colitis. The complications are paralytic ileus, toxic megacolon, perforation and peritonitis. It is difficult to establish clear criteria for prognosis and severity of CDI. The aim of this study was to determine factors that influence disease severity and final outcome in patients with CDI.

Methodology: This retrospective study included 510 patients treated at the University Hospital for Infectious and Tropical Diseases in Belgrade, Serbia, over a six-year period (2008-2013). We analyzed duration of illness, incidence of complications and outcome of disease in our patients.

Results: In most of the patients, diarrhea lasted not more than a week after specific therapy was introduced. We demonstrated that there are certain baseline clinical and laboratory factors associated with prolonged diarrhea and more severe disease such as leukocyte count above 15x109/l (P=0.000), initial number of stools frequent than 5/day (P=0.005), increased C-reactive protein (above 100 mg/l) (P=0.001) and hypoalbuminemia (below 30 g/l) (P=0.001). 14 patients had complications (ileus or toxic megacolon). The disease recurred in 8.4% of patients. 32 (6.3%) patients died, mostly because of the preexisting diseases. The age of 65 years or older was significantly associated with poor outcome (OR 1.523, 95% CI 1.347-1.721; P= 0.027).

Conclusions: CDI is mainly affecting elderly patients with comorbidities. The incidence of complications and recurrent disease is low and prognosis is mostly dependent of age and the preexisting diseases.

## P35: Determining the risk factors for Vancomycin-Resistant Enterococcus (VRE) colonization and infection in intensive care units and haematology wards in a Training Hospital

Arıcan RK[1], Karagöz E[1], Öncül O[1], Ülçay A[1], Turhan V[1], Erdem H[1], Acar A[1], Görenek L[1]

[1]*GATA Haydarpasa Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*

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Keywords: Vancomycin-resistant Enterococcus, intensive care unit, risk factors

Introduction and aim: We evaluated the risk factors of VRE colonization and infections in intensive care units (ICUs) patients.

Materials and method: This prospective study was carried out between 01 January and 31 December 2010 in a training hospital. All the cases were followed by active surveillance. To determine colonization for VRE, rectal, nasal, axillary, inguinal swabs and patient's bedside and table region samples were taken at the first day and then every seven days after admitted to ICU.

Results: A total of 170 cases were evaluated in this study. Of those 102 (60%) were female, the average age was 65,2 ± 24,4 (20-97); and the mean ICU length of stay (LOS) was 14,5 ± 21,9 (2-146) days. Totally, 2136 culture samples were obtained from 170 cases and their regions. Enterococcus spp was isolated 364(17%) of these samples. 14 patient had got 25 (%6.86) colonized VRE. The most frequently isolated agent was E. faecium (19%). The incidence of VRE isolation was significantly higher in the spring season (53%) than other seasons. The most frequently isolation were recorded from rectal (63%) and inguinal (21%) swabs. On the other hand, a total of 646 invasive culture samples were taken from 170 patients, 12 had got 15 (8.82%) invasive VRE infection. Although 156 of the cases had no VRE colonization, invasive VRE infections were detected in 9 of 156 (5.8%) cases. Additionally, invasive VRE infection was detected in 3 (21.4%) of 14 VRE colonized patient. It was significantly higher in patients with VRE colonization ($p = 0.028$). VRE infections were closely associated with Candida spp., Methicilline resistant coagulase negative Staphlycoccus spp. infections and antibiotic (meropenem, vancomycin, fluconazole, ciprofloxacin ($p = 0.011$) usage. Peripheral catheter usage ($p = 0.001$, OR = 6.24) and under 65 years of age ($p = 0.002$ OR = 6.35) were identified as independent risk factors for VRE colonization, whereas utilizing bronchoscopy was an independent risk factor for VRE infection.

Conclusion: VRE colonization and infection are still life-threatening problem for all ICU patients. Continuous infection control measurements, educational programs and patient screening cultures should be recommended to help control of VRE colonization and infection.

## P36: Impact of infection control program on Intensive care unit acquired infections: A quasi experimental study in an Egyptian University Hospital

El-Sokkary RH[1, 2]

[1]*Zagazig University, Egypt*
[2]*Zagazig University Hospitals, Egypt*

Keywords: Infection control, ICU, HAI

Introduction: The preventive impact of implementation of infection control program is difficult to assess. Our objective was to investigate the effect of infection control activity disruption on incidence rates of intensive care unit acquired infections.

Methods: A quasi-experimental study was conducted during the period between 1 May 2013 and 31 May 2014 in two intensive care units (ICUs) of a university hospital. Two groups were included: an intervention group and a control group. Infection control activity was interrupted during the study period in unit a (intervention group) and was continuous in unit b (control group). The intervention was disrupted infection control activity because of infection control team re-organization in unit a. Patients hospitalized ≥48 hours and developed infections during their stay were included. In both pre-intervention phase and post intervention phase, data was collected for both units and analyzed about rates of HAI "Blood stream infection, urinary tract infection, ventilator associated pneumonia and surgical site infection.

Results: In Unit a (intervention group), a significant increase in incidence rates of HAI over that of unit b (control group) was observed

Conclusions: HAI incidence rise after infection control activity disruption in ICU, which suggests a specific effect on HAI prevention and reinforces the role of infection control team support and promote role of counselling as a mechanism to facilitate performance improvement in ICU.

## P37: Adequate use of antibiotic prophylaxis in surgery, University Clinical Centre Ljubljana, Slovenia

Zidar Zupan A[2], Saje A[1], Gomišček B[1], Beović B[2]

[1]*Department of Infectious Diseases and Febrile Illnesses, University Medical Centre Ljubljana, Slovenia*
[2]*Faculty of Organizational Sciences, University of Maribor, Slovenia*

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Keywords: Antibiotic Prophylaxis in Surgery

Objectives: Antibiotic prophylaxis (AP) is application of antibiotic before, between or after a diagnostic, therapeutic or surgical procedure in order to prevent an infection. Inconsistent application of guidelines can have serious consequences. The goal of our study was to determine and compare the consistency of following guidelines between surgical departments in University Clinical Centre of Ljubljana (UCLJ).

Methods: A retrospective cross-sectional survey was conducted studying variables: the choice of AP; time of application; dose; number of doses; and length of hospitalization. Surgical departments included were: abdominal surgery UCLJ; abdominal surgery in subsidiary hospital of Peter Držaj (HPD); neurosurgery; orthopaedics; gynaecology; plastic, reconstructive and aesthetic surgery; thoracic surgery; and traumatology.

Results: We examined the documentation of 451 patients. 16 procedures were selected and for each procedure 20 - 30 patients were included.

Conclusions: Most surgical departments in UCLJ follow the AP guidelines in more than 80 % but there is still a lot of possibility for improvement. The most common inconsistency in AP application in UCLJ is prolonged duration of AP, while the choice of AP, dose and time of application are mostly correct. There is no significant decrease in hospitalization length when prolonging the AP.

Table 1: The percentage of consistency with guidelines for each variable according to department and procedure / operated organ

| **Department** | **Organ/ procedure** | **AP given** | **Correct AP*** | **Correct dose*** | **Correct time*** | **Correct duration*** | **100 % correct*** |
|---|---|---|---|---|---|---|---|
| Abdominal Surgery UCLJ | Colon | 100 % | 100 % | 100 % | 93 % | 0 % | 0 % |
| | Pancreas | 96 % | 74 % | 63 % | 100 % | 19 % | 3,7 % |
| Abdominal surgery BPD | Colon | 78 % | 100 % | 88 % | 85 % | 25 % | 16 % |
| Gynaecology | Hysterectomy | 62% | 46 % | 46 % | 33 % | 46 % | 4,7 % |
| Neurosurgery | Expansive process | 92 % | 100 % | 100 % | 75 % | 88 % | 43,3 % |
| | Subdural hematoma | 72 % | 100 % | 100 % | 100 % | 93 % | 64,7 % |
| | Hernia disci | 80 % | 100% | 100 % | 96 % | 95 % | 43,3 % |
| Plastic, aesthetic & reconstructive surgery | Risarthrosis | 86 % | 100 % | 96 % | 81 % | 33 % | 24,1 % |
| | Mastectomy | 86 % | 100 % | 100 % | 96 % | 17 % | 14,3 % |
| | Mamaplastic | 97 % | 100 % | 100 % | 97 % | 0 % | 0 % |
| Thoracic surgery | Lungs | 90 % | 100 % | 100 % | 67 % | 10 % | 10,5 % |
| | Oesophagus | 90 % | 100 % | 100 % | 81 % | 65 % | 51,8 % |
| Orthopaedics | TEP coxae | 90 % | 100 % | 100 % | 100 % | 30 % | 16,6 % |
| Traumatology | Humerus fracture | 89 % | 100 % | 100 % | 88 % | 83 % | 68,0 % |
| | Femur osteosynthesis | 85 % | 100 % | 100 % | 81 % | 96 % | 70,0 % |
| | TEP coxae | 96 % | 100 % | 100 % | 96 % | 33 % | 19,2 % |
| Average | | 86,81 % | 95,00 % | 93,31 % | 85,56 % | 45,81 % | **28,14 %** |

Legend: AP - antibiotic prophylaxis, TEP coxae – total endoprothesis oh hip joint, 100 % correct – percentage of patients that received the antibiotic prophylaxis consistent with guidelines in all variables.
*Consistency with guidelines in percentage was studied only in patients that received AP.

## P38: The share of cytomegalovirus (CMV) in congenital and early postnatal infections in northeastern Bulgaria

Stoykova ZH, Ivanova L, Kostadinova T, Czankova G

*Medical University Varna, Medical Faculty, Dept. of Microbiology and Virology, UMBAL St Marina Varna, Laboratory of Microbiology and Virology*

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Keywords:

Background: Human cytomegalovirus (CMV) is an ubiquitous large enveloped DNA β-herpesvirus that, like other members of the herpesvirus family, establishes lifelong latency following primary infection. The virus is the most frequent cause of congenital infections, which can cause permanent disabilities such as hearing loss, vision loss and mental retardation. Aims: To asses the role of CMV in congenital and early postnatal infections in Northeastern Bulgaria.

Study population: 304 children (newborns to 3 months of age) with mental or physical retardation, neurological symptoms, hepatitis or other disabilities were studied by single serum samples. They are divided in two groups: Group A - 129 newborns and Group B - 175 children 1 – 3 months of age. Methods: Commercial ELISA test kits for detection of specific anti CMV IgM and IgG (EUROIMMUN - Germany, VIRCELL – Spane, Dia Pro - Italy, Adaltis - Italy) was performed. Results: A total of 304 children investigated, 57 (18.75 %) were anti CMV IgM positive, 207 (68.1%) were only anti CMV IgG positive. In Group A - 11 (8.5 %) were anti CMV IgM positive. In Group B - 46 (26.2 %) were anti CMV IgM positive indicating acute infection. IgG positive results only were detected in 80.6% of Group A and 58.9% in Group B. Conclusion: CMV is etiological agent in 8.5% of the newborns disabilities and in 26.2% of the early postnatal disorders.

## P39: Seroprevalence of Syphilis among pregnant women in Region Varna (Bulgaria)

Tsankova G[1], Todorova T[1], Kostadinova T[2], Ivanova L[3], Ermenlieva N[1]

*[1]Department of Preclinical and Clinical Sciences, Faculty of Pharmacy, Medical University Varna*
*[2]Education and Research Sectors of Medical laboratory assistant, Medical college*
*[3]Department of Microbiology and Virology, Faculty of Medicine*

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Keywords:

Syphilis is a sexually transmitted disease, caused by the spirochaete Treponema pallidum. During the course of pregnancy it may lead to serious fetal disorders and to intrauterine death.

Aim: Analysis of the frequency of syphilis among pregnant women in Varna, Bulgaria.

Material and methods: The study comprises 2702 pregnant women. The syphilis screening was performed on blood samples by ELISA (Enzyme Linked Immunosorbent Assay), VDRL (Veneral Disease Research Laboratory) and TPHA (T. pallidum haemagglutination assay).

Results: The specific treponemal antibody was detected in 27 pregnant women by using ELISA, in 15 pregnant women by VDRL and in 16 women by TPHA.

Our results showed no significant relation between the age of the patient, pregnancy trimester and the susceptibility to the disease. In contrast, the ratio positive/negative samples was three-fold higher in the group of women from rural regions compared to these of urban origin.

Conclusions: Serological syphilis screening with different methods is necessary for better protection and prevention of possible congenital transmission and habitual abortions.

The unsufficient number of physicians in rural regions and therefore the limited accessibility to health care is pertinent for higher syphilis prevalence in less urbanized regions.

## P40: Neuroimaging features in HIV positive patients with AIDS defining diseases of central nervous system (CNS)

Šoštarič N

*General Hospital "Dr.Franc Derganc" Nova Gorica, Slovenia*

Keywords: HIV/AIDS, neuroradiological/MRI imaging, CNS infection/disease

Objective: In Slovenia, approximately 500 people are currently diagnosed with HIV. Annually, 30 to 50 patients are newly identified with more than 25% of them having AIDS at the time of the diagnosis.

Aim: The aim of this article is to present newly identified patients with central nervous system AIDS defining opportunistic infection/disease who were diagnosed in 2013/2014.

Results: Out of 43 newly identified HIV patients, 11 showed signs of an AIDS defining illness, 5 of these patients presented with neurological signs. Cerebrospinal fluid (CSF) examination and neuroimaging were used to diagnose central nervous system disease. 3 patients had cerebral toxoplasmosis which was confirmed by the presence of IgG in serum and positive PCR in CSF. One patient presented with progressive multifocal leukoencephalopathy (PML). The diagnosis of PML was established by PCR detection of JC virus DNA in CSF. The fifth patient had positive PCR for Epstein Barr virus which helped establish the diagnosis of primary CNS lymphoma. Head MRI was used in the diagnosis of all five patients. Due to the unknown HIV infection at the time of presentation, the diagnosis of toxoplasmosis and lymphoma was difficult on the basis of neuroimaging. Lack of data on patients' primary infection can represent a diagnostic challenge in neuroradiology.

Conclusions: The neuroimaging features found in the newly identified HIV/AIDS patients with central nervous system disease are presented in this poster.

## P41: Concordance between isolates from blood cultures, intraoperative tissue specimens and graft cultures (sonicates) in vascular graft infections

Kokošar Ulčar B[1], Lakič N[2], Jeverica S[3], Logar M[1], Pečavar B[1], Lejko Zupanc T[1]

*[1]Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia*
*[2]Department of Cardiovascular Surgery, University Medical Centre Ljubljana, Slovenia*
*[3]Institute for Microbiology and Immunology, Faculty of Medicine, University of Ljubljana*

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Keywords: vascular graft infection; sonication method; pathogen detection

Objectives: To assess the concordance among the microbiological results of the blood cultures (BC) (obtained preoperatively), cultures from intraoperative tissue specimens (ITS) and bacterial growth after sonication of graft material (SGM), and to reveal the potential benefits of sonication methods for deciding about appropriate antimicrobial therapy.

Methods: Retrospective analysis of patients (n=25) treated for vascular graft infections at University Medical Centre Ljubljana from 2011 to mid-2014. Explanted vascular grafts were flooded with sterile Ringer solution, sonicated for 1 minute at the frequency of 40 kHz and inoculated on solid and liquid media (blood, chocolate and schaedler agar, thioglicolate and blood culture system). Aerobic and anaerobic cultures were performed and incubated for 14 days. Any significant bacterial growth was quantitatively evaluated in cfu/ml units.

Results: BC were taken in 60% of cases, in 84% of cases ITS were submitted for bacterial and fungal culture. Identification of the causative organism (irrespective of the method) was achieved in 20 cases (80%). Obtained BC were positive in 47%, the results of SGM were positive in 64% (n=16). In 24% (n=6) the same microorganisms were isolated from ITS and SGM. Only in one case the same pathogen (S. aureus) was isolated from BC and by SGM. In 6 cases (24%) the pathogen was isolated only with SGM. In 52% (13/25) the isolation by SGM significantly contributed to appropriate antimicrobial treatment.

Conclusion: Our data demonstrates that sonication of the graft material may have a significant impact on the optimized antimicrobial treatment and to the final outcome, consequently.

## P42: Comparison of clinical and laboratory findings that could predict the need for hemodialysis in patients with Puumala hantavirus hemorrhagic fever

Robnik B, Erjavc N, Unuk S, Zamuda M, Baklan Z, Ekart Koren K, Gorišek Miksić N, Kotnik Kevorkijan B, Novak Z, Rejc Marko J, Saletinger R

*Department of Infectious Diseases, University Medical Centre Maribor, Slovenia*

Keywords: hantavirus, Puumala, haemodialysis

Introduction: Puumala virus, the most common hantavirus in Slovenia, causes hemorrhagic fever with renal syndrome. It is a mild form of disease, manifested by acute fever syndrome, often combining leukocytosis, elevated CRP, hepatopathy, thrombocytopenia without clinical manifestations and variable degree of acute kidney failure. Occasionally, treatment requires haemodialysis (HD).

Objective: The aim of our study was to define possible predictors for HD need in Puumala virus infection. We expected statistically significant difference between HD and non-HD patients in at least two basic laboratory markers. Our aim was also to analyse time-sequence of observed parameters among infected patients.

Methods: Our retrospective study included 76 patients (65 hospitalised) with serologically verified disease, who were treated in UKC Maribor from January 2012 to July 2014. We compared common symptoms and basic laboratory findings (leukocytosis, CRP, thrombocytopenia, creatinine, hepatopathy, proteinuria).

Results: Majority had typical clinical picture. 17 % (13/76) required HD. They had significantly higher leukocytosis. No statistically important difference was observed in other parameters but in average HD patients tended to be younger men, had shorter episode of fever and lower platelet count (table 1). Time-sequence of laboratory changes is presented in diagram 1.

Conclusion: Time-curve analysis suggested thrombocytopenia and elevated CRP to be the earliest laboratory changes in the studied population. Among observed parameters, only the difference in maximal leukocytosis was statistically significant for HD patients. No combination of clinical or basic laboratory markers could be made for the studied population in order to predict the need for HD.

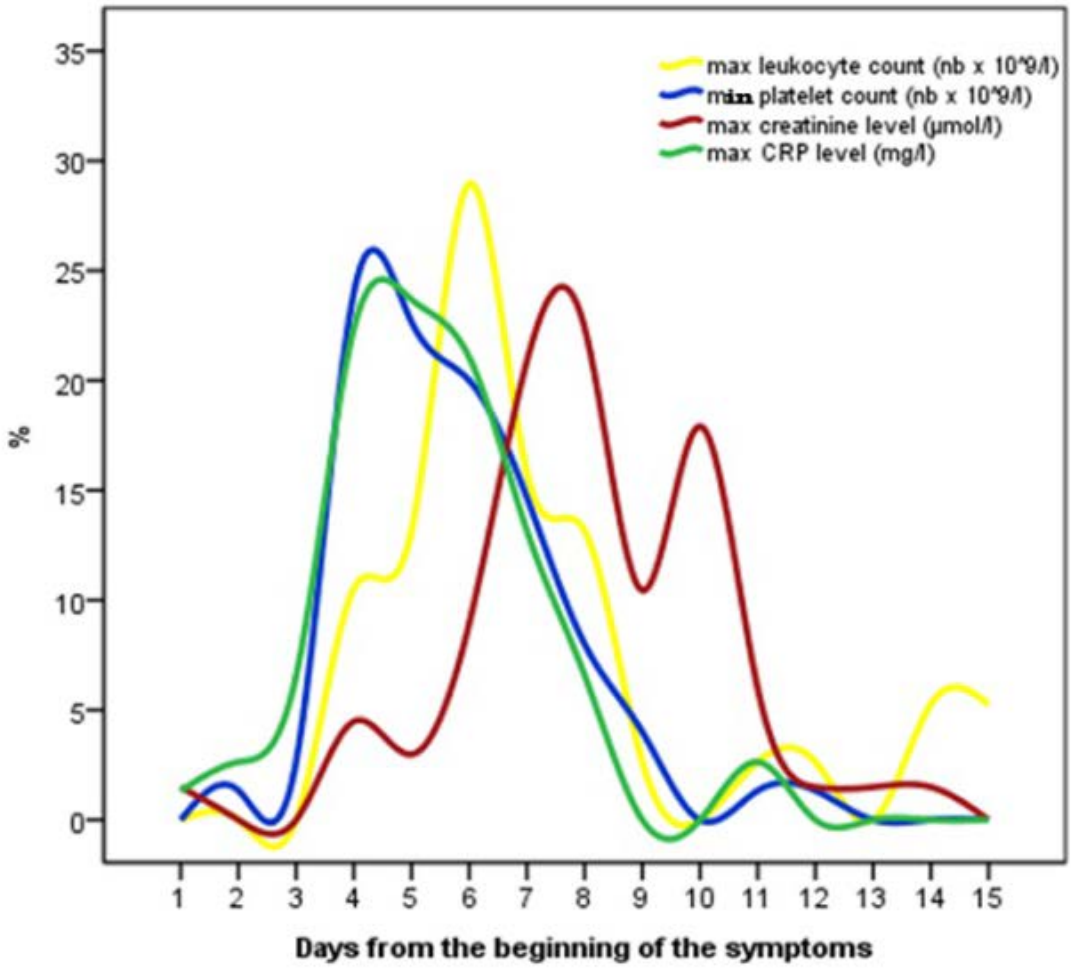


Diagram 1: Time-sequence of laboratory changes – percent age of patients (HD and non-HD) who reached maximal or minimal value of observed parameters on each consequent day

**Table 1: Presence of common symptoms and laboratory pathologic findings in the two groups of patients (HD and non-HD)**

| | Haemodyalysis | | p* |
|---|---|---|---|
| | Non-HD n=63 | HD n=13 | |
| Fever | 62 (98,4) | 12 (92,3) | 0,315 |
| Chills | 28 (44,4) | 10 (76,9) | 0,065 |
| Headache | 49 (77,8) | 12 (92,3) | 0,444 |
| Nausea/vomiting | 29 (46,0) | 10 (76,9) | 0,066 |
| Abdominal pain | 15 (23,8) | 4 (30,8) | 0,726 |
| Back pain | 28 (44,4) | 6 (46,2) | 1,000 |
| Diarrhea | 11 (17,5) | 3 (23,1) | 0,697 |
| Blurred vision | 21 (33,3) | 8 (61,5) | 0,068 |
| Leukocytosis (>10x^9/l) | 26 (41,3) | 12 (92,3) | 0,001 |
| Platelets (<140x10^9/l) | 62 (98,4) | 13 (100,0) | 1,000 |
| Elevated creatinine (>100µmol/l) | 46 (73,0) | 13 (100,0) | 0,033 |
| Hepatopathy | 36 (64,3) | 10 (76,9) | 0,520 |
| Proteinuria | 46 (78,0) | 13 (100,0) | 0,107 |

*Fisher's exact test

Table 2: Gender distribution among HD and non-HD patients

| | | HD | | Cohort |
|---|---|---|---|---|
| | | no | yes | |
| male | n | 43 | 13 | 56 |
| | % | 68,3% | 100,0% | 73,7% |
| female | n | 20 | 0 | 20 |
| | % | 31,7% | 0,0% | 26,3% |
| cohort | n | 63 | 13 | 76 |
| | % | 100,0% | 100,0% | 100,0% |

hi-kvadrat=5,601; p=0,018 (only men were in the HD group)

Table 3: Numerical clinical and laboratory characteristics for the two groups of patients (HD and non-HD patients)

| | Haemodyalysis | | | | t | p |
|---|---|---|---|---|---|---|
| | Non-HD (n=63) | | HD (n=13) | | | |
| | PV | SO | PV | SO | | |
| Age | 45,6 | 14,2 | 33,5 | 9,6 | 2,918 | 0,005 |
| Hospitalisation days | 7,0 | 3,1 | 12,0 | 2,1 | -5,475 | <0,001 |
| Duration of symptoms | 10,9 | 3,0 | 15,6 | 2,1 | -5,313 | <0,001 |
| Maximal temperature | 39,1 | 0,7 | 39,3 | 0,6 | -0,979 | 0,331 |
| Duration of fever | 5,5 | 1,9 | 4,2 | 2,3 | 2,106 | 0,039 |
| Day of max. leukocytosis | 7,1 | 2,8 | 8,0 | 3,6 | -0,853 | 0,400 |
| Minimal platelet count | 71,2 | 40,4 | 46,4 | 26,9 | 2,118 | 0,038 |
| Day of minimal platelet count | 5,7 | 1,9 | 5,8 | 1,6 | -0,078 | 0,938 |
| Maximal creatinine | 170,9 | 103,9 | 737,5 | 100,5 | -17,999 | <0,001 |
| Day of maximal creatinine | 8,0 | 2,2 | 8,5 | 2,1 | -0,789 | 0,433 |
| Maximal leukocytosis | 9,8 | 3,3 | 16,0 | 5,3 | -4,090 | 0,001 |
| Maximal CRP | 89,5 | 47,4 | 119,8 | 34,8 | -2,185 | 0,032 |
| Day of maximal CRP level | 5,4 | 1,8 | 5,1 | 1,7 | 0,591 | 0,557 |

t: independent samples t test
95% confidence interval for the mean value of maximal leukocytosis in HD and non-HD patients
non-HD: 9,8 (95%CI 8,9 – 10,6)
HD: 16,0 (95%CI 12,8 – 19,2)

## P43: Risk factors for failure of empirical treatment in patients with community acquired pneumonia (CAP)

Beović B, Žagar M, Peterlin L, Paladin M

*University Medical Centre Ljubljana, Slovenia*

Keywords: community acquired pneumonia (CAP), antibiotic treatment, risk factors

Objectives: Many hospitalized patients with CAP are old and have many co-morbidities which may influence the efficacy of the empirical antibiotic treatment.

Methods: In retrospective research we included 240 patients with CAP. We checked their hospital documentations and record risk factors that may have influence on empirical antibiotic treatment success and patients intrahospital mortality. These factors include colonisation with extended spectrum betalactamases (ESBL) producing enterobacteria, McCabe index, intrahospital oxygen need, age, gender, associated diseases. For statistical analysis we used logistic regression.

Results: Risk factors for failure of empirical antibiotic treatment of CAP were colonisation with ESBL producing bacteria (p=0,001), higher McCabe index (p<0,001), male gender (p=0,031) and associated chronic respiratory disease (p=0,042).

Risk factors for intrahospital mortality in patients with CAP were colonisation with ESBL producing enterobacteria (p=0,002), higher McCabe index (p<0,001), higher age (p=0,029), intrahospital oxygen need (p=0,029) and associated chronic respiratory disease (p=0,023).

Conclusion: Colonisation with ESBL producing bacteria, higher McCabe index and associated chronic respiratory disease may be crucial for failure of empirical antibiotic treatment and intrahospital mortality within patients with CAP.

## P44: Health problems of Slovenian travellers

Biasizzo H, Kotar T

*Section for Tropical and Travel Medicine, Slovenian Medical Association*
*Department of Infectious Diseases, University Medical Centre Ljubljana*

Keywords: Travel, travel medicine, health problems

Objectives: Many Slovenians have medical problems during travel or after return from abroad. The purpose of this study was to identify demographic, travel and medical characteristics among travellers who seeked medical assistance at Clinic for Infectious Diseases in Ljubljana upon return from abroad.

Methods: In this prospective study we analysed data of patients who were seen at Clinic for Infectious Diseases in Ljubljana for travel-related problems between June 2012 and July 2014. Part of the information was collected by questionnaire filled in by patients during their first visit at the Clinic.

Results: A total of 214 patients were studied (109 women, 105 men). The main destinations were Sub-Saharan Africa (38,8%), Indian subcontinent (18,2%) and Southeast Asia (17,8%). The majority of patients travelled for more than 29 days (45,3%) and most of them were backpacking (35%). 38,7% of them belonged to the age group of 25-34 years. The commonest diagnosis was travellers' diarrhoea (37%), followed by febrile illness (21%). Malaria was diagnosed in 5% of cases.

Conclusion: Our study showed similar results to other studies about travel related medical problems. Individuals who were backpacking and travelling for longer period developed more medical problems than other groups. The commonest complaint was travellers' diarrhoea. Since international travel is increasing, studies about travel related medical problems provide valuable information for the clinicians who treat returning travellers and should be encouraged to obtain more information about risk factors, vulnerable travelling groups and medical problems related to travel.

## P45: Hospital acquired influenza in University Medical Centre Ljubljana form 2011 to 2014

Pečavar B[1], Mrvič T[1], Petrovec M[2]

[1]*Department of infectious diseases, University Medical Centre Ljubljana*
[2]*Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana*

Keywords: Influenza, nosocomial, hospital

Objectives: To identify patients with hospital acquired influenza, study their clinical characteristics and disease outcome.

Methods: By cross referencing databases of University Medical Centre Ljubljana and Institute of Microbiology and Immunology (Faculty of Medicine, University of Ljubljana) we identified patients who met criteria for hospital acquired influenza (symptoms ≥72 hours after admission and microbiological confirmation of infection) in the seasons 2011/2012, 2012/2013 and 2013/2014. Patient characteristics were obtained from medical records and in the season 2013/2014 using a questionnaire for hospital acquired influenza.

Results: In the observed period we identified 319 (season 2011/2012, 76; season 2012/2013, 110; season 2013/2014, 133) patients who met the criteria for hospital acquired influenza. Of them 176 (55.2%) were male, 40 (12.5%) died. Average age was 66.0 years and age distribution was as followed: <18 years 18 (5.6%), ≥65 years 207 (64.9%). Most death cases occurred in the age group ≥65 years, 35/40. Time elapsed from admission to disease was 16.5 days and from confirmation of infection to discharge/death 12.5 days. For 24 cases in the season 2013/2014 questionnaire for hospital acquired influenza was completed. The clinical characteristics of the disease for these patients were as followed: body temperature ≥38°C 12/18 (66.7%), cough 12/18 (66.7%), fatigue 3/18 (16.7%), myalgia/arthralgia 2/18 (11.1%), sore throat, headache, nasal discharge each in 1/18 (5.6%) and in 6 cases no symptoms were specified.

Conclusions: Hospital acquired influenza is associated with serious morbidity and mortality and has to be considered in patients who develop symptoms.

## P46: Antibiotic prescribing attitudes and beliefs among Slovenian medical residents

Klešnik M[1], Nadrah K[1], Uhan S[2], Beović B[1]

[1]*Department of Infectious Diseases, University Medical Centre Ljubljana*
[2]*Faculty of Social Sciences, University in Ljubljana*

Keywords: Antibiotic stewardship, antibiotic resistance, education

Objectives: Antibiotic resistance is on the rise lately, which poses a significant public health threat. With very few new drugs in the antibiotic pipeline, steps should be taken to preserve the efficacy of the existing antibiotics. Current rise in bacterial resistance is largely a consequence of antibiotic misuse and overuse. New strategies to improve antibiotic prescribing are necessary. During residency young physicians prescribe antibiotics, often independently, without proper education on rational antibiotic use.

Methods: As a part of a pilot study, we asked 40 residents of various specialties to participate in an on-line survey on resident physician's knowledge and attitude toward rational antibiotic use and prescribing habits. Data were collected using a standardized survey instrument. Respondents expressed their views on the topics discussed by using Likert type scale.

Results: 23 residents completed the survey. The biggest group were infectious diseases residents (70 %), followed by pediatrics, neurology, rheumatology and family medicine residents. Infectious diseases residents felt better informed on the antibiotic prescribing guidelines, and used them more often in their work than other residents. They assessed their level of knowledge on prescribing of the right antibiotic and interpreting microbiological data higher than other residents, and deescalated antibiotic therapy more often based on microbiological data. Furthermore, they reported to share their concerns on inappropriate prescribing of antibiotics to their supervisors more than residents of other specialties. Infectious disease residents also reported to have more exposure to multi-drug resistant bacteria in their institution.

Conclusions: Together, the data shows that infectious diseases education leads to better prescribing practices.

## P47: Usefulness of additional microbiological tests to improve the aetiological diagnosis in patients with infective endocarditis

Ciglar T[1], Kolšek M[1], Lejko Zupanc T[1], Logar M[1], Pečavar B[1], Klokočovnik T[2]

[1]*Department of Infectious Diseases, University Medical Centre Ljubljana*
[2]*Department of Cardiovascular Surgery, University Medical Centre Ljubljana*

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Keywords: Infective endocarditis, microbiology, surgery

Objective: To analyze determination of causative agent of infective endocarditis (IE) by using various microbiological methods in patients who needed surgery during the course of the disease.

Methods: Retrospective study of patients with definite IE by Duke's criteria who needed surgery and were hospitalized at the Department of Infectious Diseases, University Medical Centre Ljubljana in the years 2012 and 2013. Clinical features, results of microbiological tests and outcome were evaluated using medical records.

Results: 36 patients, 25 (69.4%) were male, mean age 58.7 years were identified. Mitral valve was affected in 13 (36.1%), aortic valve in 12 (33.3%), aortic/mitral valve in 4 (11.1%), aortic/tricuspid valve in 2 (5.6%) and cardiac device electrodes in 5 patients (13.9%). Native valve was affected in 22 (61.1%), prosthetic valve in 8 (22.2%) and in 1 case data is not available. Six patients died.

The causative agent was identified in all patients; Staphylococcus aureus was the most prevalent pathogen. Blood cultures were positive in 30 (83.3%), negative in 3 and in 3 cases they were not withdrawn before a surgical procedure. In 6 (16.7%) cases the etiology was determined by additional microbiological tests (conventional culture, sonication culture, broad-range polymerase chain reaction) from material obtained by surgical procedure. Blood cultures showed concordance in all cases where multiple microbiological tests were done.

Conclusion: Determination of causative agent in IE is crucial for the optimal therapy. In case of negative blood cultures additional microbiological tests should be done, especially when surgically obtained material is available.

## P48: Tolerability of anti-tuberculosis therapy: a retrospective study of risk factors for hepatotoxicity

Rosin C, Chiarandini E, Maurel C, Monticelli J, Fabris C, Luzzati R

*Infectious Diseases and Microbiology Departments, University Hospital of Trieste, Trieste, Italy*

Keywords: tuberculosis, antibiotics, hepatotoxicity

Introduction: The therapy of tuberculosis (TB) causes high rates of adverse effects (up to 40%). It is challenging to predict which patient will develop hepatotoxicity during anti-TB treatment.

Methods: We performed a single-centre retrospective study on 213 consecutive patients with active TB, admitted to the Infectious Diseases Unit in Trieste (Italy) between January 2004 and December 2013. The primary aim of this study was to evaluate the tolerability of anti-TB therapy, focusing on hepatotoxicity in order to identify risk factors for the occurrence of liver toxicity.

Results: The study population included young foreigners (mean age 35 ± 13 years) and old Italians (mean age 67 ± 19 years). Only 5 patients had multidrug-resistant TB. Most of patients received the common anti-TB regimen. Concomitant liver disease was present in 24% of patients. The overall incidence of adverse effects was 43%. Hepatotoxicity was demonstrated in 19.2% of cases. The concomitant use of other potentially hepatotoxic drugs was demonstrated to play a role on bivariate analysis (p= 0.0035). Multivariate analysis reveals that baseline compromised liver function, by MELD Score (p= 0.0252) and low nutritional state by BMI (p=0.0407) represent independent risk factors for the onset of hepatotoxicity.

Conclusion: Our findings suggest that hepatotoxic-sparing anti-TB regimens should be considered for patients affected by low BMI, liver failure by MELD score or receiving concomitant hepatotoxic drugs.

## P49: Increased tumor necrosis factor alpha and interleukin-6 serum levels in patients with imported malaria

Poluga J[1,2], Dopsaj V[3], Pelemiš M[1,2], Dulović O[1,2], Dakić Z[4], Lavadinović L[1,2], Pavlović M[1,2]

*[1]Clinic of Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade*
*[2]School of Medicine, University of Belgrade*
*[3]Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade*
*[4]Parasitological Laboratory, Department of Microbiology, Clinical Center of Serbia, Belgrade*

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Keywords: Malaria, cytokines, parasitemia

Objectives: In malaria, especially in severe forms, blood concentrations of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), are increased.

The aim of this study was to determine the serum levels of TNF-α and IL-6, and to establish their correlation with other laboratory parameters.

Methods: The study included 34 patients with imported malaria who were treated from 2007 to 2010 at the Clinic of Infectious and Tropical Diseases, Clinical Center of Serbia. The following data sources were included: medical records, hematological, biochemical, parasitological and immunological analyses. The immunological analyses of the cytokines (TNF-α and IL-6,) were using the method of Quantikine ELISA Kit.

Results: TNF-α and IL-6 were examined in two phases, immediately after the admission, and at the end of antimalarial therapy. The results show a significant increase of TNF-α and IL-6 in the first phase, before the effects of antimalarial therapy. A very strong correlation between TNF-α and IL-6 is also confirmed, which suggests their coordinated production. Increased TNF-α values were correlated with an older age, the level of parasitemia, the number of platelets and leukocytes, elevated values of procalcitonin, D-dimer and lactate dehydrogenase, and lower values of serum iron and antithrombin. Increased values of IL-6 were correlated with the level of parasitemia, the number of platelets and leukocytes, and elevated values of D-dimer and lactates.

Conclusions: Determination of cytokines levels may represent the early diagnostic procedure that will provide more effective therapeutic approach, especially in severe forms of malaria.

**Figure 1. Correlation of TNF-α with IL-6, the age and laboratory parameters in the first phase (1)**

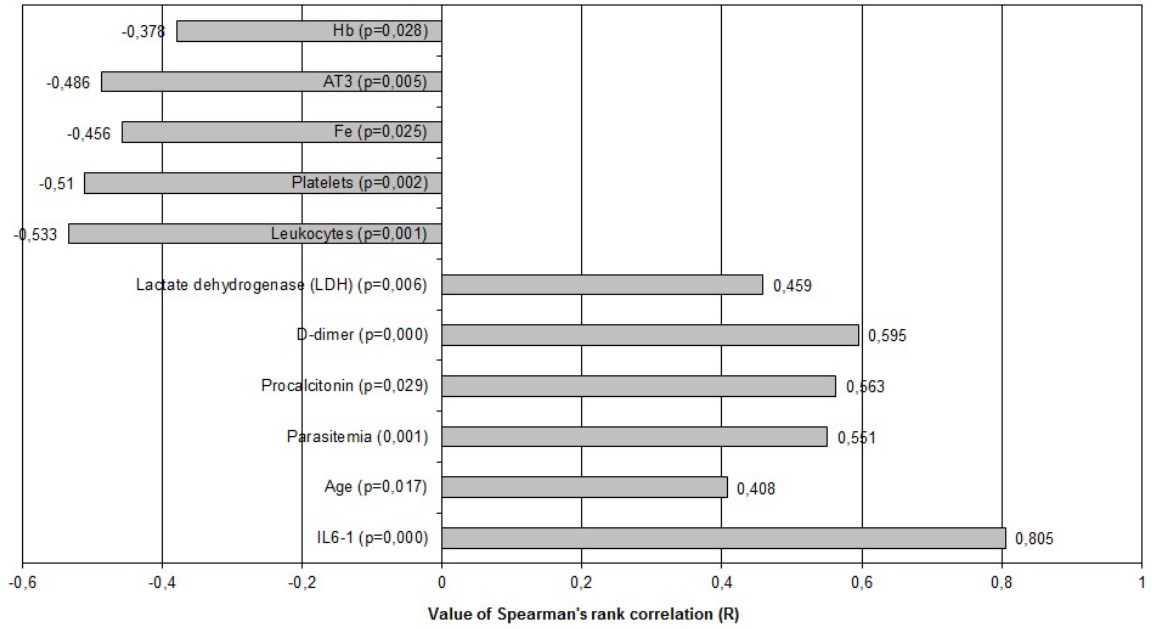
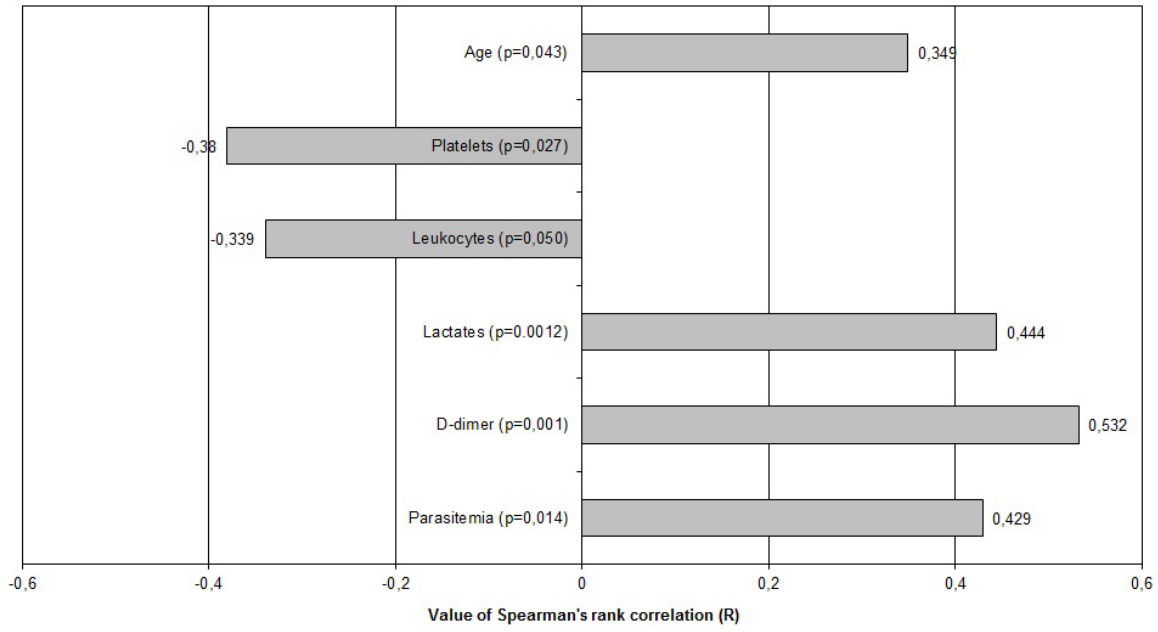



**Figure 2. Correlation of IL-6 with TNF-α, the age and laboratory parameters in the first phase (1)**



# P50: Evaluation of the cystic Eccinococcosis cases diagnosed in Dr. Lütfi Kırdar Kartal Education and Research Hospital Pathology Laboratory between 2007 and 2013

Selek A[1], Selek MB[2], Karadayi N[1]

[1]*Dr.Lütfi Kırdar Kartal Training and Research Hospital, Istanbul, Turkey*
[2]*GATA Haydarpaşa Training Hospital, Medical Microbiology department, Istanbul, Turkey*

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Keywords: Cystic echinococcosis, demographic features, atypical localization

Background: The aim of the study was a retrospective evaluation of 299 cases that were histopathologically diagnosed as cystic echinococcosis (CE) in the pathology laboratory of our hospital in a seven years period.

Material and methods: All specimens sent to the laboratory were examined microscopically following macroscopic and hematoxylin eosin (H-E) staining. 299 cases diagnosed as CE were reviewed according to age, gender and organ affected by the cyst, more than one specimen of the same organ was evaluated once.

Results: Of the 299 CE cases, 44.5% (133) were male whereas 55.5% (166) of them were female. Additionally, %5 (15) of the cases were between 0-15 ages, %31.8 (95) of them were between 16-30 ages, %29.4 (88) of them were between 31-45 ages, % 24.4 (73) of them were between 46-60 ages and %9.4 (28) of them were older than 61. Cysts were mostly localized in liver, lungs and peritoneal cavity, % 71.9 (215), %11.4 (34) and %4.7 (14) respectively.

Conclusion: Demographic features of our cases were mostly in line with the previous literature. Because of the presence of atypical localized cases, during pathologic evaluation of all surgical cystic specimens, elements of this parasite should be searched and evaluated carefully.

## P51: The adequacy of antibiotic therapy in chronic rhinosinusitis

Pandak N[1], Pajić-Penavić I[1], Mahovne I[1], Čabraja I[1], Baličević M[1], Miklaušić B[1]

[1]*General Hospital "Dr. Josip Benčević", Slavonski Brod, Croatia*

Keywords: Chronic rhinosinusitis, bacteria, antibiotic therapy

Objectives: Chronic rhinosinusitis (CRS) is a symptomatic inflammation of the mucosa of the nose and paranasal sinuses lasting for at least 12 weeks. The aetiology and pathogenesis of CRS are still the matter of numerous investigations. Some authors support the role of bacterial or fungal infection as the cause of CRS while others consider the disease to be the result of immune response. Empiric antimicrobial therapy is an accepted method of treatment for CRS supported by the current guidelines. The purpose of this study was to determine bacteria present in sinus cavities of CRS patients and to establish if these bacteria cause an infection or just colonize sinuses.

Methods: 62 consecutive patients with CRS who underwent the functional endoscopic sinus surgery (FESS) were included in the study. During the FESS sinus was irrigated with sterile 0.9% NaCl solution and this lavages were used to detect bacteria and fungi. Excised sinus mucosa was sent to the pathologist for the histopathology analysis.

Results: Bacteria and fungi were detected in 45 % of sinus lavage samples. The most commonly isolated bacteria were Staphylococcus epidermidis and Staphylococcus aureus. Chronic inflammation was detected in each mucosa sample. Half of patients had the inflammation with lymphocytic predominance while others had eosinophilic granulocyte predominance.

Conclusions: Results of this study show that bacteria detected colonize sinus mucosa and don't cause the infection. According to this CRS should be considered a chronic inflammatory condition rather than bacterial infection so routine antibiotic therapy should be avoided as frequent and prolonged antibiotic therapy could induce bacterial resistance.

## P52: Retrospective assessment of 210 diabetic foot infection patients in a tertiary care hospital

Ülçay A[1], Karagöz E[1], Karaahmetoğlu G[1], Öncül O[1,2], Turhan V[1,2], Erdem H[1], Görenek L[1], Diabetic foot Study Group[2]

[1]*GATA Haydarpasa Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*
[2]*GATA Haydarpasa Training Hospital, Diabetic Foot Council, Istanbul, Turkey*

Keywords: Diabetic foot infection, retrospective, Wagner grade

Background: The aim of this study is to assess the clinical and laboratory characteristics of diabetic foot patients and to show reduction of amputation rates by close follow-ups and multidisciplinary approaches.

Material and method: This was a retrospective study in which 210 patients were evaluated in diabetic foot council of Gata Haydarpasa Training Hospital between May 2011-April 2014.

Results: 210 patients were included in the study. 31% of the patients were female and 69% were male. The mean age of the patients was 66.6 (35-97) with an average 17.3 year history of diabetes mellitus. Of the 169 cases whose data were available, 55% of them had a history of antibiotic use. 86% of the patients were being administered insulin while 12% of them were using oral anti-diabetics. The mean white blood cell level was 10.500 (4000-10.500 cells/ml), C-Reactive-protein was 62.2 (0-8 mg/dl), sedimentation rate: 65.4 (0-20 mm/h), the mean glucose level: 178 (65-107 mg/dl), HbA1c:8.42, respectively. 0,1% of the patients were Wagner grade 0; 2,1% of the cases were Wagner 1; 23% of the patients were Wagner grade 2; 36% of the patients were Wagner 3; 13,5% of the patients were Wagner grade 4 and 0,1% of the patients were Wagner 5, respectively. 54% of the patients had osteomyelitis while 46% of the patients had skin infections without bone involvement. 62.5% of 160 patients whose data were available underwent to debridement limited to soft tissue while 32.5 % of them underwent to amputation. 40 % of the patients were administered hyperbaric oxygen therapy.

In gram positive microorganisms; Staphylococcus aureus (47%), Enterococcus spp.(42%), Streptococcus pyogenes(4%) were the most frequently isolated microorganisms while Pseudomonas aeruginosa (31.8%), Escherichia coli(13%), Proteus mirabilis (13%), Acinetobacter baumannii(10%) Klebsiella oxytoca (8.6%), Enterobacter spp. (8.6%) and were the most frequently isolated microorganisms in gram negatives. Rate of Candida spp. was 7.2% of all agents.

Conclusion: Despite high Wagner grade of these patients, multidisciplinary approaches could prevent severe infections and reduce amputation rates.

## P53: Administration of a triple vs. standard double antibiotic regimen to brucellosis patients efficiently eliminates bacterial DNA load

Vrioni G[1], Bourdakis A[2], Pappas G[3], Pitiriga V[1], Kapsimali V[1], Pournaras S[1], Tsakris A[1]

[1]*Department of Microbiology, Medical School, University of Athens, Athens, Greece*
[2]*Department of Internal Medicine, General Hospital of Trikala, Trikala, Greece*
[3]*Institute of Continuing Medical Education of Ioannina, Ioannina, Greece*

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Keywords: Efficient treatment, Brucella DNA load, real-time PCR, Brucella DNA eradication

Introduction: Brucellosis is a common zoonotic infection, being endemic in many countries worldwide, including Greece. The optimal antibiotic regimen for the treatment of human brucellosis still remains to be determined. We compared and present herein the effect of a triple versus a standard double regimen on residual bacterial DNA load, in patients suffering from acute brucellosis.

Methods: Thirty-six patients without previous brucellosis history, visiting an outpatient clinic in a highly endemic region, were treated with a standard doxycycline-streptomycin regimen or with a triple doxycycline-streptomycin-rifampicin scheme when having severe clinical presentation or osteoarticular complications. An arsenal of serological tests was performed to all patients at the initial examination and at the follow-up visit, including Rose Bengal Plate (RBP) agglutination and Wright seroagglutination tests. A quantitative real-time PCR assay using the LightCycler instrument (Roche Diagnostics, Manheim, Germany) was applied for measuring bacterial DNA load in brucellosis-affected patients prior to treatment initiation and at a follow-up visit 12-24 months post-treatment. The diagnosis of acute brucellosis was based on clinical findings confirmed by the presence of specific antibodies at high titers (Wright seroagglutination test titers ≥ 1/160, RBP agglutination test from 2+ up to 4+) and/or detection of Brucella DNA with real-time PCR assay.

Results: At the time of diagnosis, the RBP agglutination test was positive in all 36 patients (100%), the Wright seroagglutination test titers were within the diagnostic range in 32 (89%) cases and all patients were positive by the real time-PCR assay. The mean load (± SD) at diagnosis was overall 1948 ± 1037 copies/5μL DNA extract. The mean load of those selected to be treated with the triple regimen was 1939 ± 1051 copies/5μL extract and of those treated with the standard regimen 1958 ± 958 copies/5μL extract. At the second visit, 12 to 24 months after the end of treatment, 19/22 patients (86.4%) treated with the triple scheme became negative [mean time (± SD) 17,6 ± 2,7 months, range 14-24 months], while only 7/14 patients (50%) treated with double therapy became negative [mean time (± SD) 16,6 ± 3 months, range 12-24 months] ($P$=0.026). Demographic parameters such as gender and age did not influence considerably bacterial loads ($P$=0.842 and 0.146, respectively). Among 10 patients who continued to have positive real time-PCR results post-treatment, only three patients experienced symptoms indicative of active disease (two of them received triple and one received double treatment), while the rest remained asymptomatic.

Conclusion: *Brucella melitensis* DNA commonly persists after double antibiotic treatment while is efficiently eliminated when receiving triple regimen, indicating its appropriateness for the treatment of acute brucellosis.

## P54: Complex Regional Pain Syndrome Type I Following Diphtheria-Tetanus (Di-Te) Vaccination

Karagöz E[1], Ersöz E[2], Akarsu S[2], Turhan V[1]

*[1]GATA Haydarpasa Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*
*[2]GATA Haydarpasa Training Hospital, Department of Physical Therapy and Rehabilitation, Istanbul, Turkey*

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Keywords: Complex regional pain syndrome; immunization; tetanus toxoid vaccine

Complex regional pain syndrome type 1 (CRPS-1) is a clinical syndrome affecting one or more extremities, characterized with severe pain, stiffness, swelling and discoloration that affects the limbs. This syndrome was formerly known as causalgia or reflex sympathetic dystrophy. Although the exact cause of CRPS is unknown, its onset is often precipitated by a physical injury, such as minor trauma, fracture, infection or a surgical procedure. In the literature, there are reports of CRPS-1 following immunization with rubella and hepatitis B vaccines. Here we present a case of 20-year-old male patient who was diagnosed as CRPS type 1 of the right upper extremity following an injection with tetanus toxoid vaccine into the same extremity.

A 20-year-old male patient was admitted to Infectious diseases outpatient clinic with 25 days' history of pain and swelling of the left hand and forearm. He had received a single dose of diphtheria tetanus-acellular pertussis (dTap) vaccine in the left deltoid muscle 25 days ago after joining the army as a soldier. He had a small localized reaction at the injection site; however 2 days later developed pain in the left arm and difficulty with movement. Symptoms were progressed and were associated with weakness and inability to use the affected extremity. He had a normal neurological exam and simple analgesia was prescribed. EMG had also been performed to exclude the brachial plexus pathologies but found to be normal. Due to his ongoing complaints, he visited our infectious diseases outpatient clinic. On his physical examination, his left hand and forearm were diffusely swollen and very sensitive to touch. He had also allodynia and hyperalgesia. Physical therapy and rehabilitation consultation was taken and the patient was diagnosed as Complex regional pain syndrome type 1. Gabapentin and physiotherapy was initiated.

Although complex regional pain syndrome type 1 (CRPS- 1) is a rare entity, it should be recognized in patients with severe pain, swelling and restricted extremity movement that occurred after immunization. It is vital to initiate early treatment and physiotherapy to avoid trophic extremity alterations and to preserve limb functions either in the pediatric population or in adults.

## P55: Acute extensive airspace pneumonia by Legionella pneumophila

Krietsepi V, Fletsios D, Damianaki A, Athanasiadou A, Parlamenti K, Kastanakis S

*Respiratory department of Chania General Hospital, Crete, Greece*

Keywords: Legionella, serogroup, pneumonia

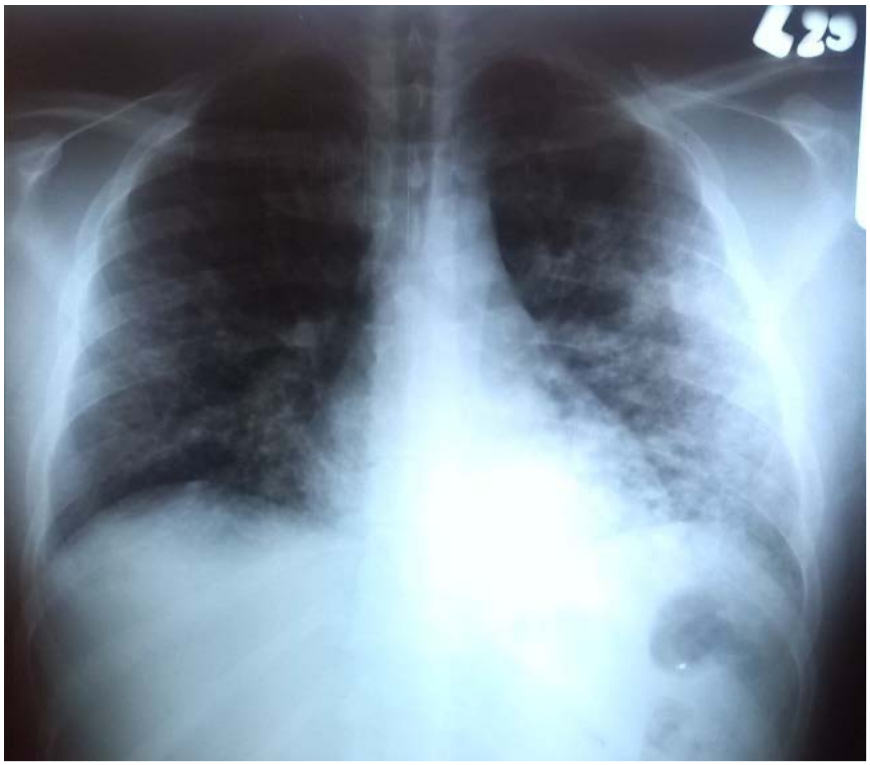
Objectives: We report a case of Legionella pneumophila pneumonia in a young immunocompetent male.

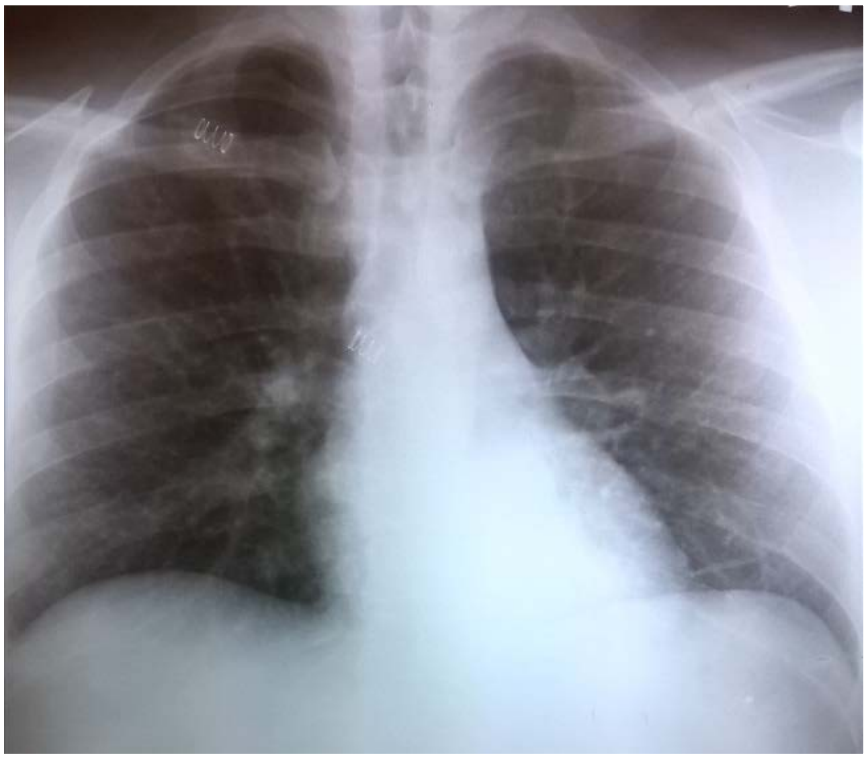
Methods: A 26 year old man, with negative clinical history, non-smoker was admitted in the pulmonary department due to high fever for three days, hemoptysis and acute respiratory failure of hypoxemic type.

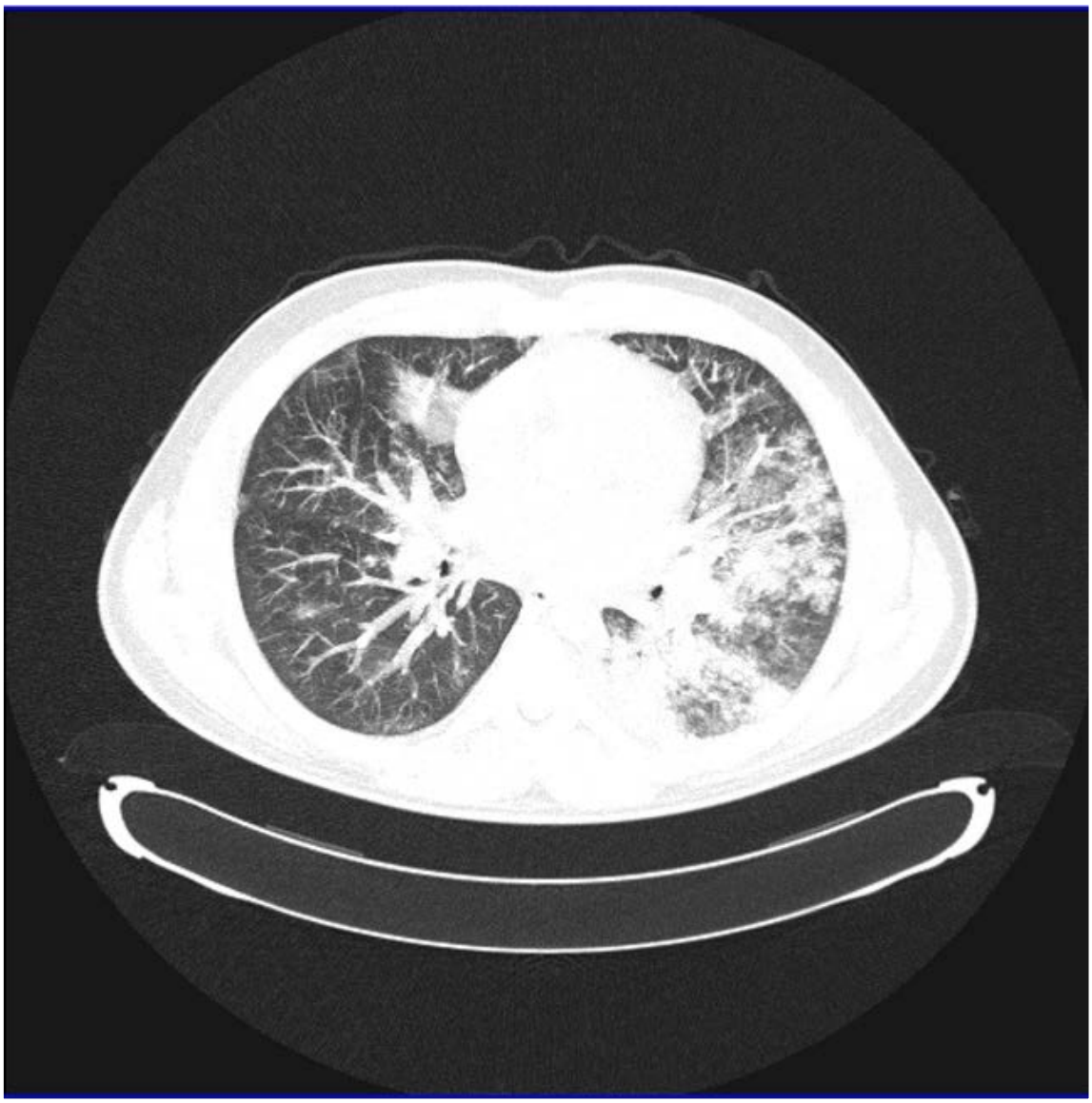
Results: The auscultation revealed bilateral crackles and the chest X-ray indicated diffuse infiltrates regionally gathering predominantly in the middle and lower lobes. The chest C/T presented extensive consolidation and ''tree in bub'' pattern of the left upper lobe and small bilateral pleural effusions. We was treated with Teicoplanin and Levofloxacin and remained febrile for six days.

Urine antigens for S.pneumoniae and Legionella were negative as well as viral antibodies .Serological tests using the IFA method were done on the 7th day from the beginning of the symptoms: Positive serum test for Legionella pneumophila serogroups 2-15: IgG 1/480, IgM 1/800 confirmed the diagnosis of the Legionnaires' disease.He was clinically and roentgenographically improved and was discharged after ten days.

Conclusions: Legionella pneumophila infection may not be detected in the urine specimen as the method applies for serogroup 1 only. The serological tests are necessary in addition.







## P56: First diagnostic case of Dirofilariasis in Montenegro

Andrić B[1], Radoman L[2], Dupanovic B[1], Terzic D[1], Dragaš S[1], Nikcevic D[1]

[1]*Clinic for Infectious Disease, Clinic Center of Montenegro, Montenegro*
[2]*General Hospital Kotor, Department of infectious Disease, Montenegro*

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Keywords: Diroflariasis, diagnosis, treatment

Objectives: Montenegro is endemic country for a significant vector borne diseases (VBD). Natural condition and geographical position (Mediterranean area) allow of disease existence. In Montenegro first case of dirofilariasis is registered to 2014.

Methods: Our patient is resident of Kotor, a civil servant, who never left Montenegro. The first polymorphic symptoms occurred in January 2014. After numerous tests for gastroenterologist, pulmologist, ophthalmologist, reason not disclosed problems. He was admitted in surgical ward of the General Hospital of Kotor with suspected impacted epigastric hernia.

Results: To the 10.01.2014 the surgeon intraoperatively entirely extirpated solid fibrous granuloma, site epifascial, the midline supraumbilicaly. In the excised granuloma is indeterminate thread parasite length 9.7 cm. Histopathological examination of the worm was identified undifferentiated filaria, based on morphological exclusion to a Wuheria bancrofti, Loa loa and Onhocerca volvulus. Serologically are negative results for antibodies to Toxocara, Trichinella.

Conclusions: Dirofilariasis is systemic parasitic zoonosis. In most of cases the infective larvae (microfilaria) injected through mosquito bites. Some species of fleas (black flea), lice, and ticks are also presumed to act as vectors. Among Dirofilariae, the most prevalent are two species (D. immitis and D. repens). Dirofilariasis has to be considered as a differential diagnosis in patients presenting with subcutaneous or pulmonary disturbances (pneumonia). Effective therapy is possible by surgical treatment and oral treatment with ivermectine (150 mg per kg) plus diethylcarbamazine (DEC) (2 mg per kg t.i.d.) over a period of 4 weeks was added to the surgical treatment.

## P57: Late exacerbation of frontal bone osteomyelitis in a patient with persistent acute rhinosinusitis

Kastanakis S[1], Chimona TS[2], Proimos EK[2], Asimakopoulou P[2], Papadakis CE[2]

[1]*Internal Medicine Department, Chania General Hospital, Chania, Crete, Greece*
[2]*ENT Department, Chania General Hospital, Chania, Crete, Greece*

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Keywords:

Objective: Case presentation of frontal bone osteomyelitis as late disease exacerbation in an adult patient, 2 ½ months after her hospitalization for acute complicated pansinusitis on the right.

Method: A 16 years old female presented with symptoms of acute rhinosinusitis and preseptal cellulitis on the right. Due to orbit complication the patient underwent imaging with CT scan of the nose and sinuses, which revealed pansinusitis on the right and confirmed the presence of preseptal cellulitis without any abscess formation. Mucopurulent sample was sent for culture after right maxillary sinus puncture. Patient was treated with intravenous ceftriaxone and nasal decongestion.

Results: Culture of the aspirated sinus material revealed Streptococcus pneumoniae and Bacteroides. According to bacterial sensitivities Clindamycin was added to intravenous antibiotic therapy for a total of 14 days. The patient was discharged with oral antibiotic therapy for 14 days. On follow-up examination, 2 ½ months later, the patient which was completely free of symptoms, presented with frontal sinus opacification on a Water's view x-ray. A new CT scan of the sinuses was performed revealing defect of the front wall of the frontal sinus. This new finding was thought to be the result of bone erosion due to frontal bone osteomyelitis. Tc 99m and 67gallium scintigraphy of frontal bone was not consistent with inflammatory activity and no further antibiotic therapy was prescribed.

Conclusion: Patients with complicated sinusitis need to be followed-up carefully for 12 weeks and receive medical treatment for at least 2 weeks after discharge. Frontal sinus infection may cause serious complications without significant symptoms.

## P58: Hydatid lung disease and pneumonia

Parlamenti K[1] Damianaki A[1], Krietsepi V[1], Athanasiadou A[1], Korakas P[2], Tsirakis G[3], Kastanakis S[4]

[1]*Respiratory department St. George, General Hospital of Chania, Crete, Greece*
[2]*Radiology Department St. George, General Hospital of Chania, Crete, Greece*
[3]*Hematology Department St. George, General Hospital of Chania, Crete, Greece*
[4]*Internal Medicine Department St. George, General Hospital of Chania, Crete, Greece*

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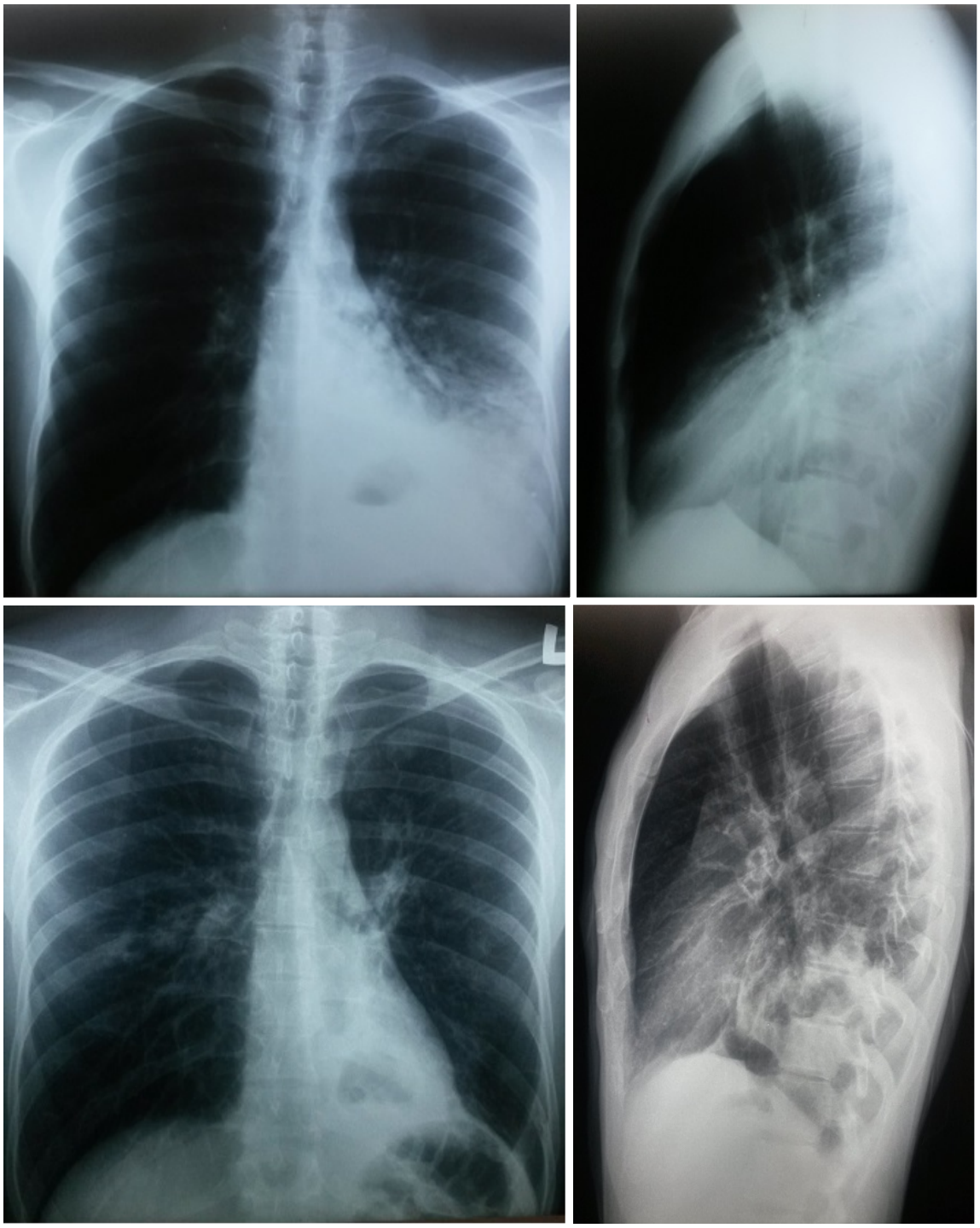
Keywords: Pneumonia, echinococcus, eosinophilia

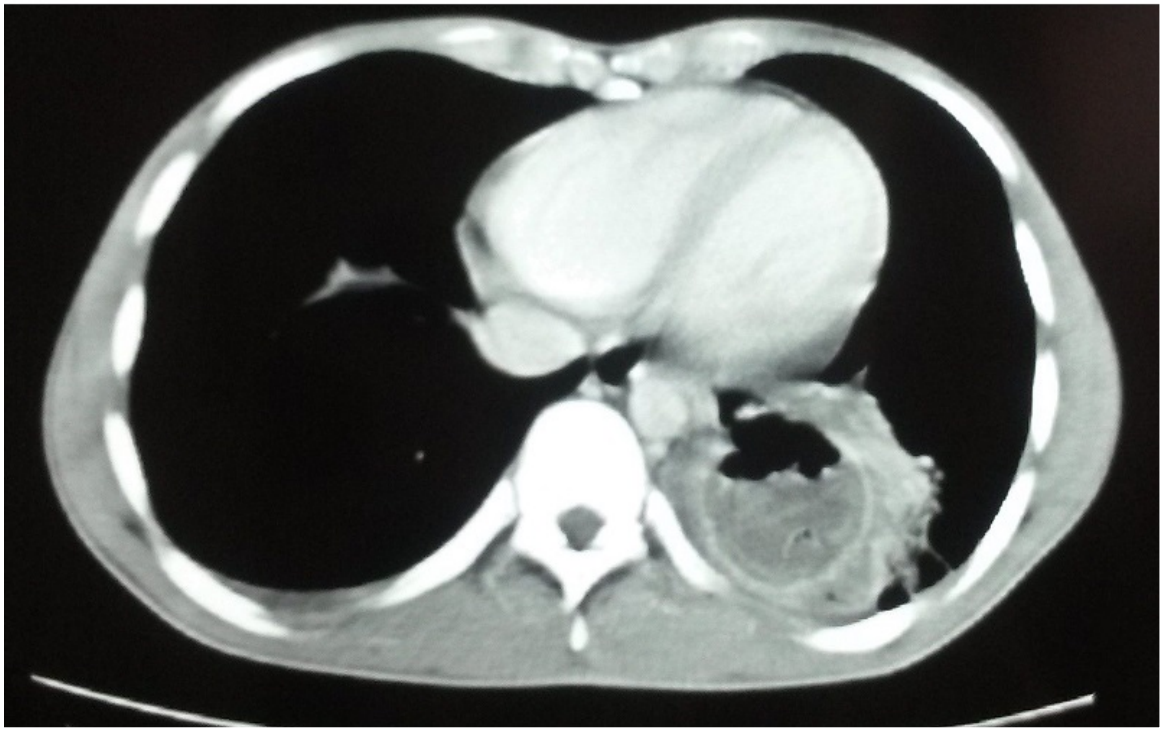
Objectives: We report a case of pneumonia in a Tunisian male on an unknown hydatid lung cyst.

Methods: A 23 years old Tunisian student in Greece was admitted to respiratory department due to pneumonia of the left lower lobe. A complete blood count was consistent with infection, however, an increased number of eosinophils (580 K/μl) was noted. The patient was treated with intravenous infusion of broad spectrum antibiotics (azithromycin, cephalosporin, teicoplanin) and was improved and afebrile after the fifth day of treatment. The number of total white blood cells was normal with an increased eosinophilic count (4.800 K/μl). He was negative for Hepatitis B and C and HIV1/2. The Mantoux test was 0mm and the gastric fluid's culture for β-Koch bacillus was negative. The stool sample was negative for parasites. He was discharged after 10 days with instructions for a new complete blood count and chest X-ray in 7 days. The new blood count showed persistent eosinophilia (1100 K/μl) and the CXR complete remission of the pneumonia, however, new opacities adjacent to the right heart border and air fluid level posterior to the heart were noted.

Results: A chest CT revealed a solitary cystic lesion in the left lower lobe. The cyst was round well-defined with meniscus sign. The indirect hemagglutination serology test was positive for Echinococcus IgG 1/1024. The patient was proposed to remove the cyst. He desired to do so in his own country.

Conclusions: A clinician should always consider underlying lung diseases in any case of pneumonia.





## P59: CMV myelitis and pneumonitis in an immunocompetent woman

Damianaki A[1], Parlamenti K[1], Krietsepi V[1], Panou E[2], Athanasiadou A[1], Kastanakis S[3]

[1]*Respiratory department St. George, General Hospital of Chania, Crete, Greece*
[2]*Intensive Care Unit St. George, General Hospital of Chania, Crete, Greece*
[3]*Internal Medicine Department St. George, General Hospital of Chania, Crete, Greece*

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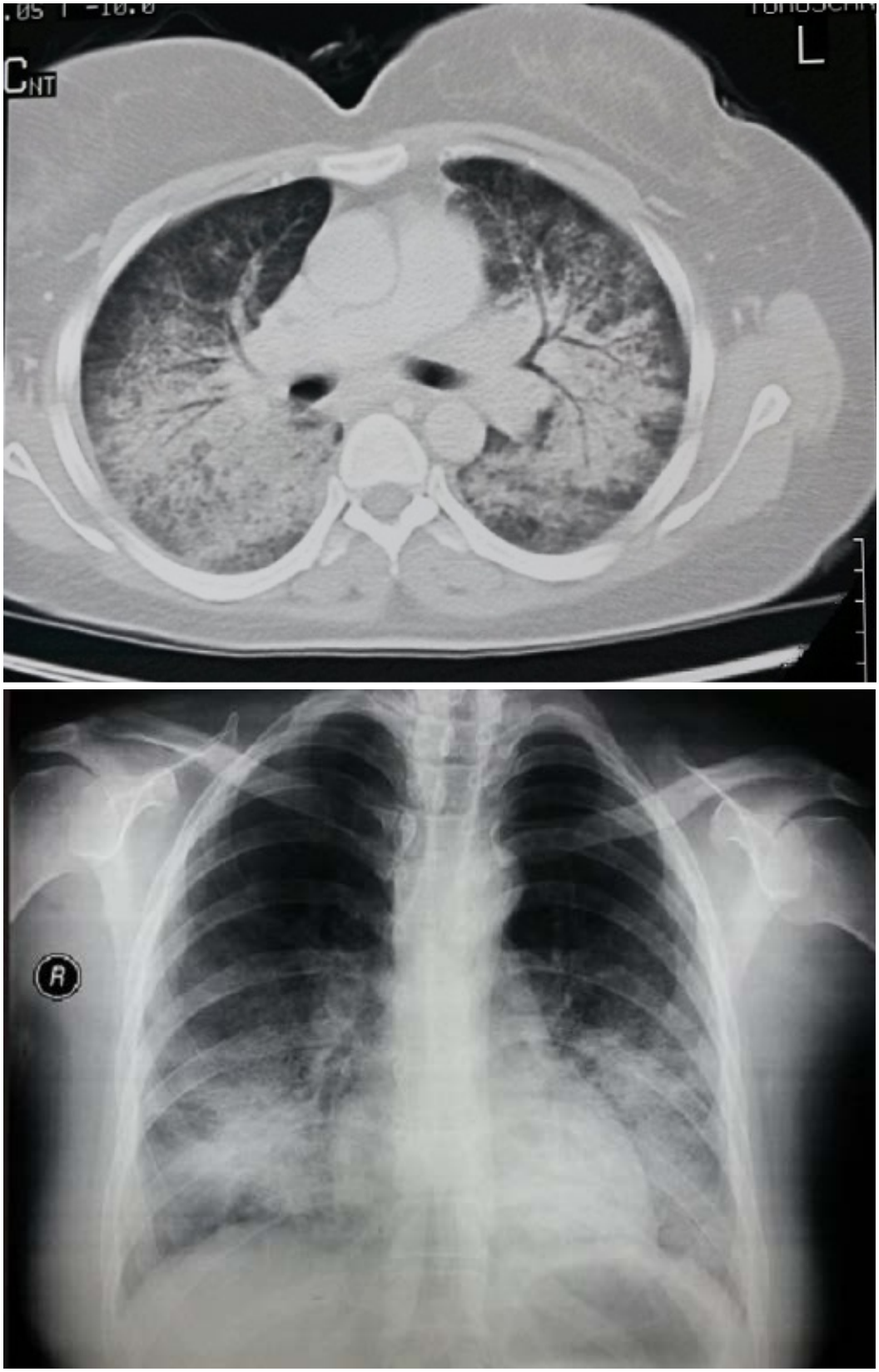
Keywords: CMV, pneumonitis, myelitis

Objectives: CMV infection is asymptomatic in the immunocompetent host but rarely can lead to severe organ dysfunction with significant morbidity and mortality. We report a case of a CMV myelitis in a 40 year old woman with no signs of immunodeficiency, followed by CMV pneumonitis after treatment with steroids.

Methods: A 40 year old woman, with a medical history of thyroid nodules, was admitted in the neurological department due to a rapid onset of weakness of the lower limps, sensory alterations and bowel and bladder dysfunctions. PCR test for CMV in cerebrospinal fluid (CSF) was positive in two consecutive tests. The HIV test was negative. Her white blood count was within normal limits. The patient was treated with ceftriaxon and aciclovir which was later substituted by ganciclovir and methylprednisolone which was given because of severe neurologic disease. She was discharged 1 month later clinically improved. After 2 months, the patient was brought back to the respiratory department with acute respiratory failure. The CRX showed butterfly pulmonary opacities. CRP was 5 mg/dl with normal white blood cells count.

Results: PCR blood test was positive for CMV DNA with high viral load. The HIV test was negative. She was treated with ganciclovir 500mgx2. Due to respiratory deterioration, the patient was intubated after 10 days and died 4 days later in the ICU. Perhaps the steroids and the resistance of the virus to ganciclovir were responsible for the unfavorable outcome.

Conclusions: CMV infections, although rare, should always be considered in the immunocompetent host.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## P60: A case of tuberculosis in parotid

Damianaki A[1], Krietsepi V[1], Parlamenti K[1], Athanasiadou A[1], Zouglos A[1], Panou E[2], Kastanakis S[3]

[1]*Respiratory Department St. George General Hospital of Chania Crete, Greece*
[2]*Intensive Care Unit St. George General Hospital of Chania Crete, Greece*
[3]*Internal Medicine Department St. George General Hospital of Chania Crete, Greece*

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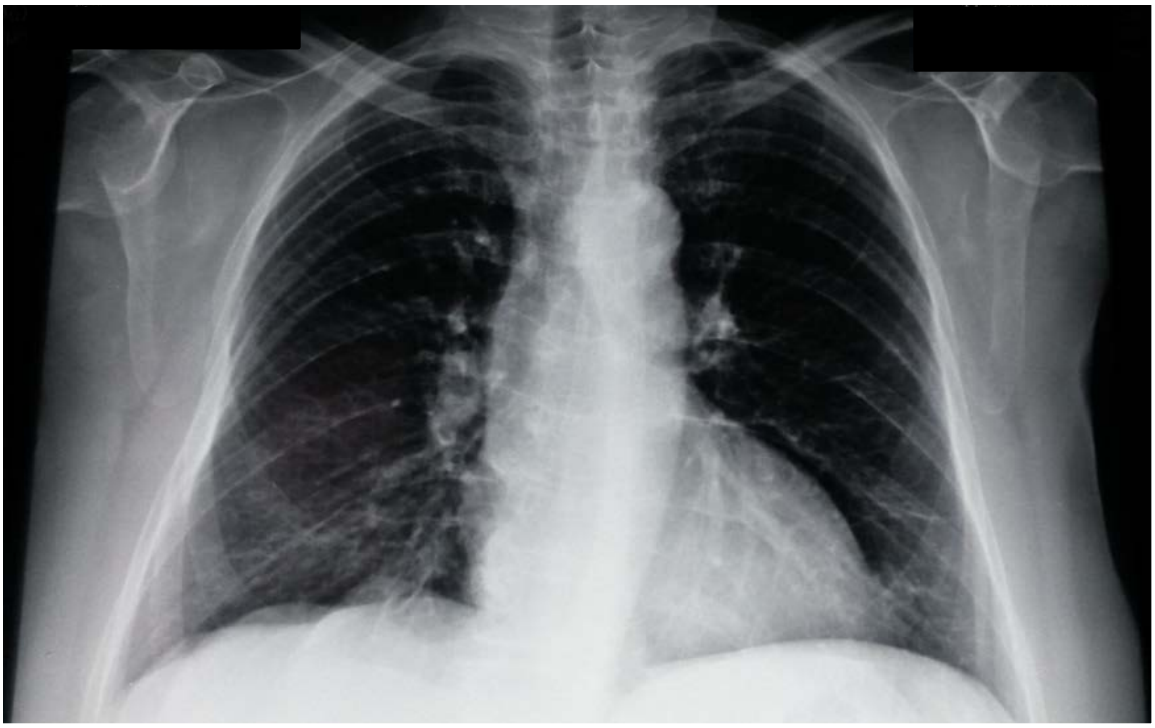
Keywords: Tuberculosis, parotid, mass

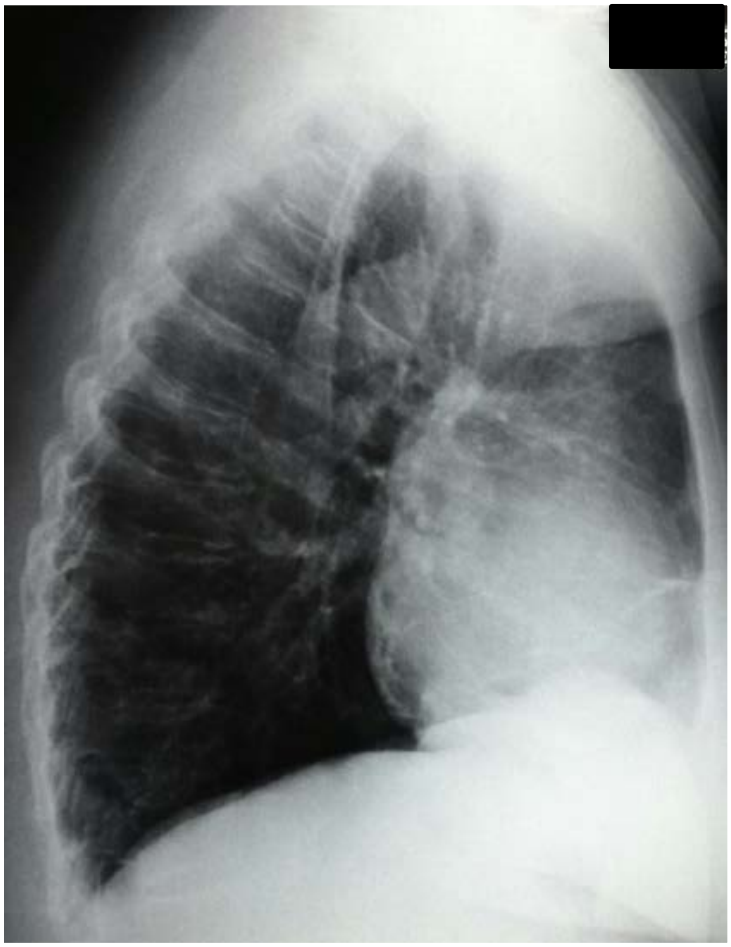
Objectives: tuberculosis is chiefly pulmonary although extrapulmonary cases make sometimes doctors have this disease in mind in case they confront diagnostic difficulties. We present a case of tuberculosis localized in the right parotid.

Methods: A 66 years old woman, nonsmoker, with a negative medical history complained of a palpable slightly painful lump under her right ear which was growing gradually in a period of time of 3 years. She referred no other annoying symptoms as fever, weight loss, night sweats and this was the reason she didn't ask for medical advice all these years. Her laboratory tests were normal except for an elevation of erythrocyte sedimentation rate (48) and C-reactive protein (16,89mg/l). Mantoux test was positive (15mm).The chest x-ray revealed some sparse calcified tiny nodules near the right hillum and lung parenchyma. MRI confirmed the presence of a parotid mass. Sputum for Ziehl Nielsen stain and culture was negative for Koch bacillus. The patient was sent for surgical intervention and resection part of the parotid contained the mass.

Results: caseous granulomatous lesions with central necrosis and a surrounding zone of epithelioid cells and giant cells and peripheral zone of lymphocytes and fibroblasts were depicted indicative of active tuberculosis. The patient immediately was given 9INH+9RIF+2ETH+2PZ with no serious side effects. CRP and ESR revert to normal gradually. The patient has no relapse after 1.5 year of follow up.

Conclusions: Tuberculosis is sometimes insidious and can imitate other diseases. That’s why the clinicians must be alert of this possibility.





## P61: Pantoea agglomerans septic arthritis of the knee in the adult, diagnosed with eubacterial polymerase chain reaction

Čurić K[1], Beović B[1], Meglič-Volkar J[1], Videčnik Zorman J[1], Malovrh T[2], Sluga B[2], Ružić Sabljić E[3], Cerar Kišek T[3]

*[1] Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia*
*[2]Department of Traumatology, University Medical Centre Ljubljana, Slovenia*
*[3]Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia*

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Keywords: Pantoea agglomerans, septic arthritis, eubacterial polymerase chain reaction

We present a case of culture negative, but polymerase chain reaction identified Pantoea agglomerans septic arthritis of the knee in a 31-year old male patient.

After sustaining blunt trauma to his right knee while tearing down a greengage tree without any local visible skin injury patient had to be treated twice because of recurrent septic arthritis of the injured knee despite initial apparently successful treatment with levofloxacin. Cultures of the joint fluid remained sterile, while Pantoea agglomerans was identified from joint aspirate and tissue sample collected on second arthroscopy using eubacterial polymerase chain reaction. Patient recovered only after a migrating greengage thorn under the lateral meniscus became visible during the fourth arthroscopy and was removed in combination with antibiotic therapy.

Pantoea agglomerans, a plant pathogen, is a gram negative aerobic bacillus from the family of Enterobacteriacae. Only eight cases of Pantoea agglomerans septic arthritis have been reported, predominately in children. A delay in identifying the causative pathogen is a consequence of inflammation developing long after the thorn injury, because of the indolent nature of Pantoea agglomerans, low levels of clinical suspicion and inappropriate imaging techniques.

All strains of Pantoea agglomerans reported were uniformly susceptible to aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, broad-spectrum cephalosporines and imipenem.

Our case shows that molecular diagnostic technics may be useful in clinically suspicious, but culture negative septic arthritis. When history of joint plant injury is reported, arthroscopic inspection and synovectomy should be considered, because small plant particles are not visible with imaging techniques, otherwise recurrence is probable.

## P62: Neuroborreliosis mimicking Multiple Sclerosis: A Case report

Karagöz E[1], Demir S[2], Turhan V[1], Ülçay A[1], Toğrol E[2]

*[1]GATA Haydarpasa Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*
*[2]GATA Haydarpasa Training Hospital, Department of Neurology, Istanbul, Turkey*

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Keywords: Lyme neuroborreliosis, multiple sclerosis, Borrelia burgdorferi

Lyme neuroborreliosis (LNB) is one of the chronic manifestations of Lyme disease and is caused by a tick-borne spirochete, Borrelia burgdorferi. This disease may manifest with episodic focal neurological involvement and differential diagnosis of LNB may be difficult and may not be distinguished from Multiple sclerosis (MS) in some instances. Herein, we report a 28 year old male patient with typical clinical features of MS who was found to have serum antibodies to Borrelia burgdorferi. Since there was no respond to 10-day cortisone therapy, determining oligoclonal band negativity in cerebrospinal fluid (CSF) and presence of serum and CSF antibodies for Borrelia burgdorferi; the patient was diagnosed as LNB. Magnetic Resonance Imaging (MRI) of the brain with Contrast demonstrated a T1 hypointense and T2 hyperintence well defined, 6x6 mm sized nodular periventricular and corpus callosal multiple lesions, calloseptal and periventricular white matter involvement in the the brain. Additionally, postcontrast sagittal and axial T1-weighted MR images show diffuse this cervical cord involvement including C3-C5 level. The patient was treated with ceftriaxone 1 gr twice a day for the first 3 weeks. After this therapy since his complaints were still present, an additional three months of treatment with 100 mg of doxycycline twice a day and 500 mg of azitromycin three times a day were administered. After this antibiotic treatment, his complaints were reduced and physical examination and laboratory were found to be normal despite there is only a few improvement in his control MRI.

We conclude that lyme neuroborreliosis (LNB), which is a neurotropic spirochete infection, should always be considered in the differential diagnosis of MS.

## P63: Case presentation - Chikungunya virus infection

Sredojević M, Harej M

*Section for Tropical Diseases in Slovenia*

Keywords: Travel, arthralgia, Chikungunya

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus. The disease is clinically presented with abrupt fever illness (>38,9°C), maculopapular rash and polyarthralgia. It has caused devastating epidemics in Indian Ocean Basin and Asia in the past. Most recently local transmission had been identified in 31 countries in Americas, especially in the Caribbean in 2014. Herein we present the clinical case of 44-years old woman that attended the Clinic of Infectious Diseases in Ljubljana in February 2014, after returning from 3-weeks travelling through Indonesia and Malaysia. The patient noticed the first signs of illness in her second week of travel. It all started with a nonpruritic rash on her forearms and thighs and was quickly followed with a rise in temperature up to 40°C which lasted for 3 days, but was still remitting in the following weeks. The fever was accompanied with severe general joint pain. The rash spread over the entire body after 2 days. After one week she noticed problems with her peripheral vision (it was foggy and tingling). Based on clinical features and epidemiological data we suspected Dengue/Chikungunya infection. PCR and serology were positive for Chikungunya virus. Since there is no specific antiviral drug treatment for Chikungunya, she was put on symptomatic treatment with nonsteroidal antirheumatic drugs. The arthralgia persisted for months, with difficulties to walk and write, but it gradually subsided and after five months it was completely resolved.

## P64: Imported filariasis in Serbia

Dakić Z[2,3], Lavadinović L[2,3], Stevanović G[2,3], Pelemiš M[1], Jovanović S[2,3], Milošević B[2,3], Urošević A[2,3], Dulović O[2,3], Poluga J[4]; Džamić A[5], Indjic N[1], Ofori-Belić I[2,3], Pavlović M

*[1]Department of Microbiology, Clinical Center of Serbia,*
*[2]Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Serbia*
*[3]Faculty of Medicine, University of Belgrade, Serbia*
*[4]Department of Parasitology-Micology, Faculty of Medicine*
*[5]Center of Medical Preventive Care, Belgrade*

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Keywords: imported filariasis, diagnosis, Serbia

Objectives: Most patients with suspected filariasis in Serbia are referred to the Clinical Center of Serbia for diagnosis and treatment. The goal of this study was to examine the prevalence and trend in the occurrence of imported filariasis in Serbian travelers returning from tropical and subtropical areas.

Methods: The study involved all travelers returning from endemic areas, who presented at the Clinic for Infectious and Tropical Diseases in Belgrade between January 2003 and December 20012. Clinical findings and epidemiological data defined suspicion of filariasis. Knott's concentration method and Giemsa-stained blood smears were used for diagnostics.

Results: The study series involved a total of 2667 travelers, including 187 tested for filariasis.

A total of 11 (5.9%) patients were diagnosed with filariasis, respectively 0.4% of all travelers. Visited countries were Equatorial Guinea, Gabon, Cote d'Ivore, Nigeria. All patients had eosinophilia and ten swellings on the extremities. Microfilaremia was confirmed in five (45.5%) patients but, identification of filaria species was performed in only three patients. Bifilarial infection (Loa loa and Mansonella perstans) had two and L. loa one patient. One case of bifilarial infection and concurrent disseminated renal cell carcinoma was resulted in death. Patient with confirmed loiais had coinfection with Plasmodium malariae. In six amicrofilaremic patients, the clinical diagnosis of loiasis was confirmed indirectly, by a successful therapeutic effect.

Conclusions: The presented results show a relatively low presence of filariasis in the traveler population as a whole. The high levels of amicrofilaremic travelers necessitates improving diagnostic protocols for filariasis.

## P65: A rare case of Streptococcus pneumoniae tubo-ovarian abscess

Papadogianni M, Papadomanolaki E, Aleuraki G, Tzouganakis E, Tsouri A, Kastanakis S

*Microbiology Department, Saint George General Hospital of Chania, Crete, Greece*

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Keywords:

Objective: We present a rare case of tubo-ovarian an abscess caused by Streptococcus pneumoniae occurred in a previously healthy 52-year-old woman.

Materials and methods: A 52-year-old previously healthy woman was admitted to our hospital because of high temperature (40°C) and acute abdominal pain. Her clinical condition required urgent surgery. The laparotomy revealed a tubo-ovarian abscess which had ruptured. Samples of pus were sent to the laboratory for direct observation, which was negative for microorganisms, and culture. All cultures were processed using the usual aerobic and anaerobic culture media and standard microbiological methods and susceptibility testing was performed using the Kirby-Bauer method, E-test and the automated system Vitek-2 (bioMérieux).

Results: After 24h of incubation all cultures yielded colonies of gram (+) coccus, catalase (-), a-haemolytic, escoulin negative and susceptible to optochin which was identified as Streptococcus pneumoniae. Vitek-2 (bioMérieux) also confirmed the diagnosis.

Conclusion: Streptococcus pneumoniae is not part of the normal vaginal flora, therefore acquiring infection from this unusual pathogen is complex. Tubo-ovarian abscess resulting from such an infection is a rare occurrence, however cases like this adds to the body of knowledge concerning genital infections and Streptococcus pneumoniae pathogenesis.

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